# Quality Factors of Fruits and Vegetables

In Quality Factors of Fruits and Vegetables; Jen, J.;
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# Quality Factors of Fruits and Vegetables

## Chemistry and Technology

**Joseph J. Jen,** EDITOR
*The University of Georgia*

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# Foreword

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# Preface

QUALITY OF RAW AND PROCESSED FRUIT AND VEGETABLE PRODUCTS are sometimes elusive factors and may differ from person to person based on individual tastes. However, there is no doubt that consumers want high-quality foods in our fast-moving society. Consumers want convenient, fresh, light, and nutritious products for their diets. Fruits and vegetables fit in the healthy diet. The scientists and technologists who work with fresh and processed fruits and vegetables must deliver high-quality products to consumers economically. It was with this thought in mind that I organized the symposium on which this book is based.

I had a second motive in organizing this symposium on quality factors of fruits and vegetables. Literature and research reports on the subject appear in many different places: in journals and books that cover food science, food technology, plant physiology, plant pathology, horticulture, agricultural sciences, agricultural engineering, and packaging. One cannot gather the vast amount of information on the quality of fruits and vegetables in one volume. However, I hope this book can serve as an important starting reference for researchers working in this field. It may also serve as a textbook for students interested in fresh and processed fruits and vegetables.

The book is separated into six sections and 30 chapters. An overview chapter is provided to introduce the subject. The first three sections deal with the chemical aspects of the three major quality factors of fruits and vegetables, that is, color, flavor, and texture. The next three sections deal with the technological aspects of quality factors of fresh and processed fruits and vegetables: Section four deals with storage and light processing of fresh produce; section five deals with quality improvement of further processed fruits and vegetables; and section six deals with some of the newer technology used in this field.

With 30 chapters and multiple authors in this book, it is unavoidable that some minor duplication of coverage may occur. The comprehensiveness of each chapter will differ. Nevertheless, all authors are well-known researchers in their respective fields or have published extensively in the subject. I hope that cross-fertilization among the authors can help the growth of this scientific field.



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I want to thank many people for assisting me in bringing this book to fruition. I want to thank the authors for presenting the papers and for their cooperation in completing the chapters in a timely manner. Also, more than 50 scientists served as reviewers. Their comments contributed greatly to the quality of the book. The editors of the ACS Books Department worked patiently with me. My wonderful, diligent secretary, Beth Knight, assisted in the handling of the manuscripts. Last, but not least, I appreciate the financial assistance provided by the Financial Committee of the Division of Agricultural and Food Chemistry of the American Chemical Society, the Agricultural Research Division of Campbell Soup Company, the Basic Research Division of Kraft, Inc., and the Research and Development Division of R. J. Reynolds Tobacco Company.

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# Chapter 1

# Chemical Basis of Quality Factors in Fruits and Vegetables

## An Overview

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Color, flavor, texture, and nutritive value are generally recognized as the four quality factors of fruits and vegetables. The natural pigments, chlorophylls, carotenoids and anthocyanins, form the chemical basis of color. Enzymatic and non-enzymatic browning contribute to coloring of certain processed fruits and vegetables. Various volatile aroma and nonvolatile compounds give fruits and vegetables special flavors. Cell wall components and turgor pressure are the two entities that provide the texture of fruits and vegetables. Pectic substances and pectic enzymes are closely related to firmness and softening of many fruits and vegetables. Celluloses and lignins are associated with toughness and woody texture. The roles of hemicelluloses and extinsins in fruits and vegetables are not clear. Vitamin C and minerals are the major nutrients of fruits and vegetables. Processing often alters the quality of fruits and vegetables but does not change the chemical basis underlining the factors.

Fruits and vegetables constitute an important part of the human diet. It is one of the four major groups of food our body needs to ingest daily. The current consumer trend is towards fresh, natural, minimally processed, and, yet, convenience foods. This situation increases the opportunities for this segment of diet to play an increased role in our health and well-being. Consumers purchase foods on the basis of quality. To improve quality of fruits and vegetables, we must understand the chemical basis of the quality factors. Many books and chapters have been written on food quality although few have been devoted solely on fruits and vegetables (1, 2). Brief summaries of the subject matter can usually be found in food chemistry books (3). The purpose of this chapter is to provide an overview of the chemical bases of quality

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factors in fruits and vegetables. Subsequent chapters will provide detailed information on many specific topics. The flavor and nutritive value of foods are often the subject of many symposia and books. This volume will emphasis color and texture of fruits and vegetables.

## Color

With few exceptions, the coloring matters of fruits and vegetables belong to one of the following four categories of chemical compounds (4):

1. Tetrapyrrole derivatives: chlorophylls, pheophytins
2. Isoprenoid derivatives: carotenoids, xanthophylls
3. Benzopyran derivatives: anthocyanins, flavonoids, and related compounds
4. Certain artifacts: caramels, melanins, etc.

The first three groups are natural pigments while the last group is reaction products of enzymatic and non-enzymatic browning reactions from natural physiological changes or from processing of fruits and vegetables.

The chlorophylls are responsible for the green color of nearly all fruits and vegetables. The isomers, chlorophyll *a* and chlorophyll *b*, exist in a 3:1 ratio in higher plants. They have slightly different visible spectra and color shades. Chlorophylls are magnesium-chelated tetrapyrroles with an esterified 20 carbon alcohol, phytol. The phytol gives the pigments a unique ability to be embedded in the cell organelle chloroplasts. The naturally existing chlorophyllases can convert chlorophylls to water-soluble chlorophyllides, without the phytol, but do not significantly alter the green color. Acidic conditions can cause the replacement of hydrogen for magnesium and make the chlorophylls into pheophytins. The pheophytins are brown in color and are normally undesirable in most foods. The loss of green color in green vegetables is an important problem in certain thermal processing operations.

The chlorophyll chemistry has been the subject of several investigations. The conversion of chlorophylls to pheophytins follows first-order reaction kinetics (5). The photooxidation of chlorophylls have slow reaction rates (6). Organic acids released from the destruction of intact cells can cause conversion of chlorophylls to pheophytins in processed plant foods. Acids are also formed during processing of certain green leafy vegetables (7). Various methods developed to preserve the green color of processed food have undesirable effects of one form or another. Green metallocomplexes of chlorophyll derivatives formed during thermal processing offer a possible solution to some vegetables. A chapter dealing with this subject is included in this book.

The carotenoids are a group of lipid-soluble pigments responsible for the yellow, orange, and red color of many fruits and vegetables. Most plant foods contain a variety of carotenoids that differ mainly in their content of double bonds and oxygen atoms. The mevalonic acid pathway derived isoprenoids may also occur in combination with reducing sugars via glycosidic bonds.

Carotenoids are the major source of pro-vitamin A in our diet. Fortunately, they are relatively heat stable and do not suffer extensive loss during thermal processing. However, most carotenoids are sensitive to photoxidation and may lose their bright color under long exposure to light and oxygen. In intact fresh tomatoes, light can promote carotenoid biosynthesis (8).

Anthocyanins are a group of reddish water-soluble pigments that are widespread in plant foods. Anthocyanins, together with flavonoids, are the largest group of natural pigments due to their conjugation with glycoside compounds. The destruction of anthocyanins are pH and temperature-dependent, making it difficult to preserve during thermal processing operations. The unique feature of anthocyanins is the display of different colors at acidic and alkali pH conditions. Sometimes metal complexes and dimerization of anthocyanins may lead to different color shades from the free benzopyran bases. The use of anthocyanins as natural color additives to foods has only been partially successful due to the unstable nature of the pigments (9).

Other natural pigments existing in selected plant foods include tannin, betalain, leucoanthocyanin, quinone and xanthone. Table I lists the color and stability of natural pigments in fruits and vegetables (10).

Table I. Summary of Characteristics of Natural Pigments in Fruits and Vegetables

| Pigment Group | No. Compounds | Colors | Stable To | Sensitive To |
|---|---|---|---|---|
| Chlorophylls | 25 | Green, Brown | Alkali | Heat, Acid |
| Carotenoids | 300 | Yellow, Red | Heat | Light, Oxidation |
| Anthocyanins | 120 | Red, Blue | -- | pH, Heat |
| Flavonoids | 600 | Yellow | Heat | Oxidation |
| Betalains | 70 | Red | -- | Heat |
| Leucoantho-cyanins | 20 | Colorless, Yellow | Heat | -- |
| Tannins | 20 | Yellow | Heat | -- |
| Quinones | 200 | Yellow | Heat | -- |
| Xanthones | 20 | Yellow | Heat | -- |

Enzymes known as polyphenol oxidases cause enzymatic browning. Other names of the enzyme include phenolases and tyrosinases. The enzymes catalyze the conversion of monophenols and diphenols to quinones. The quinones can undergo a series of non-enzymatic reactions to produce brown, gray and black colored pigments, collectively known as melanins (11). Maillard reactions, caramelizations and ascorbic acid oxidations can produce similar types of colored compounds (12). For some food processing

operations, browning reactions are desirable, such as in potato chip manufacturing. However, in most fruit and vegetable products, browning reactions, whether enzymatic or non-enzymatic, are undesirable. This is to maintain the natural coloration of the plant foods. Sulfites, a very effective browning inhibitor, is experiencing declined usage due to health reasons to asthmatic consumers. Alternative browning inhibitors are described in a subsequent chapter.

Research projects in basic enzymology of polyphenol oxidase and the chemistry of non-enzymatic browning may lead to new browning inhibitors. Reviews on polyphenols oxidase (13) and on Maillard reactions (14) are available. The interest in enzymatic browning is aided by skin cancer research as melanins are the pigments involved in the coloring of human skin.

## Flavor

In recent years, the instrumentation analyses of flavor compounds in foods have given us a rather comprehensive knowledge on the chemical compounds that give the flavor sensation of fruits and vegetables (15). The biosynthetic pathway and enzymes involved in biogenesis of flavor compounds of many fruits and vegetables have also made progress (11). It is beyond the scope of this chapter to discuss identification and biosynthesis of fruit and vegetable flavor compounds. The techniques involved in the prevention of off-flavor formation during processing and storage periods is of interest to food chemists. The enzymes involved in off-flavor formation in fruits and vegetables seem to be peroxidases and lipoxygenases. These enzymes have the ability to form highly reactive free radicals and hydroperoxides. Lipid and unsaturated fatty acids are the substrates for the oxidase activities. It has been an industry standard for years to blanch vegetables to total destruction of peroxidase in shelf-stable foods. In recent years, some researchers have raised the question of whether blanching to peroxidase free condition may be over-processing. Lowering of blanching time or processing temperature can lead to superior quality products and can save energy in operation. Research on lowering the heat treatment to inactivate lipoxygenase rather than peroxidase has yielded promising results in selected vegetables. Details of this research are discussed in another chapter in this book.

Using flavor enzyme to regenerate lost fresh food flavor in processed fruits and vegetables has received limited attention (16). Flavorase can catalyze flavor regeneration of banana, raspberry, peanuts and a number of vegetables, e.g., cabbage, onions, carrots, green beans and tomatoes (11). With consumer interest in high quality products, renewed interest in the concept of flavorase is likely to occur. The rapidly spreading biotechnology techniques for mass production of high valued ingredients, including food flavor, will undoubtedly soon accelerate flavor research. Currently, scientists are working on fruit and vegetable tissue culture research (17). A chapter on the application of biotechnology to improve the quality of fruits and vegetables is included in this book.

## Texture

Texture is probably the most elusive quality factor of fruits and vegetables. Scientists have different definitions for food texture (18, 19). In food chemistry, texture of fruits and vegetables represent the biomolecules involved in the cellular structure of cell walls. The degradation during natural physiological transitions or artificial processing operations may alter textural properties of foods. The size and shape of the cells and the turgor pressures are factors in determining textural parameters of fresh fruits and vegetables. Some of the most important parameters are hardness, firmness and crispness.

The classes of macrobiomolecules in cell walls of fruits and vegetables that have received the most attention are pectic substances and pectic enzymes. A recent book provided an in depth review of the chemistry and function of pectin (20). Considering the importance of pectins and their close relationship with textural properties of fruits and vegetables, more information on the subject will continue to be gathered.

Pectic substances are the glues to plant cells and exist predominately in middle lamella between cell walls. The American Chemical Society defined the terms protopectin, pectinic acid and pectic acid (21). The native protopectin is a long chain, alpha-1, 4-D-galacturonic acid polymer, interspersed with alpha-1,2-L-rhamnose residues. Shorter polymers, galactans and arabinans exist and are bonded covalently with the main chain (22). Many types and different amounts of side chains exist in various plant tissues. In apple pectin, hairy regions and smooth regions were observed (23). The hairy region has a backbone of rhamnogalacturonan carrying arabinogalactan and xylogalacturonan side chains. The smooth regions have homogeneous galacturonan chains of 70-80% methylation. Other fruit or vegetable protopectin fractions probably have similar but different structures. The nature of bonding between pectin molecules and other polysaccharides and structural protein, extinsins, have been proposed in cultured plant tissue (24).

The solubilization of insoluble protopectin to water-soluble pectinic acid is a phase of the natural ripening process in many fruits and vegetables. Besides several polysaccharides and structural proteins, other substances such as calcium and magnesium may play a role in the firmness of raw and processed fruits and vegetables. The divalent ions can form a bridge between free carboxyl groups of pectic chains to give rigidity to the cell structure. Many plant cell walls contain pectins with high degree of methylation and few free carboxyl groups for such bonding to occur. Polygalacturonases (PG) are enzymes that can break down the free pectic acid chain into lower molecular weight pectins. Highly methylated pectins must be de-esterified by pectin methyl esterase (PME) before PG action. For certain vegetables, such as tomatoes, a direct correlation between pectic substances or pectic enzyme activities and textural softening do not exist (25). Since the blossom end of a tomato ripes faster than the stem end of the fruit, a non-uniform sample exist in each tomato fruit. In one experiment carried out in our laboratory, the tomatoes were cut

into three equal sections and analyzed as separate samples. Linear correlations between PME activities and texture were obtained for each of the three sections (Fig. 1). However, in the same experiment, no correlation of textural properties of tomato fruits with different fractions of pectins or PG activiaties were observed.

The diversified fruit and vegetable cells make direct correlation between cell wall compositions and textural properties difficult to achieve. However, within a single fruit or vegetable, many such correlations are possible. These correlations can provide useful information for process optimization to produce high quality products with desirable textural properties.

Other cell wall polysaccharides, the celluloses, hemicelluloses and lignins, have received less attention in comparison with pectins. Celluloses and lignins are normally associated with woody, tough and other undesirable textural qualities. The hemicelluloses are difficult to study in fruits and vegetables. The structural proteins of the cell walls, the extinsins, have received little or no attention in fruits and vegetables. However, the extinsins could very well provide information needed to fully explain many phenomena observed in the ripening and senescence of fruits and vegetables. More basic information on extinsins and their interrelationships with fruit and vegetable cell wall polysaccharides are needed.

## Nutritive Values

The most important nutritive values of fruits and vegetables are their content of vitamin C, minerals and fiber. Various amounts of other vitamins and nutrients exist in fruits and vegetables which contribute significantly to the balance of our diet (26).

It is a general belief that fresh, raw fruits and vegetables contain the highest amount of vitamins and minerals. This belief is not always true. The nutritive value of fruits and vegetables vary due to many factors. Some of the factors are varieties, maturities, cultural practices, climate and location of the growth of the plant foods. Home storage time and method of preparation before consumption has significant bearing on nutrient retentions (27).

Processing does have some destructive effect on the nutritive value of foods. This topic is reviewed in book form (28). Most national brand processed foods have tight ingredient specifications. Thus, most frozen and canned fruits and vegetables contain as much nutrients as fresh, raw products. Other processed foods contain enriched or fortified vitamins and minerals. Ascorbic acid is often higher in processed foods than in non-processed products. Consumers are interested in fresh, natural, raw and minimally processed foods. Fortification and enrichment of nutrients are more difficult in these foods.

## Effect of Processing on Quality Factors

The food industry consistently seeks new and improved processing technology to produce high quality products at a low cost. The

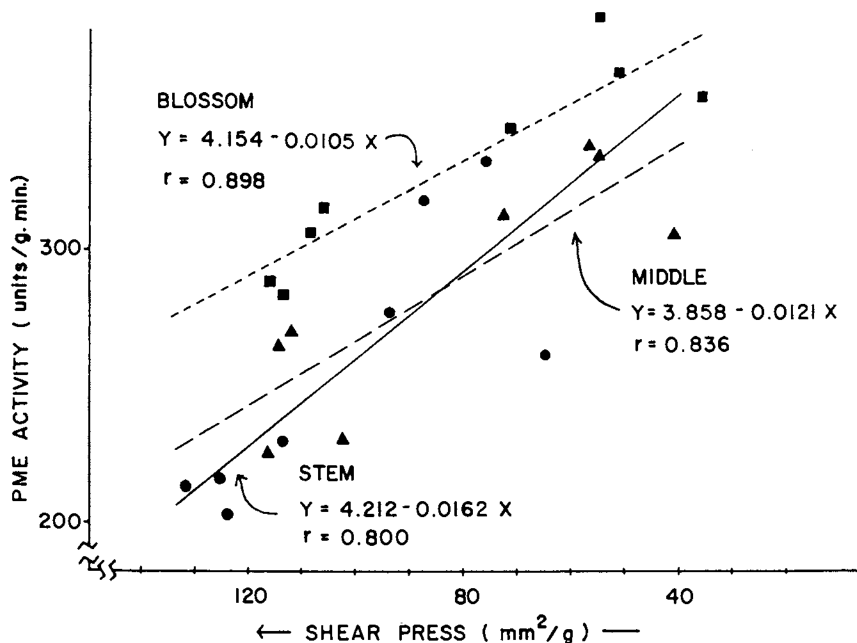


Figure 1. Correlation between the firmness values and PME activities of ripening tomatoes. Mature green tomatoes were held at room temperature for seven days ripening periods. Each day several tomatoes were sectioned longitudinally into three equal sections (■-----■ blossom section, ▲– –▲ middle section, ●——● stem section). Half of the sectioned samples were used for PME assays and the corresponding other half sectioned samples were used for textural measurement on an Instron Universal Tester. Each mark on the figure represents the average of three samples. Statistical analyses were performed to obtain the linear regression lines for each section.

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research and development departments of food industry, academic and other research institutions are all interested in this research. In addition to the traditional canning, freezing, pickling and dehydration processes, several new processing techniques have surfaced in recent years. Irradiation with gamma rays, storage with controlled and modified atmospheric environment, reverse osmosis and ultrafiltration will be discussed in subsequent chapters. The emphasis will be on chemistry of quality improvement of the processed fruit and vegetable products.

When fresh fruits and vegetables are thermally processed, alteration to color, flavor, texture and nutritive values may occur. It is the intention of the food technologists to either lessen these alternations or to enhance quality factors by processing.

Raw fruits and vegetables are living organisms that undergo physiological changes after harvest. All processing technologies are to stop the physiological and enzymatic activities with minimum disruption of cellular structures. Stoppage of physiological functions means the freezing of chemical compositions in fruit and vegetable cells. Total inactivation of enzyme activities means that no chemical reactions occur in processed foods. However, some non-enzymatic chemical reactions may occur during processing, e.g., the formation of acid (7) and non-enzymatic browning (12). Other examples are non-specific hydrolyses of macromolecules, interconversion of sugars, aggregation of monomers, dissociation of polymers and changes of ionic bonding in cell walls (1, 2). Various literature reports are available concerning the chemical changes in fruits and vegetables during processing. Several chapters in this book will deal with this subject in depth. With the raise in consumer demand of quality products, more research funds are needed to investigate the chemistry of quality improvement of fruits and vegetables.

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Chapter 2

# Chemistry of Color Improvement in Thermally Processed Green Vegetables

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Chlorophyll degradation during thermal processing of green vegetables leads to losses in color and consumer appeal. Rapid formation of olive-colored pheophytin and pyropheophytin during heating is initiated by the release of cellular acids and continues during storage. Previous efforts to maintain the color of heat processed vegetables have met with limited success. Green metallocomplexes of chlorophyll derivatives formed during thermal processing have been observed and offer a possible solution to the problem of color loss. In this review, the chemistry of chlorophyll and its derivatives formed during thermal processing are presented. Also discussed is the technology of color preservation of green vegetables including recent developments on regreening of commercially canned green vegetables through the formation of metallocomplexes.

Color of foods is often the first of many quality attributes judged by the consumer and is, therefore, extremely important in overall product acceptance. The importance of color in green vegetables is demonstrated by USDA quality standards where as much as 60% of the total quality score is assigned to color. The decreasing market share of canned relative to frozen green vegetables can be attributed to the dramatic color difference between the two products (1).

Attempts to improve the color quality of thermally processed green vegetables have included chlorophyll retention through pH control of process solutions, high temperature short time processing, and enzymatic alteration of chlorophyll within plant tissues to a more stable structure. However, none of these methods have been entirely successful. Prolonged storage of canned vegetables has occasionally resulted in the development of a non-uniform, but desirable, green color. This spontaneous regreening process has been attributed to the formation of

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metallocomplexes of chlorophyll derivatives. Recent FDA approval for the addition of zinc salts to process solutions has led to renewed interest in color improvement through complex formation in commercially canned green vegetables.

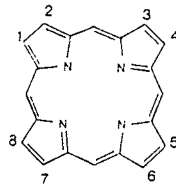
In this chapter, the chemistry of chlorophyll and its derivatives formed during thermal processing and the technology of color preservation in green vegetables will be reviewed.

Chemistry of Chlorophyll. Chlorophylls are magnesium complexes derived from the compound porphin which consists of four pyrrole rings joined by methine bridges. Substituted porphins are named porphyrins. The porphyrin, phorbin, (Figure 1) is considered the basic nucleus of chlorophyll and is formed by the addition of the isocyclic ring (2). Chlorophyll (Figure 1) is further characterized by the hydrophobic C-7 phytol chain and a carbomethoxy group at the C-10 position. Chlorophyll *a* and *b* differ in structure only by the presence of a methyl or formyl group, respectively, at the C-3 carbon and, in green vegetables, are present in an approximately 3 to 1 ratio. Chlorophyll derivatives formed during thermal processing can be directed into two groups; magnesium-containing and magnesium-free derivatives. Magnesium-containing derivatives have retained the magnesium atom in the tetrapyrrole nucleus and are green in color. Magnesium-free derivatives include olive-brown, hydrogen-containing compounds and the green metallocomplexes.

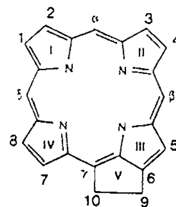
## Magnesium-containing Chlorophyll Derivatives

Chlorophyll isomers. Isomerization of chlorophyll is caused by inversion of the C-10 carbomethoxy group. It occurs rapidly in heated plant tissues and in organic solvents. Ten percent conversion of chlorophyll *a* and *b* to *a'* and *b'* isomers was reported by Bacon and Holden (3) when leaves were held in boiling water for 5 minutes.

Chlorophyllide. Green chlorophyllides are formed from chlorophylls by hydrolysis of the C-7 propionic acid phytol ester bond. Formation of chlorophyllides may occur within intact plant tissue or when plant extracts are held in aqueous acetone solution (4). Chlorophyll hydrolysis is catalyzed by the enzyme chlorophyllase, believed present in all plants in varying amounts. Formation of chlorophyllides in fresh leaves does not occur until the enzyme has been heat activated. Reports of optimum temperatures for chlorophyllase activation in vegetables range from 60° to 82.2°C (5-8). Jones et al. (6) measured the amount of phytol-free pigments formed in cucumbers, okra pods, turnip greens, and green beans held in water at 180°F (82.2°C) for 4 minutes. Conversion of chlorophyll to chlorophyllide in the first three vegetables was 64.1, 49.6, and 11.2%, respectively. No phytol-free pigments were detected in green beans. In the same study, chlorophyllase activity was induced in unheated cucumbers by holding them in 7.5% sodium chloride solution at room temperature.




PORPHIN

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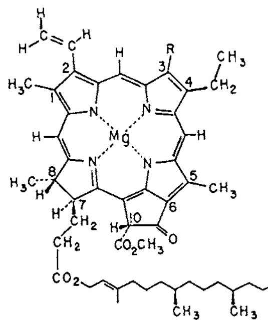


CHLOROPHYLL
a R = $CH_3$
b R = CHO

Figure 1. Structure of porphin, phorbin and chlorophyll a, b.

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In this laboratory, the conversion of chlorophyll to chlorophyllide in heated spinach leaves (cv. Melody) was followed using high performance liquid chromatography (HPLC). Figure 2(a-c) shows typical HPLC chromatograms of fresh spinach (a) and spinach blanched at 160°F (71°C) (b) or 190°F (88°C) (c). Activation of chlorophyllase in spinach blanched at 160°F is clearly illustrated by the formation of chlorophyllide while nearly complete inactivation of the enzyme was found at 190°F. Isomerization of chlorophyll with the application of heat was also detected. Chlorophyllase activity was followed throughout the growing period (Figure 3). Maximum activity was observed at the time the plant started flowering (solid line). Post-harvest storage of spinach at 5°C decreased enzyme activity compared to activities measured at the time of harvest (broken lines).

## Magnesium-free Chlorophyll Derivatives

Pheophytin. Displacement of the magnesium atom in chlorophyll with two hydrogen ions results in the formation of olive-brown pheophytin. The reaction is irreversible in aqueous solution (2). Degradation of chlorophyll *a* in acidified acetone occurs as much as 8 times faster compared to chlorophyll *b*, with activation energies for the two reactions ranging from 10 to 13 and 11 to 13 Kcal/mole, respectively (9,10).

Cho (11) studied the kinetics of pheophytin formation in acetone acidified with HCl and buffer salts. The reaction rate was found to be proportional to the square of both hydrogen ion and undissociated organic acid concentration. The author proposed that pheophytinization is a two-step reaction that begins with the rapid bonding of two hydrogen ions to two pyrrole nitrogen atoms forming an intermediate. Subsequent removal of magnesium is the slower, rate determining step. The greater stability of chlorophyll *b* was attributed to the electron withdrawing effect of the C-3 formyl group. The increased positive charge on the pyrrole nitrogens reduces the equilibrium constant for the formation of the reaction intermediate.

Rate studies have been conducted on the formation of pheophytin in vegetable purees under thermal processing conditions. Gold and Weckel (12) studied chlorophyll degradation using pea puree heated at temperatures between 100° and 138°C for up to 210 minutes and calculated an Arrhenius activation energy of 12.6 Kcal/mole for the reaction. Observed first order reaction kinetics was attributed to the 100-fold or more excess of hydrogen ions over chlorophyll molecules within plant cells. Chlorophyll degradation was directly proportional to the titratable acidity of pea puree adjusted with an acid or base before processing. Rate constants obtained using 300-gram samples of puree with 20 and 40 milliliters of 0.1 N sodium hydroxide added were 1.6 and 2.2 times lower than the value obtained for normal puree. Gupte et al. (13) confirmed the greater stability of chlorophyll *b* using spinach puree heated at temperatures between 260°F (127°C) and 300°F (149°C) for between 20 and 320 seconds. Arrhenius activation energies for the degradation of chlorophyll *a* and *b* within this

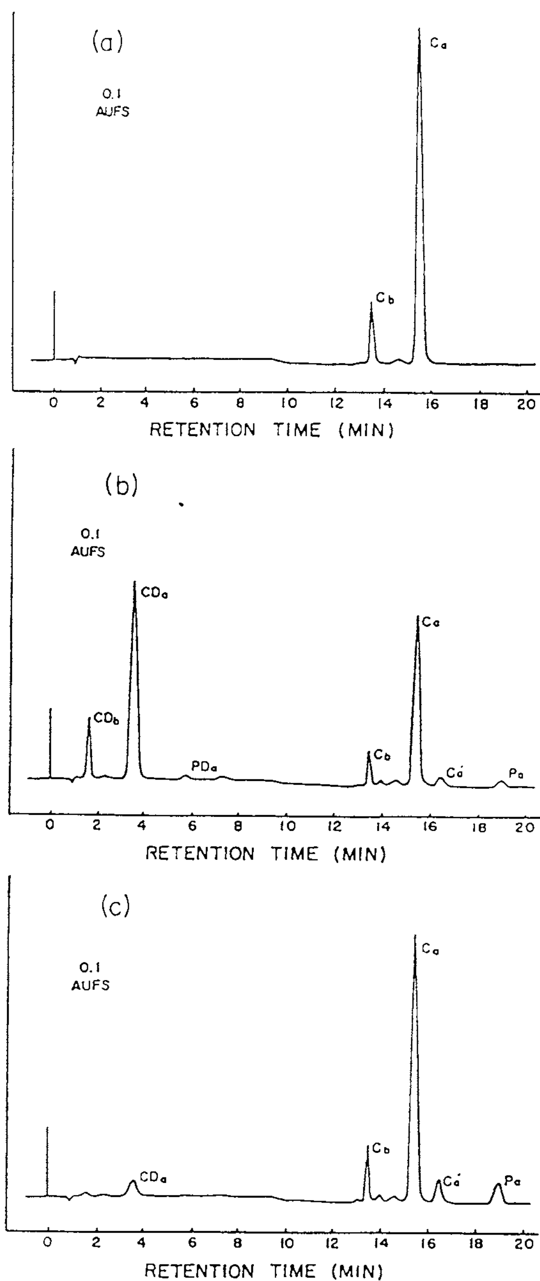


Figure 2. Chlorophyll and chlorophyll derivatives of spinach a) unblanched, b) blanched 3 min at 160°F (71°C) c) blanched 3 min at 190°F (88°C); C = chlorophyll, CD = chlorophyllide, P = pheophytin, PD = pheophorbide.

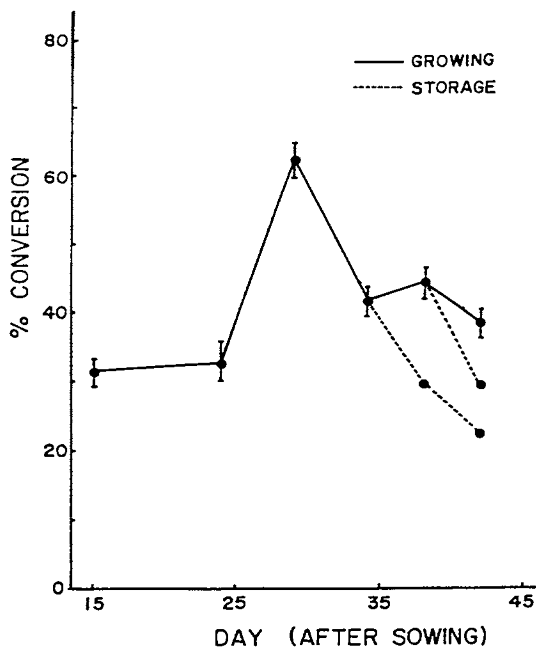


Figure 3. Chlorophyllase activity expressed as percent conversion of chlorophyll to chlorophyllide, in spinach during growth (solid line) and after storage at 5°C (broken line).

temperature interval were 143 Kcal/mole and 35.2 Kcal/mole, respectively.

Chlorophyll retention in cooked vegetables was reported by Sweeney and Martin (14) to vary with tissue pH levels. Spinach with an initial tissue pH of 6.8 retained over 60% of its original chlorophyll content after boiling in water for 20 minutes. Brussels sprouts with a pH of 6.3 retained less than 30% of their initial chlorophyll after the same treatment. Chlorophyll retention in green beans was 41% greater when boiled in citrate-phosphate buffer with a pH value of 6.9 instead of water.

Haisman and Clarke (15) studied the effects of heating time and temperature, pH value, species, and chemical additives on chlorophyll degradation in heated leaves. Formation of combined pheophytin *a* and *b* tended to be greater in plant tissues with low pH values when immersed in water at 60°C. The rate constant for the reaction in Pelargonium leaves (pH = 4.1) was 14 times greater than for pea seeds (pH = 6.9). The rate of chlorophyll conversion to pheophytin in tobacco leaves held in acetate buffer at 90°C was found to vary linearly with the square root of the hydrogen ion concentration and approached zero at a pH value of 7.8. The relationship was attributed to mediation of the transfer of hydrogen ions to chlorophyll molecules by the chloroplast membrane.

Compared to chloride ion, citrate, phosphate, and acetate ions, present in 1 molar solutions of their sodium salts increased the rate of pheophytinization in sugar beet leaves, heated to 90°C, 1.4, 1.5, and 2.5 times, respectively. No significant increase was reported using sulfate or nitrate anions. In contrast, increased chlorophyll retention was observed in tobacco leaves heated at 90°C using certain cations. Sodium, magnesium, and calcium cations as 1 molar chloride solutions decreased the rate of pheophytinization by 47, 70, and 77%, respectively, compared to heating in water. The effect of added anions and cations was increased when surface-active ions were used. Conversion of chlorophyll in brussels sprouts boiled in water for 15 minutes increased from 85 to 98% when 0.1% sodium dodecylsulfate (SDS) was added. The same concentration of cetyl trimethyl ammonium bromide (CATB) decreased the rate by 31%. Formation of pheophytin in tobacco leaves was decreased by 79% when 0.0005 molar CATB was used. Ionic effects were attributed to adsorption of anions or cations on the chloroplast membrane. Negatively charged ions catalyze the transfer of protons across the membrane while positively charged ions inhibit their transfer and decrease the rate of pheophytinization.

Schwartz an von Elbe (16) used high performance liquid chromatography (HPLC) to study chlorophyll degradation using spinach puree heated between 116° and 126°C. In contrast to previous studies, both the formation of pheophytin and its degradation product, pyropheophytin, were monitored. The first order rate constant for chlorophyll *a* degradation at 116°C was 2.1 times larger than that obtained for chlorophyll *b*. Rate constants for chlorophyll *a* and *b* increased 2.4 and 2.2 times within the range of increasing reaction temperatures, yielding activation energies of 25.2 and 22.5 Kcal/mole, respectively. The wide variation in reported values for activation energy was attributed

to various pH values of reaction media, differing temperature ranges, and that previous studies considered only pheophytin as a degradation product.

Pyropheophytin. Replacement of the C-10 carbomethoxy group of pheophytin with a hydrogen atom results in the formation of olive-colored pyropheophytin. Schwartz, et al. (17) reported the formation of pyropheophytin in green vegetables during heat sterilization. The amount formed is dependent on the heat severity of the process. The ratio of pyropheophytin to pheophytin in canned spinach, a product heated primarily by conduction, was approximately five times greater than that obtained for peas, heated more efficiently by convection. In the study previously mentioned (16), first order rate constants for the degradation of pheophytin *b* at 116° and 126°C were 1.6 and 1.3 times greater, respectively, than for pheophytin *a*. Activation energies of 20.7 and 15.7 Kcal/mole, respectively, were reported for the two reactions.

Pheophorbide. Replacement of the magnesium atom in chlorophyllide with hydrogen ions results in the formation of olive-brown pheophorbide. The degradation of chlorophyllide *a* and *b* and their respective methyl and ethyl esters in acidified acetone was studied by Schanderl et al. (10). Substitution of lower molecular weight alcohols decreased steric hindrance from the C-7 side chain and increased reaction rates. Ethyl-chlorophyllide *a*, methyl-chlorophyllide *a*, and chlorophyllide *a* reaction 1.08, 1.42, and 1.56 times faster than chlorophyll *a* at 55°C. The *b* derivatives of all chlorophyllides reacted approximately 5 times slower at the same temperature. Dependence of reaction rate on temperature is nearly unaffected by length of the C-7 side chain with activation energies of 10.4, 10.6, and 10.8 Kcals/mole reported between 25°C and 55°C, respectively for the 3 reactions.

Rate studies using plant tissues have been conducted under processing and storage conditions (7,8). In contrast to the previous study, pigment analyses and visual rankings have suggested that chlorophyllides are slightly more stable than chlorophylls at processing temperatures. However, these studies have not been conclusive because of the relatively small amounts of chlorophyllide initially available for kinetic studies and their rapid degradation under processing conditions (18).

Metallocomplexes of Chlorophyll Derivatives. The two hydrogen atoms within the tetrapyrrole nucleus of magnesium-free chlorophyll derivatives are easily displaced by zinc or copper ions to form green metallocomplexes. Zinc and copper complexes of pheophytin are more stable than chlorophyll in acidic solution. Lamort (19) ranked metal chelates of pheophytin *a* in 50% aqueous propanol according to the minimum pH value at which they are stable. Magnesium was easily removed in slightly acidic solution while zinc pheophytin *a* was stable in solution adjusted to a pH value of 2. Removal of copper was achieved only at pH levels sufficiently low to begin degradation of the porphyrin ring. Copper pheophytin formation in ethanol was found to be first order

with respect to copper(II) ions and pheophytin by Berezin and Koifman (20). Second order rate constants did not vary significantly when calculated at copper ion concentrations 15, 30, and 60 times in excess of pheophytin concentration. The rate constant for the formation of copper pheophytin a was 7 times greater than for copper pheophytin b at 25°C. Arrhenius activation energies were 14.5 and 14.0 Kcal/mole for copper pheophytin a and b, respectively.

Dempsey et al. (21) studied the kinetics and thermodynamics of the incorporation of metal ions into neutral porphyrins and concluded that the reaction is bimolecular and that the mechanism is of the $S_n2$ displacement type. It is proposed that the reaction begins with attachment of the metal ion to a pyrrole nitrogen atom followed by the immediate and simultaneous removal of two hydrogen atoms (22).

Comparative studies on the formation of organometallic complexes in vegetable purees indicate that chelation of copper is faster than zinc. Copper complexes were detected by Schanderl et al. (23) in pea puree with between 1 and 2 parts per million (ppm) copper after refluxing 1 hour. Zinc complex formation under the same conditions did not occur in puree containing less than 25 ppm zinc. Jones et al., (24) confirmed the greater reactivity of copper in complex formation. Chelation of zinc was 51.1% complete after 30 minutes of heating at 100°C when zinc chloride concentration was 40 times greater than chlorophyll concentration. The same reaction rate was obtained when copper sulfate concentration was only 1.5 times greater than chlorophyll concentration.

In the same study (24), the effect of pH on complex formation was studied. Spinach puree adjusted to a pH value of 8.5 had 38% more zinc complex formed than puree with an unadjusted pH value of 6.8. Formation of copper complexes was unaffected by hydrogen ion concentration between pH values of 3.8 and 8.5.

Substituent groups affect the stability and rate of metal incorporation because of the highly resonant structure of the tetrapyrrole nucleus. Dempsey et al. (21) reported that increasing the electron withdrawing character of groups substituted on mesoporphyrin decreased the rate of zinc complex formation. Replacement of two ethyl groups with two vinyl or two acetyl groups decreased the reaction rate in 2.5% aqueous sodium dodecylsulfate by 64 and 86%, respectively, while increasing the stability of the metal chelate (22). The smaller rate constants for the formation of copper pheophytin b relative to copper pheophytin a have similarly been attributed to the electron withdrawing effect of the C-3 formyl group (20). Migration of electrons through the conjugated porphyrin ring system causes pyrrole nitrogen atoms to become more positively charged and therefore less reactive to metal or hydrogen ions.

Steric hindrance, attributed to the phytol chain of pheophytin, may decrease the rate of complex formation. In the study by Berezin and Koifman (20), pheophorbide a reacted 4 times faster with copper(II) ion than did pheophytin a in ethanol at 25°C. Moreover, electrostatic interaction of the C-7 propionic

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acid group with copper(II) ions caused the reaction to deviate from second order kinetics.

Incorporation of metal ions into porphyrins is affected by other compounds in solution. Lowe and Phillips (25) found that copper(II) ions were chelated with dimethyl protoporphyrin ester 20,000 times faster in 2.5% sodium dodecylsulfate (SDS) than in 5% cetyl trimethyl ammonium bromide (CATB). The increased activity of SDS treated porphyrin was attributed to electrostatic attraction between anionic micelles formed around the tetrapyrrole nucleus and the metal cation. The authors also reported the influence of certain chelating agents on the rate of copper complex formation. Equimolar concentrations of copper and 8-hydroxyquinoline or sodium diethylthiocarbamate in 2.5% SDS increased the reaction rate 38 and 165 times, respectively, above the control. Secondary chelators may act by removing the hydration sphere on the metal ion increasing its attraction to pyrrole nitrogens (26).

## Technology of Color Preservation in Thermally Processed Vegetables

**Acid Neutralization.** Past efforts to preserve green color in canned vegetables have concentrated on retaining chlorophyll or green magnesium-containing derivatives of chlorophyll with the addition of alkalizing agents to process solutions. Calcium, magnesium, and sodium buffer salts were first used to raise the pH value of vegetables during sterilization, however, the product was often soft and had an alkaline flavor (27-30).

Blair (31) recognized that excessive concentrations of calcium and magnesium ions toughened processed vegetables and that sodium ions had a softening effect. Vegetables held in 0.1 N sodium hydroxide or sodium chloride solutions before or after blanching could be depleted of calcium and magnesium ions allowing for their controlled replacement in subsequent steps. Blanch and brine solutions of 0.005 M calcium hydroxide and 0.02 M magnesium hydroxide, respectively, were sufficient to raise the pH of peas to 8.5 immediately after processing as the calcium and magnesium ions restored tissue firmness (32). The use of hydroxide ion as an alkalizing agent eliminated the problem of off-flavors associated with the use of buffer salts.

Modifications of these procedures were patented in later years. Stevenson and Swartz (33) proposed holding vegetables in 0.2 N sodium hydroxide and enough calcium salts to maintain texture as the pH value of the product was raised above 8. The higher incidence of objectionable magnesium ammonium phosphate crystals (struvite) in peas processed with magnesium hydroxide led Geiseker (34) to use ammonium salts in holding solutions. Bendix et al. (35) raised the pH of canned vegetables with sodium hydroxide. Texture was controlled by adding a soluble calcium complex of sucrose to the brine.

Malecki (36) applied a coating of ethylcellulose and 5% magnesium hydroxide to the interior lining of cans to maintain the pH of vegetables between 8 and 8.5. It was claimed that the slow leaching of magnesium hydroxide from the lining would maintain the pH of the brine above 8 for extended periods of time and therefore

increase the shelf life of the product. Later, the author increased the buffering capacity of canned vegetables by blanching them in 0.005 M calcium glutamate and 0.05 M monosodium glutamate as well as calcium hydroxide solutions (37).

Commercial application of the these processes has not been successful because of the inability of alkalizing agents to effectively neutralize tissue acids over long periods of time. Malecki (38) noted that the pH value of vegetables canned with alkalizing agents decreased to between 7.0 and 7.5 after 10 months of storage thereby increasing the formation of pheophytin. Peas processed by the Blair method and stored at temperatures between 85°F (29.4°C) and 90°F (32.2°C) lost most of their original color in less than 2 months, necessitating the use of refrigerated warehouses to maintain consistent product quality (39).

Increasing the pH value of canned vegetables may lead to the formation of off-flavors (40). Hydrolysis of amides such as glutamine or asparagine at elevated pH values can result in formation of ammonia-like odors. Moreover, free fatty acids formed by lipid hydrolysis may oxidize to form rancid off-flavors.

High Temperature Short Time Processing. The rate of chlorophyll degradation is less dependent on processing temperature compared to the destruction of certain foodborne microorganisms and spores (13). Therefore, equivalent sterilization of green vegetables at higher than normal temperatures and for the shorter times (HTST) can increase chlorophyll retention.

Tan and Francis (42) measured the amount of chlorophyll retained in spinach puree processed between 240°F (116°C) and 280°F (138°C). Process times at each temperature were calculated to achieve the same destruction of *Clostridium botulinum* spores that would be obtained at 250°F (121°C) for 4.9 minutes ($F_0$ = 4.9). Retention of chlorophyll *a* increased from 8.0% at 116°C to 55.5% at 138°C. Colorimetric measurements indicated a simultaneous increase in green color with higher temperatures and shorter times.

Luh et al. (43) compared the color of lima bean puree processed at 116°C for 6.3 minutes and at 149°C for 13 seconds. The color of HTST puree immediately after processing was rated superior to that of conventionally processed puree by a panel of judges. However, the color was ranked only slightly better after 2 weeks of storage at 20°C. Loss of color during storage of HTST puree was accompanied by a decrease in pH from 6.59 shortly after processing to 6.25 after storage for 240 days. The pH value of conventionally processed puree was only 6.16 after heating and decreased to 6.08 after the same storage period.

Gupte and Francis (41) attempted to preserve the chlorophyll retained using HTST processing by increasing the pH of vegetable tissue. Spinach puree was adjusted to a pH value of 8.50 with magnesium carbonate and processed at temperatures between 121° and 149°C ($F_0$ = 4.9). Samples processed at higher temperatures and a high pH value were initially greener and contained more chlorophyll than control samples processed at lower temperatures. Color loss was nearly complete in control samples processed at all temperatures after 6 months of storage. However, samples

processed with an initial pH value of 8.5 and at 149°C were judged to be considerably greener at all storage times. Nevertheless, samples processed at a high pH level for all temperatures were only slightly greener than normal pH samples after 18 months of storage.

Buckle and Edwards (44) reported that chlorophyll retention in pea puree processed at temperatures between 116° and 149°C ($F_0$ = 6) was dependent on process temperature, pH, storage time, and storage temperature. Chlorophyll retention in puree with an initial pH of 6.95 increased from 16.8% when processed at 116°C to 83.7% at 149°C. Puree adjusted to a pH value of 8.45 with magnesium carbonate retained 65.1 and 91.9% at the same respective temperatures. Low pH puree processed at 116°C contained no chlorophyll after 3 months of storage at 20°C and less than 10% of the initial chlorophyll was detected 12 months after processing at 149°C. The alkaline purees remained greener throughout storage at 20°C although the difference was only slight after 18 months of storage. Moreover, the color of the acidic and alkaline purees was not significantly dependent on HTST process temperatures when stored for 18 months.

Differences in initial retention of chlorophyll and subsequent degradation rates were attributed to changes in pH of the purees during processing and storage. The pH of the slightly acidic puree decreased from 6.95 to 6.47 after processing at 116°C and to 6.09 after 18 months of storage at 20°C. The pH of HTST puree increased to 7.16 after processing at 149°C and then decreased to 6.30 after the same storage period. Similar reductions in the pH of alkaline puree were noted although the final pH value of puree processed at 119° and 149°C were 7.79 and 8.10, respectively. In contrast to room temperature conditions, pH values were only slightly decreased and color loss was not observed in frozen HTST purees stored for 18 months at -23.3°C.

Formation of Chlorophyllide. Blanching at temperatures lower than conventionally used has been suggested as a means of retaining color in green vegetables in the belief that the chlorophyllide produced is more thermally stable. Early investigators claimed that some vegetables retained more of their green color during thermal processing if previously blanched at temperatures between 130°F (54.5°C) and 168°F (75.6°C) (45,46). Loef and Thung (7) later concluded that the improved color of processed spinach blanched at 65°C for up to 45 minutes was due to the heat induced conversion of chlorophyll to chlorophyllide by the enzyme chlorophyllase. Quality increases were greatest in vegetables processed in the spring or fall.

Clydesdale and Francis (8) attempted to increase the amount of chlorophyllide produced in spinach during blanching in order to study its role in color retention. Blanching for 10 minutes at 155°F (68.3°C) and at a pH of 8.5 resulted in a 60% conversion of chlorophyll *a* to chlorophyllide *a* while inhibiting the formation of pheophorbide. However, the amount of chlorophyllide formed represented only 29.7% of the total pigments and decreased to 8.2% after processing at 240°F (116°C) ($F_0$ = 4.9). None remained after 2 weeks of storage at 20°C. Spinach processed after enzyme

conversion was thought to be slightly greener by a panel of judges but the effect was not significant enough to warrant the development of a commercial process. Later, Clydesdale et al. (18) combined the previous treatments with HTST processing at 300°F (149°C) ($F_0$ = 4.9). As was expected, chlorophyllide retention immediately after processing was improved. However, there was no color difference between HTST and conventionally processed samples after 3 months of storage.

## Commercial Application of Metallocomplexes in Foods

Copper Chlorophyll. Current efforts to improve the green color of processed foods include the use of copper complexes of chlorophyll derivatives. Copper complexes of pheophytin and pheophorbide are available commercially under the names copper chlorophyll and copper chlorophyllin, respectively. Their use in canned foods, soups, candy, and dairy products is permitted in most European countries under regulatory control of the European Economic Community (47). The Food and Agriculture Organization (FAO) of the United Nations (48) has certified their safe use in foods provided that no more than 200 ppm of free ionizable copper is present. Use of copper-containing chlorophyll derivatives in foods is not allowed in the United States under the Color Additive Amendment to the Food, Drug, and Cosmetic Act of 1938.

Commercial production of the pigments in England was described by Humphrey (49). Chlorophyll is extracted from dried grass or alfalfa with acetone or chlorinated hydrocarbons. Sufficient water is added, depending on the moisture of the plant material, to aid in penetration of the solvent while avoiding activation of chlorophyllase enzyme. Pheophytin may be acid hydrolyzed before copper ion is added resulting in the formation of water soluble copper chlorophyllin.

The stability of commercially prepared copper chlorophyllin in water was measured by Kearsley and Katsaboxakis (50). Color, measured spectrophotometrically, was approximately 80% reduced after 1 hour at 100°C. In samples stored at 7°C, rapid color loss at pH values lower than 4.0 was attributed to precipitation of the pigment. The color of blanched peas was improved after holding them in copper chlorophyllin solution, however, use of the pigment in canned foods is limited because of its instability at high temperatures.

Regreening of Thermally Processed Vegetables. Regreening of commercially canned vegetables has been observed when zinc or copper ions are present in process solutions. Fischback and Newburger (51) reported that commercially processed okra retained its original green color when zinc chloride was added to brine solutions. It was determined that the green pigment in regreened okra contained up to 1.91 ppm zinc on a fresh weight basis whereas no zinc was detectable in pigment extracted from unprocessed okra (52). Okra processed without zinc chloride contained no pigment zinc unless it was allowed to ferment overnight. It was thought that the low pH value of the brine solution contributed to contamination from galvanized canning equipment. Sweeney and

Martin (53) confirmed the role of zinc in the improvement of color in processed vegetables. Acetone extracts of cooked broccoli regained the color of the fresh vegetable after addition of saturated zinc chloride solution.

Zinc contamination was also implicated by Decleire (54) in the spontaneous regreening of commercially processed green beans. Metal concentrations of extracted pigments ranged from 7.3 ppm to 16.0 ppm zinc and 0.4 ppm to 4.2 ppm copper on a fresh weight basis. Swirski et al. (55) reported that Brussels sprouts picked before autumn frosts regreen when heated at 115°C for 30 minutes. The pigment was reported to be a heat stable and water soluble protein complex containing zinc, copper, and chlorophyll derivatives. Regreening of vegetables has also been observed during storage. Schanderl et al. (23) detected zinc and copper complexes of pheophytin *a* and *b* in pea puree stored at 36.7°C for 6 months. The authors concluded that there were sufficient amounts of naturally occurring zinc and copper ions in the samples to account for the change of color.

Recently, a patent was issued for the commercial canning of vegetables with metal salts in blanch or brine solutions (56). The process, given the name Veri-Green, calls for blanching vegetables in water containing sufficient amounts of zinc (II) or copper (II) salts to raise tissue concentrations of the metal ions to between 100 and 200 ppm. Peas, spinach, and green beans treated in such a manner prior to sterilization were claimed to be greener than conventionally processed vegetables. Color improvement was marginal when manganese (II), chromium (III), cobalt (II), nickel (II), or iron (II) ions were used in blanch solutions. No effect was observed with aluminum (III), cadmium (II), or iron (III) ions after processing.

Zinc chloride in brine solutions had no significant effect on the color of regreened vegetables. Peas blanched in water containing 200 ppm zinc ion and then packed in brines containing up to 200 ppm zinc ion were not rated greener than peas receiving the zinc blanch only. Similar trials with green beans illustrated that incorporation of zinc ion into the vegetable is primarily accomplished during blanching.

Further improvement in color is possible when the pH of the brine solution is maintained above 7. Peas blanched in 100 ppm zinc ion and then packed in brine containing 250 ppm calcium oxide were rated greener when packed in cans lined with magnesium oxide. The alkalizing effect of the lining continued to retain the green color after 4 months of storage at 23°C. However, peas processed without lining were rated only slightly greener than those processed conventionally.

Differences observed in the color quality of regreened vegetables may be attributed to varietal differences. Processed Early Bird and Blue Lake cultivars of green beans were superior to Early Gallitin and Slenderette when blanched in water containing 190 to 200 ppm zinc ion. The green color of Blue Lake 53 was rated superior to all others tested although the blanch solution contained only 125 ppm zinc ion.

Pigments contained in canned green beans processed by conventional methods and by the Veri-Green process were determined

by von Elbe et al. (57) using HPLC. Comparison of chromatograms for the two treatments revealed the presence of zinc pheophytin *a* and zinc pyropheophytin *a* in Veri-Green samples. Veri-Green beans were quantitatively greener than those processed conventionally as determined colorimetrically. Formation of zinc complexes continues during storage as evidenced by the increased amount of green color measured in samples stored for 1 year or more.

The Food and Drug Administration (FDA) has responded to this new technology by limiting the amount of added zinc (II) ion in canned vegetables to 75 ppm (58,59). This has led to further study in our laboratory on metallocomplex formation in vegetable tissue during thermal processing. Using HPLC as a method of analysis vegetable purees containing only pyropheophytin *a* were prepared to study the dependence of zinc ion and pigment concentrations and pH value on metallocomplex formation during thermal processing (LaBorde, L.F. and von Elbe, J.H. *J. Agric. Food Chem.*, in press). The study was limited to zinc pyropheophytin *a* formation in order to eliminate zinc complex formation of pheophytin and the conversion of chlorophyll to pheophytin to pyropheophytin during heating. Pea puree with 150 and 300 ppm added zinc (II) ion had 3 and 9 times, respectively, more zinc pyropheophytin *a* than puree containing 75 ppm after heating at 250°F (121°C) for 60 minutes. The effect of pigment concentration was demonstrated by comparing the amount of zinc complex formed in spinach and pea puree containing 75 ppm zinc (II). Unheated spinach puree, with an approximately 12 times greater concentration of pyropheophytin *a* compared to pea puree, contained approximately 43 times more zinc complex after heating for 60 minutes. Furthermore, an 11-fold increase in the reaction rate was observed as the initial pH value of the puree was raised from 4.0 to 8.5. The green color of purees as measured by Hunter color values (-a) followed zinc complex formation. Additional work in the authors' laboratory suggests that to obtain an acceptable green color, approximately 40% of the chlorophyll derivatives must complex with zinc.

These data suggest that since the amount of zinc in processed green vegetables is restricted to 75 ppm, satisfactory green color is best achieved using vegetables containing higher amounts of chlorophyll (i.e., spinach, beans). Vegetables containing lower amounts of chlorophyll (i.e., peas) may require processing at higher pH values for adequate color development. However, this solution may be complicated by the fact that production of pheo-derivatives for the complexing reaction is reduced under alkaline conditions.

Green vegetables processed with zinc salts are only now appearing in the marketplace. The Food and Drug Administration (FDA) has recently been petitioned to include zinc chloride in the list of optional ingredients in the standard of identity for canned green beans (60,61). Temporary permits have been granted to several canners for test marketing of regreened spinach and green beans containing no more than 75 ppm zinc ion (58,59).

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# Chapter 3

# Inhibition of Enzymatic Browning in Fruits and Vegetables

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Alternative means of controlling enzymatic browning in raw fruits and vegetables are required by the food industry due to restrictions in the use of sulfites by the U.S. Food and Drug Administration. The use of sulfite substitutes and other means of preventing browning are reviewed, and new approaches are presented. Ascorbic acid-2-phosphates are highly effective in preventing browning in cut apple. Sporix acts synergistically with ascorbic acid in apple juice and on cut apple. Ascorbic acid-6-fatty acid esters, combinations of ascorbic acid with cinnamate or benzoate, and β-cyclodextrin are effective browning inhibitors in apple juice but not on cut surfaces. Browning inhibitor performance can be improved if cut apple and potato are treated by pressure infiltration rather than by dipping at atmospheric pressure. Variation in the tendency of potato cultivars to brown can be exploited by using slower browning cultivars in processing situations where sulfites cannot be employed.

Enzymatic browning occurs in many fruits and vegetables when certain phenolic compounds in cut or bruised tissues undergo oxidation to *O*-quinones, a reaction catalyzed by the enzyme polyphenol oxidase, or PPO. Quinones then polymerize to form dark brown, black or red polymers. These reactions, the properties of PPO, and the distribution of this enzyme and its substrates in various commodities have been the subject of numerous investigations and review articles (1-7). The problem of controlling enzymatic browning in fruits and vegetables has been accorded much attention by researchers because of its importance to the food processing industry (1-2, 8-9).

## Current Means of Controlling Enzymatic Browning

**Sulfites and Ascorbic Acid as Browning Inhibitors.** Historically, enzymatic browning was controlled by the application of sulfites

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($SO_2$, sulfite, bisulfite, metabisulfite), which inhibit PPO and may combine with quinones or reduce quinones to phenols, thereby preventing pigment formation (4, 9-10). Sulfites can produce acute allergic reactions in some asthmatics, however, with serious if not lethal consequences (11-12). Therefore, the Food and Drug Administration has banned the use of sulfites in fruits and vegetables served or sold raw to consumers (13) and has proposed restrictions on the use of sulfites in certain fresh potato products (14).

L-ascorbic acid (AA) and its isomer D-erythorbic acid (EA) (also called D-isoascorbic acid) have been used as inhibitors of enzymatic browning in fruit and vegetable products for at least 50 years, (15-17). These compounds prevent quinone accumulation and subsequent pigment formation by reducing the O-quinones generated from the phenolic substrates of PPO back to O-dihydroxyphenolic compounds (17-18). AA also can act as a PPO inhibitor (19-20). AA and EA are used interchangeably although there are indications that AA is more effective in some systems (21-22).

Alternatives to Sulfites. To meet the needs of the food industry for alternatives to sulfites, a number of browning inhibitor formulations have been marketed. These products are mostly combinations of AA, EA, or their sodium salts with such adjuncts as citric acid, sodium or calcium chloride, phosphates, cysteine and potassium sorbate (23-29). Commercial browning inhibitor formulations vary widely both in AA or EA content and in recommended use levels (Sapers, G. M., Eastern Regional Research Center, Philadelphia, PA, unpublished data). These sulfite substitutes are considered to be less effective than sulfites because they do not penetrate as well into the cellular matrix (11). Furthermore, AA is easily oxidized by endogenous enzymes (18) or by autoxidation, and in the course of its intended role as a browning inhibitor, may fall into a concentration range where it exerts pro-oxidant effects (30). To enhance their effectiveness, the sulfite substitutes may be used in conjunction with modified atmosphere or vacuum packaging (29,31).

Other Inhibitors of Enzymatic Browning. In addition to the aforementioned compounds, a number of other inhibitors of enzymatic browning, including reducing agents such as 2-mercaptoethanol, 2-mercaptobenzthiazole, and thioglycollate; quinone couplers such as glutathione, sodium diethyldithiocarbamate, and benzenesulphinic acid; chelating agents such as cyanide, carbon monoxide, and diethyldithiocarbamate; inorganic ions such as fluoride and borate; aromatic acid inhibitors of PPO such as benzoic acid and various substituted cinnamic acids; polyvinylpyrrolidone (PVP), which binds phenolic compounds; and the enzyme O-methyl transferase, which converts PPO substrates into inhibitors have been described (3-4). Some of these inhibitors are toxic, and none have found commercial application as food additives (4).

Among the more promising compounds showing anti-browning activity are cinnamic and benzoic acids, which were tested

successfully in apple juice (34) and on apple and potato slices (35-36). Cinnamic acid has been shown to inhibit PPO, either competitively or non-competitively, depending on the substrate and treatment of the enzyme (32), while benzoic acid was reported to be a non-competitive inhibitor of the mixed type (33).

Sporix, an acidic polyphosphate which is described as a potent chelating agent (Friedman, S., International Sourcing, Inc., South Ridgewood, NJ, personal communication, 1986), has been tested as a sulfite substitute in apples (36) but is not approved for food use in the U.S. A blend of food grade phosphates with citric acid and dextrose has been marketed as a browning inhibitor for fruits and vegetables (26). A browning inhibitor formulation containing sodium acid pyrophosphate has been introduced by Monsanto (29).

Cyclodextrins (CD's), cyclic oligosaccharides containing 6 or more glucose units with $\alpha$-1,4 linkages, form inclusion complexes with various organic compounds and have been used to debitter grapefruit juice by removing naringin, a flavanone, and limonin, a terpene (37). Szejtli (38) has observed that the discoloration of some fruits, induced by reactions of polyphenols, may be retarded by CD's. Labuza (39) reported that proteolytic enzymes such as those used in meat tenderizers can prevent enzymatic browning by destroying PPO.

Blanching and Deaeration. Blanching may be used to prevent enzymatic browning in some fruit products (40) since PPO is heat labile (4). Blanching will result in the destruction or loss of flavor components in fruits (40-41), however, and would not be suitable for products expected to have the flavor and texture characteristics of the raw commodity. A process has been described for preparing light colored raisins without sulfiting, which entails a 2 minute dip in 93°C water to inactivate PPO and improve mass transfer of water vapor through the skin (42).

Vacuum deaeration and infiltration techniques have been used as freezing pretreatments to prevent enzymatic browning in apple slices by replacing tissue gases with aqueous solutions of sucrose and/or AA (43-44). Vacuum infiltration of solutions into apple slices resulted in a translucent or waterlogged appearance which would not be acceptable in a raw product (45).

## New Approaches to the Control of Enzymatic Browning

A program to overcome the limitations of current alternatives to sulfites and develop new approaches to the control of enzymatic browning in fruits and vegetables was undertaken by the U.S. Department of Agriculture at its Eastern Regional Research Center in 1985. These studies yielded improved quantitative methods to determine the efficacy of browning inhibitors. A number of promising new approaches to the control of browning were identified, including the use of stable AA derivatives, individually or in combination with complexing agents and PPO inhibitors; the application of browning inhibitors by pressure infiltration; and the utilization of slow-browning cultivars of apples and potatoes.

Methodology to Evaluate Browning Inhibitors. In order to evaluate the effectiveness of treatments to inhibit enzymatic browning, quantitative procedures to measure browning were developed (46). These included the use of tristimulus colorimetry (decrease in L- and increase in a-value), spectrocolorimetry (decrease in percent reflectance at 440 nm), and UV-visible spectrophotometry (increase in absorbance of clarified juice at 420 nm), all of which correlate with the extent of browning. Treatments to control browning were evaluated in two model systems: the raw juice from Granny Smith apples, to which browning inhibitors could be added, and plugs, cut from Delicious apples, Winesap apples or Russet potatoes with a cork borer. Plugs were cut in half so that one half could be dipped in the treatment solution while the other half could be used as a control, receiving only a water dip. Eight pairs of plug halves could be obtained from apples, and 12 from potatoes, permitting replicated experiments that could compensate for the high degree of variability seen in individual fruits and vegetables. Samples were stored for as long as 24 hr at room temperature or several weeks at 4°C, and browning in control and treated juice samples or at the common cut surface shared by treated and control plug halves was measured periodically. In both the juice and plug systems, experimental treatments were compared with standard ascorbic acid treatments, selected to provide partial protection against browning, so that more effective treatments could readily be recognized. The ascorbic acid derivatives were compared with equimolar concentrations of ascorbic acid.

Quantitative estimates of treatment effectiveness were obtained from the data, plotted as a function of storage time (Figure 1). The lag time (interval before the onset of browning), seen as an initial flat region, and the slope of the linear region (browning rate) were determined from such curves. In addition, the changes in L-value, a-value, percent reflectance or absorbance over specified time intervals were calculated. These $\Delta$-values were used to compare treatments by calculating the percent inhibition, a value defined as the difference between the control and treatment $\Delta$-values, expressed as a percentage of the control $\Delta$-value. Treatments that completely inhibited browning would give percent inhibition values of 100, while ineffective treatments would give percent inhibition values near zero. Large negative values would indicate that the treatment induced browning.

Ascorbic Acid-2-Phosphates. Seib and Liao (47) described the preparation of AA-2-phosphate (AAP) and AA-2-triphosphate (AATP), compounds that are stable against oxygen and are not reducing agents *per se* but release AA when hydrolyzed by a phosphatase. Such compounds might be used as additives in foods or feeds or in pharmacological applications since they would be stable until consumed and converted to AA during digestion. These compounds were evaluated as browning inhibitors for apple juice and plugs with the expectation that they would be less subject to oxidation by oxidases or autoxidation than AA and, if hydrolyzed at a suitable rate, would provide extended protection against browning (50).

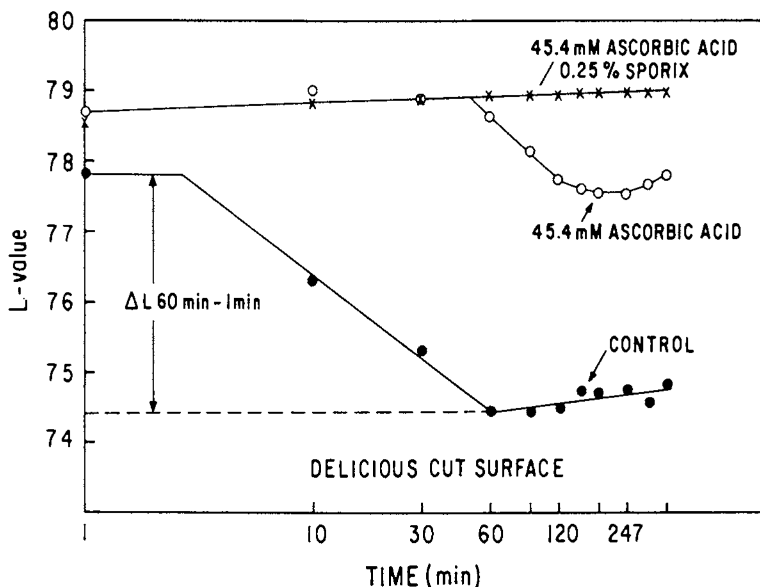


Fig. 1. Reflectance L-value at transversely cut surface of Delicious apple plugs treated with browning inhibitors or untreated (control) and stored at 20°C.

In the juice system AA-2-phosphates gave inconsistent results, and in some cases, were less effective than AA. This failure probably resulted from the loss of endogenous acid phosphatase during juice preparation. Residual acid phosphatase activity would be decreased further by the low pH of Granny Smith juice (3.3), which is substantially less than the optimal pH for acid phosphatase obtained from plant tissues (48-49).

In contrast to the juice results, both AAP and AATP were more effective browning inhibitors than equivalent concentrations of AA when applied to apple plugs. Samples treated with AAP or AATP showed little or no browning after 24 hr at room temperature or 1 week at 4°C (Figure 2). Browning inhibition was not improved significantly by the addition of AA in combination with AAP or AATP. The addition of citric acid to dips had no effect on AAP but decreased the effectiveness of AATP as a browning inhibitor.

The effectiveness of the AA-2-phosphates is due primarily to their stability. AA, applied to the cut surface of apple, may be consumed by reaction with quinones resulting from polyphenol oxidation (18) or by autoxidation (30). Seib and Liao (47) demonstrated that the AA-2-phosphates were much more stable to oxidation by $H_2O_2$ than was AA. Presumably, sufficient acid phosphatase is present at the cut surface of apple fruit to permit hydrolysis of the AA-2-phosphates at a rate sufficient to prevent browning but not great enough to generate a large excess of AA that would be subject to autoxidation. The poor performance of AATP in combination with citric acid probably resulted from acid inhibition of acid phosphatase, as in juice. Even under favorable conditions, AATP would be hydrolyzed more slowly than AAP (47). The suitability of the AA-2-phosphates as browning inhibitors for commodities other than apple will depend on their acidity and endogenous acid phosphatase activity.

Ascorbic Acid-6-Fatty Acid Esters. L-Ascorbyl-6-palmitate (AP), a fat soluble analog of AA, is an effective antioxidant for vegetable oils and other fatty products (51). AP and other 6-fatty acid esters of AA were shown to function as oxygen scavengers in an aqueous system only if solubilized by adjusting the pH to 9 (51). Because of the possibility that these esters might be more stable than AA and capable of functioning as reducing agents in aqueous systems, they were tested for anti-browning activity in Granny Smith juice.

Experiments with AP, ascorbyl laurate (AL) and ascorbyl decanoate (AD), added to Granny Smith juice at concentrations as high as 1.14 mM (equivalent to 200 ppm AA), demonstrated that these esters were less effective than or similar to AA initially but surpassed AA as browning inhibitors after longer periods of storage (50). The combination of AA with the fatty acid ester had little or no effect with AP and AL but greatly improved the performance of AD, providing protection against browning for at least 24 hr.

Mixed results were obtained when fatty acid esters of AA were applied to apple plugs as dispersions in pH 7 phosphate buffer, stabilized with lipophilic emulsifying agents such as Durkee's EC-25 or Durlac 100. The degree of inhibition was not

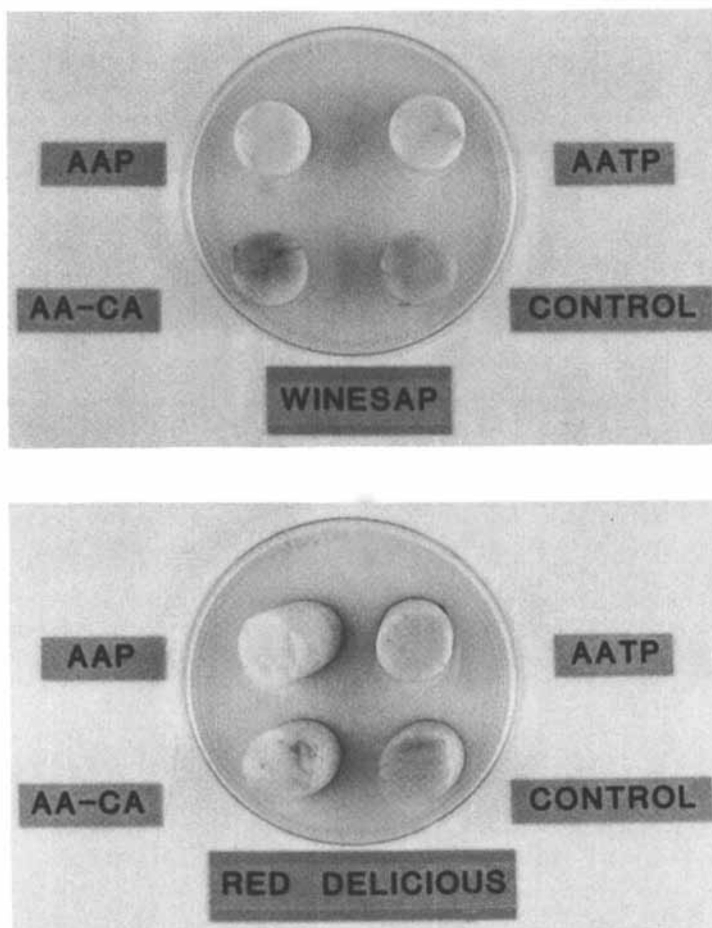


Fig. 2. Apple plugs treated with 56.8 mM ascorbic acid-2-phosphate (AAP), ascorbic acid-2-triphosphate (AATP) or ascorbic acid in 1% citric acid (AA-CA) vs untreated control, stored at 4°C for 1 week.

consistent, probably because of AP precipitation on the cut surface during storage. More stable dispersions could be prepared by substituting AL or AD for AP. Treatment of apple plugs with the former esters tended to induce browning, however. Similar results occurred when AP dispersions were prepared with less lipophilic emulsifying agents such as Tween 60 or Tween 80. These effects may have resulted from the disruption of membranes in cells near the cut surface by the emulsifying agents or esters, causing leakage of PPO and its substrates, and thereby increasing the extent of browning.

Recent patents describe new classes of AA derivatives that are claimed to have antioxidant properties and are recommended for use in high moisture foods: 6-O-phytanoyl-L-ascorbic acid and similar esters of AA or EA with tri- or tetramethyl C15-C17 alkanoic acids (52) and AA ethers in which a methoxy group with an organic residue having a molecular weight of 58 to 400 is attached to the hydroxyl group at carbon 2 (53). It is not evident whether these compounds would be effective as browning inhibitors for cut fruits and vegetables or subject to the same limitations as the 6-fatty acid esters of AA.

Cinnamate and Benzoate. Walker (34) reported that the addition of cinnamic acid to Granny Smith juice at concentrations greater than 0.5 mM prevented browning for over 7 hr. Sapers et al. (50) found that sodium cinnamate inhibited enzymatic browning in Granny Smith juice when added at concentrations between 0.67 and 2.67 mM (114-454 ppm). Combinations of cinnamate with AAP, AP or AA were similar in effectiveness, greatly surpassing AA as a browning inhibitor.

With apple plugs, 10 mM cinnamate inhibited browning for several hours but induced severe browning over extended storage times. The combination of cinnamate with AA in dips was more effective than AA alone; however, the combination of cinnamate with AAP showed no advantage over AAP alone. The tendency of cinnamate to induce browning indicates a potential problem with this compound which may undergo conversion at the cut surface to a PPO substrate by cinnamate-hydroxylase and other enzymes involved in the biosynthesis of polyphenols (54).

Sodium benzoate exhibited anti-browning activity in the juice system, the effect being concentration dependent (50). Combinations of 6.9 mM benzoate (corresponding to 0.1%, the maximum level permitted in foods as a preservative in the U.S. (21 CFR 184.1021) with 1.14 mM sodium ascorbate or AAP inhibited browning to a greater extent than either individual treatment, the effect appearing to be synergistic and due primarily to an increase the lag time.

Dips containing benzoate, alone or in combination with AA, provided short-term protection against browning in Delicious and Winesap plugs but induced browning in samples stored 6 or more hours. Induced browning by benzoate may be an indication of its conversion to a PPO substrate or its stimulation of substrate synthesis by enzymes at the cut surface. Benzoic acid in plants is derived from phenylalanine via trans-cinnamic acid (55-56) which is also a precursor of caffeic acid and other PPO

substrates (54). Benzoate formation in higher plants occurs on the thylakoid membrane; this process is apparently not reversible (56). Zenk (57) demonstrated that the addition of a large excess of benzoic acid to Catalpa hybrida leaves stimulated the hydroxylation of cinnamic acid to p-coumaric acid, a PPO inhibitor which might be hydroxylated to caffeic acid, a substrate. Since benzoic acid and cinnamic acid both may induce browning, neither PPO inhibitor is recommended as a component of anti-browning formulations at this time.

Sporix. Sporix, a commercial polyphosphate product, was the most promising browning inhibitor evaluated in the USDA study besides the AA-2-phosphates (50). Sporix is a powerful chelating agent as well as an acidulant (Friedman, S. International Sourcing, Inc., South Ridgewood, NJ, personal communications, 1986) and probably acts by inactivating or inhibiting PPO and by stabilizing added ascorbate, thereby delaying the onset of browning.

In the juice system, Sporix was an effective inhibitor at concentrations above 0.5% or at lower concentrations if combined with AA. Typically, such combinations prevented browning for more than 24 hr in samples that browned within 30 min if untreated or within a few hours if treated with AA alone. The exceptional effectiveness of Sporix-AA combinations resulted primarily from a lag time extension which appeared to be a synergistic effect. The ability of Sporix to control browning in juice was pH-dependent. Adding Sporix to Granny Smith juice decreased the pH from 3.3 to 3.1. When the pH of juice containing a Sporix-AA combination was adjusted to 3.3 by adding NaOH, the percent inhibition and lag time values were smaller than values obtained at pH 3.1 although not as small as values obtained with AA alone.

Sporix was ineffective as a browning inhibitor for apple plugs. Combinations of Sporix with AA were highly effective browning inhibitors, however, preventing browning for at least 24 hr at room temperature. Zent and Ashoor (36) reported that enzymatic browning in apple and potato could be inhibited by treatment with combinations of sodium erythorbate and Sporix.

Browning inhibition by Sporix combinations can be attributed to two effects: a greatly extended lag time compared to that obtained with the individual inhibitors as seen in juice, and a reduced rate of browning once the lag time has been exceeded. The lag time effect probably results from the inhibition of copper-containing oxidases and other copper-catalyzed oxidative processes in apple by Sporix. These oxidative reactions normally would bring about the rapid loss of AA and permit browning to occur once the added AA was depleted (18). Sporix also would inhibit PPO directly by chelation of its copper (3), thereby decreasing the rate of polyphenol oxidation and subsequent browning. The ability of Sporix to exert its effect on enzymatic browning by these two independent mechanisms probably accounts for the apparent synergism obtained with Sporix-AA combinations.

Cyclodextrins. Cyclodextrins (CD's) form inclusion complexes with various aromatic compounds (58), including cinnamic acid (59). Complexation of the phenolic substrates of PPO by CD's might

prevent browning. β-CD, but not α- or γ-CD, was an effective browning inhibitor in apple juice, especially when combined with AA, AA-2-phosphate, or AP (50). Browning in juice could be inhibited for at least 24 hr at room temperature by combinations of β-CD with Sporix or addition of the more soluble branched β-CD (which contains maltosyl branch chains) at a higher concentration than is possible with β-CD (Hicks, K. B., Eastern Regional Research Center, Philadelphia, PA, unpublished data).

PPO substrates in apple presumably can be complexed by β-CD but are too large to fit completely in the cavity of α-CD and too small to be retained strongly by γ-CD. Shaw and Buslig (37) reported that β-CD polymers were more effective than α- or γ-CD polymers in removing naringin and limonin from solution. The effectiveness of β-CD as a browning inhibitor will depend on the equilibrium between free and complexed PPO substrates and the rate of complex formation. Gradual browning of apple juice at all β-CD concentrations tested indicates that complex formation did not go to completion. Browning by the uncomplexed PPO substrates could be controlled by the addition of AA, AA derivatives, or other browning inhibitors.

Attempts to translate the favorable results obtained with juice into a β-CD dipping treatment for apple plugs were not successful. The inability of β-CD to inhibit enzymatic browning in apple plugs can be understood in terms of fundamental differences between the juice and cut surface systems. In the former, PPO substrates, PPO, $O_2$, AA and browning inhibitors are all in solution so that the rate of browning is determined by their concentrations, the temperature, stirring conditions, and perhaps the surface to volume ratio. With the cut surface system, juice released from disrupted cell layers at the freshly cut apple surface, which contains PPO, PPO substrates, and other reactants, would be removed by the dipping treatment. Browning would not occur until these species diffused from the interior of the disrupted cell layers towards the surface or reacted in situ, given sufficient dissolved oxygen. An effective β-CD dipping treatment would have to complex PPO substrates before they diffused to the surface or reacted within the disrupted cells. The diffusion of β-CD from the cut surface to the interior of the disrupted cell layers apparently is too slow to allow the complexing agent to compete with PPO for substrates.

Application of Browning Inhibitors by Pressure Infiltration. One of the disadvantages of conventional sulfite substitutes is their limited penetration into the fruit or vegetable piece, compared to that of sulfite (11). Pressure and vacuum infiltration have been used to increase the penetration of calcium solutions into whole, unpeeled apples to improve their keeping quality during storage (60-62). Pressure infiltration was used successfully to increase the uptake of browning inhibitor solutions into apple and potato plugs (Sapers, G. M., Eastern Regional Research Center, Philadelphia, PA, unpublished data). When Delicious plugs were pressure infiltrated at 5 psi for 5 min, they showed a weight gain of about 5%. This treatment resulted in some initial darkening due to waterlogging, indicated

by a decrease in L (ΔL infiltration), which was partially offset during subsequent storage by an increase in L (ΔL storage) (Table I). With the browning inhibitor formulations tested, the pressure treatment added about 1 week to the storage life of the samples at the higher concentration, and 3 or 4 days at the lower concentration, compared to dipping at atmospheric pressure. Pressure infiltration of Katahdin potato plugs at 15 psi added about 4 days to their storage life, compared to atmospheric pressure dipping, without causing waterlogging. These results are significant because they provide processors with the means to improve the performance of sulfite substitutes that are available now.

Cultivar Variation in Tendency of Apples and Potatoes to Brown. Cultivar (cv.) differences in the tendency of fruits and vegetables to brown are well known (4) and have been correlated with PPO activity and substrate concentration in various commodities (63-69). Sapers and Douglas (46) exploited such differences in selecting apple cvs. for use in evaluating new browning inhibitors. Among six cvs. compared, Idared and Granny Smith browned the least while Stayman showed the most extensive browning. The use of cvs. with a low tendency to brown in processing situations where sulfites cannot be employed may obviate or minimize the need for sulfite substitutes.

Differences in the tendency of potato cvs. to brown may be used as a rationale for selecting potatoes that can be processed without sulfite or sulfite substitutes. A comparison of Atlantic and related cvs. with Russet Burbank revealed large differences in the extent of browning which were correlated with PPO activity, total phenolics and tyrosine content (68). Atlantic potato browned much more slowly than Russet Burbank potato. A simple dip in water after slicing virtually eliminated browning at the cut surface of Atlantic, while Russet Burbank showed significant browning after dipping. Peeling, especially by steam or abrasion, tends to induce browning at the peeled surface of potato. Atlantic was much less subject to such browning than Russet Burbank.

## Future Directions

Studies reviewed herein have demonstrated the potential ability of several new approaches to control enzymatic browning in fruit and vegetable products: the use of novel AA derivatives, polyphosphates and β-cyclodextrin as browning inhibitors; the application of browning inhibitors to cut fruits and vegetables by pressure infiltration; and the use of cvs. having a low tendency to brown. It is likely that one or more of these approaches will be useful as an alternative to sulfites to control browning in affected commodities.

Before commercial application becomes a reality, additional studies are required to optimize the treatments, extend them to other commodities, establish their safety, and demonstrate economic feasibility. Furthermore, because these approaches to the replacement of sulfites address only one function of sulfites,

Table I—Application of Browning Inhibitors to Delicious Plugs by Pressure Infiltration vs Dipping

| Formulation | Method of Application[a] | ΔL Infiltration | ΔL Storage[b] | Percent Inhibition[c] Storage (days) 4 | 7 | 14 |
|---|---|---|---|---|---|---|
| 4.5% Sodium Erythorbate + 0.2% $CaCl_2$ | Pressure | -4.0[e] | 2.3[d] | 92[d] | 86[d] | 82[d] |
| | Dip | -0.7[d] | 0.6[e] | 93[d] | 84[d] | 66[e] |
| 2.25% Sodium Erythorbate +0.2% $CaCl_2$ | Pressure | -3.3[d] | 1.8[d] | 80[d] | 70[d] | -- |
| | Dip | 0.9[d] | -0.4[d] | 66[d] | 54[e] | -- |

[a] Immersion for 5 min.

[b] 7 days at 4°C.

[c] Based on change in a-value.

[d-e] Means of 4 replicate treatments; means within columns for each formulation, followed by different superscripts, are significantly different at $p < 0.05$ by the Bonferroni LSD test.

Source: Sapers, G. M. Eastern Regional Research Center. Unpublished data.

namely, the control of enzymatic browning, other functions served by sulfites must be dealt with. Among these functions are the inhibition of non-enzymatic browning in dehydrated fruits and vegetables; control of bacteria in wine, grapes and other products; and bleaching of cherries. Since no single approach is likely to meet these needs, research is needed to develop treatments combining several principles of preservation, i.e., use of browning inhibitors in combination with antimicrobials and modified atmosphere packaging. Such treatments would be applied to cvs., selected for their low tendency to brown, and processed under conditions of peeling, cutting, heating and dehydration that minimize the extent of both enzymatic and non-enzymatic browning. Due to the complexity of the problem, a systems approach is suggested to determine the optimal conditions and treatments for any given commodity and product category.

## Acknowledgments

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## Chapter 4

# Polyphenol Oxidase Activity and Enzymatic Browning in Mushrooms

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Tyrosinase activities and isoenzyme forms were examined in developing mushrooms. In extracts prepared in the absence of phenolic adsorbents, no trend was apparent in catechol oxidase activity, but dopa oxidase and tyrosine hydroxylase activity decreased from small pins to the mature mushroom. Latent enzyme activity, detected by including SDS in the assay, was associated with dopa oxidase activity more than catechol oxidase activity or tyrosine hydroxylase activity. The decrease in dopa oxidase and tyrosine hydroxylase was observed in two different strains of mushrooms irrespective of whether fresh, frozen, or freeze dried mushrooms were used as samples. Dopa oxidase activity also decreased during development in samples from each break harvested from a single compost innoculum. Concomitant with the decrease in dopa oxidase activity was an increase in latent enzyme activity. Histochemical staining of mushroom tissues blotted into nitrocellulose showed that the enzyme was localized in the skin, cap flesh, gills, and stalk regions of developing mushrooms. These results suggest that inactive, active, and latent enzyme forms exist at several stages of development and that potential browning problems in mushrooms may be more complicated than once believed.

Polyphenoloxidases have been the subject of several recent reviews (1-8). The enzyme is ubiquitous and found in a variety of vertebrate, invertebrate, plant, and fungal organisms. Numerous

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reports have specifically examined the mushroom enzyme, tyrosinase, with regard to its enzymatic, physical and molecular characteristics (1,4-7). In recent years, many investigations have used the crude enzyme obtained from commercial sources rather than enzyme obtained directly from mushroom tissues.

Tyrosinases catalyze the hydroxylation of monophenols, such as tyrosine, to ortho-diphenols and the conversion of ortho-diphenols to ortho-diquinones. The mushroom enzyme can utilize either tyrosine, catechol, or dopa as substrates and therefore exhibits tyrosine hydroxylase, catechol oxidase, or dopa oxidase activity respectively. Boekelheide et al. (10-11) and others (12-13) have proposed that mushroom tyrosinase catalyzes the conversion of gamma L-glutamyl-4-hydroxybenzene to the corresponding dihydroxyl and 3,4-dibenzoquinone derivatives during spore formation. More recently, Sugamaran (14) reported that commercial mushroom tyrosinase preparations catalyzed the unusual oxidative decarboxylation of 3,4-dihydroxymandelate to 3,4-dihydroxybenzaldehyde through a quinone methide intermediate. Sugamaran et al. (15) have also reported that mushroom tyrosinase can catalyze the oxidative dimerization of 1,2-dehydro-N-acetyldopamine to a benzodioxane derivative. While the *in vivo* substrates of mushroom tyrosinase have not been identified, N-( -L-glutamyl)-4-hydroxyaniline, tyrosine and dihydroxyphenylalanine (dopa) are available as substrate for browning reactions (16).

Many physical characteristics have been determined for the mushroom tyrosinases. These include diffusion coefficients, sedimentation coefficients, frictional ratios, Stoke's radii, isoelectric points, and apparent molecular weights (17-20). Recent evidence suggests that tyrosinase is composed of two heavy chain components (H) of approximately 43-45 kd each and two light chain components (L) of approximately 13 kd each (18-20). The heavy chains contain the catalytic sites. Podila and Flurkey (21) have suggested that the heavy chains were synthesized as smaller molecular weight precursors to the native enzyme. Different isoenzyme forms of tyrosinase apparently contain different H chains (7,17). No role for the L chain subunits have been reported.

In spite of the wealth of information available on mushroom tyrosinase with regard to its enzymatic and physical characteristics, little is known about the enzyme in developing mushrooms. Yamaguchi et al. (22) reported that only a small portion of the total enzyme activity (latent + active) was in the active form during development from the tight button stage to the flat pileus stage. Some of this enzyme existed in an inactive or latent state and could be released from latency by exposing the enzyme to sodium dodecylsulfate (SDS). This latent activity is characteristic of many polyphenoloxidases (1-7). Yamaguchi et al. (22) also reported that the latent tyrosinase activity in the cap flesh and stalk increased with development. In contrast, Burton (23) examined the tyrosinase in pre-harvest developing mushrooms and found that skin tissue had more non-latent or active tyrosinase than flesh tissue. The tyrosinase in the skin was activated more

by trypsin treatment than by SDS, but the tyrosinase in the flesh tissue was activated by SDS to greater extents. Burton also reported that nonlatent and active enzyme in the flesh showed no consistent trendsin activity with mushroom development. On the other hand, fully activated tyrosinase in the skin declined during development while nonlatent tyrosinase activity showed little change. Earlier, Burton (24) showed that the skin tissue contained three times more tyrosinase than the flesh tissue when compared on a fresh weight basis. Both Yamaguchi et al. (22) and Burton (23-24) utilized substrates which could be acted upon by other oxidative enzymes and thus may not be a true reflection of tyrosinase activity or levels of tyrosinase present in the samples.

## Materials and Methods

Commercial mushroom tyrosinase was obtained from Sigma Chemical Company (St. Louis, MO). Fresh, frozen, or freeze dried mushrooms (*Agaricus bisporus*) were supplied by the Campbell Institute for Research and Technology (Napoleon, OH).

Mushroom tyrosinase was extracted as described by Ingebrigtsen and Flurkey (*J. Food Sci., in press*). Tyrosinase activity was monitored using either catechol, dopa or tyrosine as the substrates. All assays were carried out in the presence and absence of 0.1% SDS (w/v) to detect active and latent enzyme activities. The catechol oxidase activity of tyrosinase was assayed in 50 mM phosphate (pH 6.0) containing 10 mM catechol and the absorbance monitored at 410 nm (25-26). The dopa oxidase activity of tyrosinase was assayed in 50 mM phosphate (pH 6.0) containing 5 mM L-dopa and the absorbance monitored at 475 nm. The tyrosine hydroxylase activity of tyrosinase was assayed in 33 mM phosphate (pH 6.0) containing 0.33 mM L-tyrosine and the absorbance monitored at 280 nm. Protein content was determined by the method of Lowry et al. (26).

Native and partially denaturing electrophoresis was carried out using the method described by Angleton and Flurkey (27). Denaturing electrophoresis was followed according to the method of Laemmli (28). Equivalent amounts of protein were used for each sample application. Gels were stained for dopa oxidase activity in 0.1 M phosphate (pH 6.0) containing 1 mM L-dopa (25,27). Western blotting procedures were conducted as described previously using anti-tyrosinase antibodies and goat anti-rabbit conjugated alkaline phosphatase antibodies (26).

Tyrosinase in whole tissue sections was localized by pressing tissue sections onto nitrocellulose membranes using a procedure similar to that described by Spruce et al. (29). Histochemical localization was determined by incubating blots in dopa as described by Moore et al. (30,31). Immunochemical localization of tyrosinase at the whole tissue level was carried out using the method of Moore et al. (30).

## Results and Discussion

Investigators have described novel reactions (14,15), prepared antibodies (32), or described unusual activities associated with the enzyme (33) using commercial mushroom tyrosinase. Much of the recent information on mushroom tyrosinase has relied exclusively upon the use of commercial enzyme. This is somewhat of a problem considering less than 10% of the material in commercial preparations has been estimated to be the enzyme (7). No estimates have been made concerning the amount of inactive (denatured) or latent enzyme present in the same samples. This problem is further compounded because commercial enzymes may be derived from more than a single *Agaricus* strain (M. Wach, personal communication). For example, Fig. 1 shows the SDS PAGE pattern of two lots of commercial mushroom tyrosinase preparations. Lot A was derived from a single strain of *Agaricus* while lot B was derived from a mixture of two strains. It is readily apparent that (1) both lots contain prominent protein bands at 43-45, 24-26, and 13-14 kd, (2) many other proteins are present in each sample, and (3) lot A and B differ significantly in their protein patterns at the 40-48 kd region. Presumably, the proteins in this 40-48 kd region correspond to the catalytic heavy chain subunits of tyrosinases. While the effects of different H subunit distribution on the enzymatic characteristics have not been examined thoroughly, mixtures of different H chains would give rise to different isoenzymes and would necessitate reevaluation of existing kinetic data. In addition, the isoenzyme composition of tyrosinase may change with development. Therefore, we examined the tyrosinase activities and isoenzymes in developing mushrooms.

Tyrosinase activity was monitored in four stages of mushroom development. Small pins (0-.5 cm), large pins (.5-1 cm), immature and mature mushrooms were classified by cap size, gill development, and veil covering (K. Dahlberg, personal communication). These samples were assayed for active and latent tyrosinase activity after extraction in the absence of phenolic adsorbents (Fig. 2). No apparent trend in enzyme activity was noted during development using catechol as the substrate. Latent enzyme activity was also present in all stages. In contrast, dopa oxidase activity appeared to decrease with development concommitant with an increase in latent tyrosinase activity. Tyrosine hydroxylase activity also decreased with development using tyrosine as the substrate, but no latent activity was noted using this substrate. Yamaguchi *et al.* (22) found a somewhat similar result using a different substrate (catechol/proline) and assay method for monitoring active and latent tyrosinase in mushrooms. They observed that active tyrosinase was relatively constant from tight buttons to the flat pileus stage; however, latent activity increased with development. Using catechol as the substrate, Burton (23) found that nonlatent or active tyrosinase activity was relatively constant during pre-harvest development. Burton also observed that latent activity did not change significantly during pre-harvest development.

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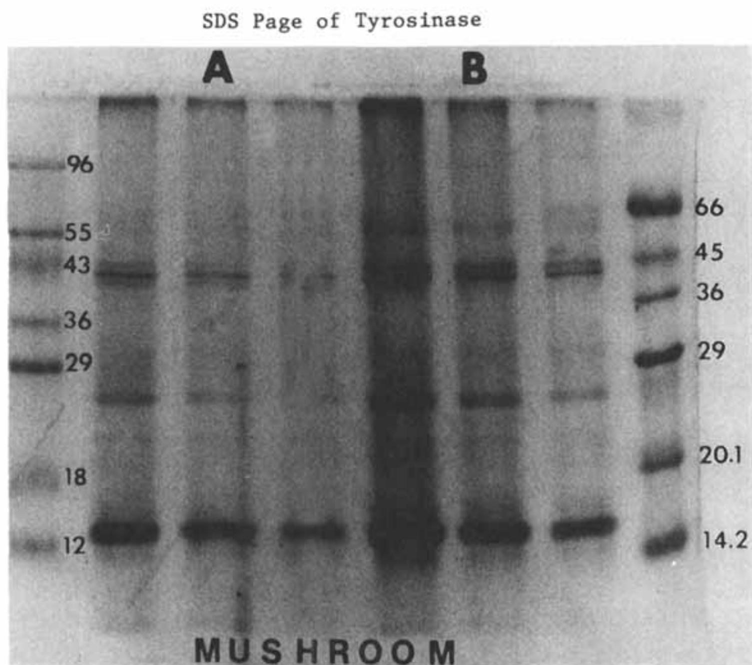


Figure 1. Letters A and B refer to two different lots of commercial mushroom tyrosinase. Numbers refer to molecular weight markers in kd.

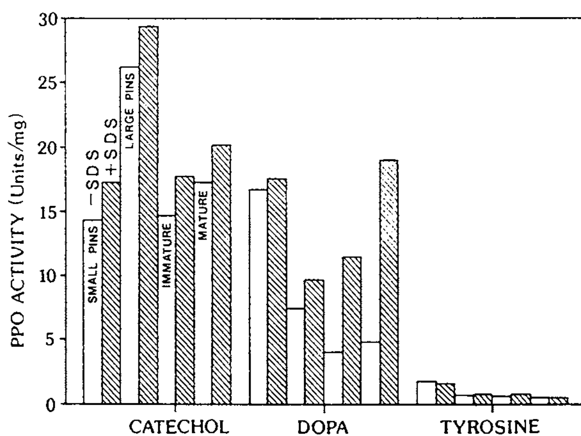


Figure 2. Clear bars represent active tyrosinase in four different developmental stages of mushrooms, while the hatched bars represent latent and active enzyme. (Reproduced with permission from ref. 35. Copyright 1989 *J. Food Sci.*)

Measurements of total tyrosinase activity do not reflect its isoenzyme composition. Therefore, we examined the isoenzyme forms of tyrosinase in small pins, large pins, immature and mature mushrooms by native electrophoresis followed by staining for dopa oxidase activity. Using dopa oxidase as an indicator of tyrosinase, one dominant isoenzyme form (I) was present in all development stages (Fig. 3). Two faster migrating forms (IIIa,b) were also apparent as well as a faint intermediate migrating form (II). No new isoenzyme forms were observed during development. All forms appeared to decrease during development with the III a and b forms decreasing more rapidly. If these same samples were extracted in the presence of phenolic adsorbents (+), the intermediate and faster migrating forms were inactivated by or completely adsorbed on to the phenolic adsorbents. Identical patterns were obtained irrespective of whether electrophoresis was carried out in the presence or absence of SDS, indicating that no new latent isoenzyme forms were made which were specifically activiated by SDS.

Because tyrosinase can be activated from a latent state, neither enzyme assays or electrophoresis can distinguish the mass amount of tyrosinase present on a protein basis. To determine the amount of tyrosinase present on a protein basis, we subjected the same samples as in Fig. 3 A to denaturing electrophoresis followed by Western blotting. Anti-mushroom tyrosinase antibodies were used to locate the enzyme in each of the samples. As seen in Fig. 3B, a broad band of immunostaining was observed in each extract. This band appeared to remain relatively constant during development. A minor band at 24-26 kd appeared to decrease with devleopment. This band was entirely absent in samples treated with phenolic adsorbents and suggests adsorption to the resins. In addition, this band cannot account for the decrease in enzyme activity, because only the H subunits are believed to contain the catalytic site. In general, the mass amount of tyrosinase detected immunologically did not appear to correlate well with the decrease in activity observed by using enzyme assays or by electrophoresis. This suggests that the decrease in enzyme activity during development may not be related to decreased enzyme synthesis but may be related to the presence of an inhibitor or some other mechanism of inactivation.

Commercial mushroom growers harvest three to four cycles of mature mushrooms from a single compost innoculum. Each cycle is called a break or flush. To determine if tyrosinase activity and content changed with the breaks harvested, freeze dried samples were obtained from small pins, large pins, immature and mature stages in the first three breaks from the same mushroom innoculum and assayed for active and latent tyrosinase activity (Fig. 4). Using dopa oxidase as an indicator for tyrosinase enzyme activity decreased in all three breaks as the mushrooms matured. Tyrosinase activity was somewhat greater in earlier developmental stages in break 1 than in breaks 2 or 3. This general pattern of activity was similar to that obtained using fresh or frozen samples and latent activity appeared to be more pronounced in later

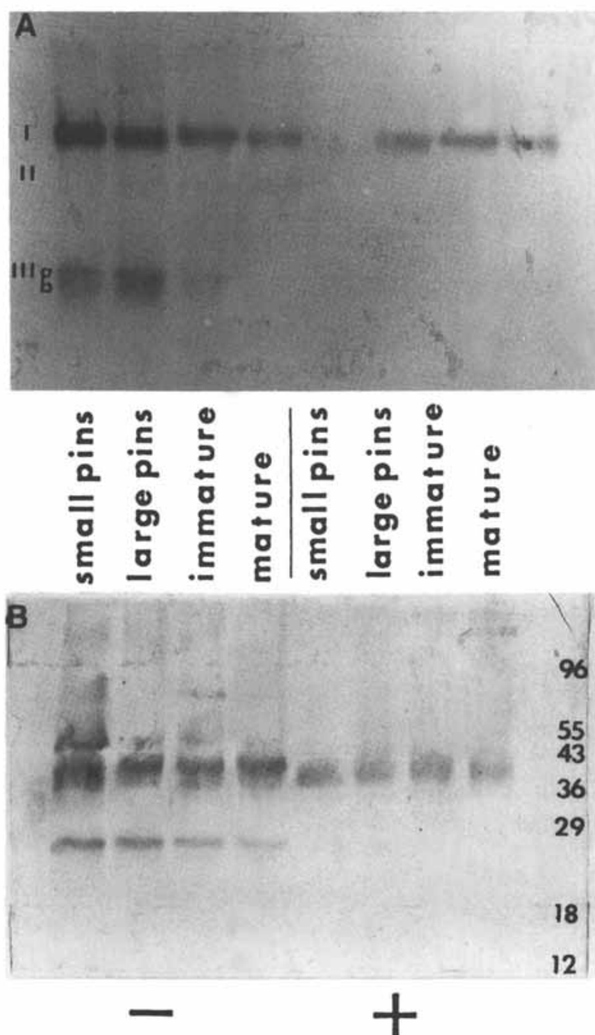


Figure 3. Extracts prepared in the absence (−) and presence (+) of phenolic adsorbents were subjected to native electrophoresis and stained for tyrosinase using dopa (A). Western blot of mushroom tyrosinase in four developmental stages after SDS PAGE (B). Samples were the same as in A. (Top photograph reproduced with permission from ref. 35. Copyright 1989 *J. Food Sci.*)

developmental stages than in earlier stages. In general, the trend in decreased tyrosinase activity and increased latent/active ratio of enzyme appeared to be similar in all three breaks.

The above samples in each break were analyzed for tyrosinase isoenzymes by native electrophoresis to determine if any changes in isoenzyme forms occurred from break to break (Fig. 5). All samples applied to electrophoresis contained similar amounts of protein (15 ug). One dominant slower form (I) was present in all three breaks. The intensity of this form appeared to decrease with development in each break. Two faster migrating forms (III a,b) were also observed in each break and these two forms also decreased in intensity with development. No apparent trend was noted in the amount of the intermediate migrating forms (II). These profiles were qualitatively similar to fresh mushroom extract profiles at different developmental stages and once again suggest that no new isoenzyme forms were made during development or in different breaks.

Using a method of histochemical localization developed by Spruce *et al.* (29), tyrosinase activity was visualized at the whole tissue level. After blotting mushroom slices onto nitrocellulose, the membranes were subsequently stained for dopa oxidase activity (31-32). As seen in Fig. 6, tyrosinase was distributed throughout the entire tissue section. However, darker areas of histochemical staining intensity were noted in the epidermis, gill, and stalk tissues compared to the cap flesh. These results are in general agreement with Burton (23-24) and Yamaguchi *et al.* (22) but give no indication of the amount of latent activity present in each tissue.

To examine the amount of active and latent activity in different parts of the mushroom, cap skin, cap flesh, gill and stalk tissues were extracted and assayed for dopa oxidase activity in the presence and absence of SDS. Based upon specific activities, greater amounts of tyrosinase were present in flesh and stalk tissues compared to skin or gill tissue (Table I). These results are qualitatively similar to those reported by Yamaguchi *et al.* (22) and Boret *et al.* (34). In fact, Boret *et al.* (34) appeared to find a concentration gradient of tyrosinase activity in the stem from bottom to top. However, they also found considerable variation in enzyme activity in different tissue sections from mushroom to mushroom. We also have found such variations between individual mushrooms and between different batches of mushrooms. When tissue samples were measured for tyrosinase activity in the presence of SDS, all tissues showed latent activity. This latent activity appeared to be slightly greater in the skin tissue than in the cap flesh, gill, or stalk tissues. Yamaguchi *et al.* (22) observed that the stipe tissue contained more active and latent enzyme and Burton (23-24) reported that skin tissues contained more tyrosinase than the cap flesh. The differences between their data and ours may be related to the different substrates used to monitor tyrosinase activity.

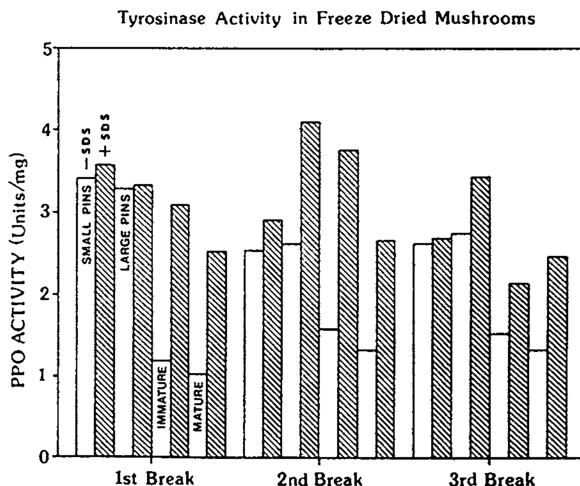


Figure 4. Clear bars represent active tyrosinase in four developmental stages in each of three different breaks. Hatched bars represent active and latent enzyme using dopa as the substrate. (Reproduced with permission from ref. 35. Copyright 1989 *J. Food Sci.*)

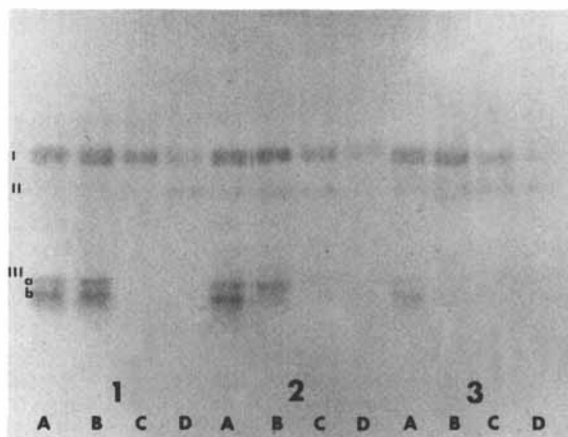


Figure 5. Tyrosinase isoenzyme forms identified in three different breaks (1–3) after native electrophoresis and staining for dopa oxidase activity. A–D represent small pins, large pins, immature, and mature mushroom samples, respectively. (Reproduced with permission from ref. 35. Copyright 1989 *J. Food Sci.*)

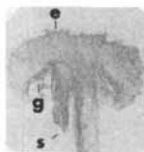


Figure 6. Histochemical localization of mushroom tyrosinase on nitrocellulose blots. Letters refer to epidermis (e), gills (g), and stalk (s). (Reproduced with permission from ref. 31. Copyright 1989 *Histochemistry.*)

Table I. Active and Latent Tyrosinase in Different Tissues of Mature Mushrooms

| | Tyrosinase Activity (Units/mg) | | |
|---|---|---|---|
| Tissue | -SDS | +SDS | Fold Increase |
| cap skin | .35 | 5.9 | 17 |
| cap flesh | .69 | 5.13 | 7 |
| gill | .34 | 3.53 | 10 |
| stalk | 1.13 | 7.71 | 7 |

Each tissue contains different amounts of tyrosinase activity and may have a different isoenzyme composition. Samples from the skin, gill, cap flesh, and the stalk were subjected to native electrophoresis followed by staining with dopa for dopa oxidase activity. In contrast to whole mushroom homogenates, each tissue appeared to contain a single dominant isoenzyme form (data not shown) but each tissue also showed a different isoenzyme pattern. This suggests that when all tissues are homogenized together some interconversion or transformation process takes place, perhaps by proteolysis, which generates the patterns we observed in different developmental stages. This also suggests the need to monitor discrete isoenzymes in individual tissue sections with development.

In conclusion, the results presented in this report and by others tend to indicate that tyrosinase activity and the isoenzyme content in mushrooms (1) are very complicated, (2) are different in pre-harvest development, (3) vary from tissue to tissue, (4) are composed of latent and active enzyme forms; and that no new isoenzyme forms are synthesized during development. The applications of these findings may be of interest to the mushroom industry but also indicate the need for further study of the enzyme during pre-harvest and post-harvest development and storage.

## Acknowledgments

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# Chapter 5

# Oxygen- and Metal-Ion-Dependent Nonenzymatic Browning of Grapefruit Juice

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Grapefruit juice was packaged in glass bottles with various headspaces, calculated to provide several different concentrations of oxygen/ml juice. The oxygen played the most important role in degradation of ascorbic acid (ASA) and non-enzymatic browning of grapefruit juice stored at 23°C. A linear correlation was found among the amount of oxygen, the degradation rate of ASA and the browning rate. The lag period of browning of grapefruit juice decreased in length with increasing oxygen concentration. Ascorbic acid oxidase, which oxidizes ascorbic acid to dehydroascorbic acid, was found capable of eliminating the lag period of the browning in grapefruit juice. Oxygen also enhanced the browning affected by dehydroascorbic acid. As the electronic structure of oxygen is in the triplet state, its reaction with molecules of singlet multiplicity, such as ascorbic acid, is spin-forbidden. This phenomenon led us to determine the involvement of metal ions in the process of non-enzymatic browning affected by ascorbic acid oxidation. The oxidative degradation of ASA and the browning of grapefruit juice were inhibited by the addition of chelating agents (EDTA). The results demonstrated the involvement of metal ions in the process of non-enzymatic browning.

Non-enzymatic browning of citrus juices and concentrates has been reported by many researchers as a major cause of quality deterioration in citrus products (1-6). It was suggested that sugar-sugar and sugar-amino reactions were unlikely to be the only contributors to the formation of brown pigments during storage of citrus products (7), and especially of those packaged in plastic metallized laminate bags

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or cartons (8). The involvement of ascorbic acid in non-enzymatic browning of citrus products was studied by many researchers (9-12). Degradation of ascorbic acid provided carboxyl compounds (4,7,13,14) that subsequently reacted via aldole condensation or with amino acids groups and polymerized to give brown pigments (10,11,12). Ascorbic acid degradation in heat-treated citrus products is due to aerobic and anaerobic reactions of a non-enzymatic nature.

The aerobic pathway of non-enzymatic browning in citrus products required oxygen, which may enter in aseptically packed products by dissolved from the headspace or penetrate through packaging material (8). Ascorbic acid destruction rates were directly proportional to the initial concentration of dissolved oxygen in several model systems (8,15,16). Storage studies of the loss of ascorbic acid potency in canned orange juices have shown an initial period of rapid loss of vitamin C that was caused by the presence of free oxygen (4,5). However, the electronic structure of oxygen has two unpaired electrons at the energy level of $\pi$ antibonding, in triplet state. The reaction of oxygen, therefore, is spin-forbidden with ground state molecules of singlet multiplicity such as ascorbic acid (17) (Fig. 1, reaction a).

Transition metals, e.g. iron and copper, with their labile d-electrons system, are well suited to catalyze redox reaction. Stable paramagnetic states, resulting from the presence of unpaired electrons, are common for transition metals and facilitate their reaction with free radicals or triplet molecules, such as oxygen, which is bi-radial in the triplet state (17) (Fig. 1, reaction b). Thus, they are able to remove this spin restriction between oxygen and ascorbic acid and thereby promote the oxidation of the singlet molecule (Fig. 1, reaction b). Oxygen could interact with other molecules in the triplet state (Fig. 1, reaction c). Activated molecules of oxygen in the singlet state (singlet oxygen) could oxidize directly with singlet molecules (Fig. 1, reaction d).

This study was conducted in an attempt to understand better the effect of oxygen on non-enzymatic browning of citrus juices and the involvement of metals in this process.

## Materials and Methods

L-ascorbic acid, EDTA-$Na_2$, metaphosphoric acid, dichlorophenol-indophenol, sodium benzoate and potassium hydroxide were purchased from BDH Chemicals Ltd. (Poole, England), and hydrochloric acid 37% and citric acid from Merck (Darmstadt, FRG); ascorbate oxidase was obtained from Sigma Chemical Co. (St. Louis, MO).

Single-strength grapefruit juice was reconstituted from a fresh APV evaporator concentrate of 58$^{o}$ Brix with distilled water to 11.2$^{o}$ Brix and poured into 200-ml glass bottles. The bottles were filled with juice to a headspace which by calculation was found to provide different amounts of oxygen per ml of juice. The juice was preserved with 600 ppm sodium benzoate.

Ascorbic acid was determined by titration with 2,6-dichlorophenol-indophenol (18). The color of the juice was determined directly with a Gardner Tristimulus Colorimeter, model KL 10. The instrument was calibrated against a white plate, L=91.6, a=1.8, b=+1.8.

Results are the means of triplicates, and in the figures each error bar (I) denotes the standard deviation.

## Results and Discussion

Non-enzymatic browning of grapefruit juice is accelerated by increasing oxygen concentration. During the first days of storage there was a lag period where the browning rate was low. After this period, the browning rate accelerated and decreased once more after all the ascorbic acid had been degradated (Fig. 2). Figure 3 illustrates the linear relationship between browning and the amount of oxygen. In contrast to juice browning, ascorbic acid degradation was rapid during the first period of storage (Fig. 4).

Figures 5 and 6 illustrate the linear relationship between ascorbic acid oxidation and oxygen concentration, and the correlation between browning and ascorbic acid degradation.

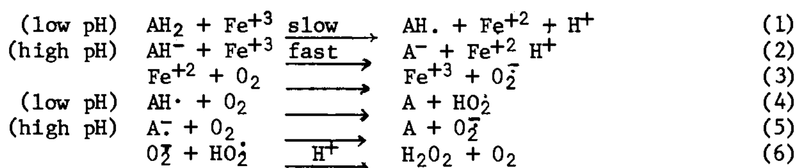
In order to better understand the effect of ascorbic acid on non-enzymatic browning of citrus juices, we oxidized endogenous ascorbic acid in grapefruit with ascorbic acid oxidase. The enzyme oxidized ascorbic acid to dehydroascorbic acid and $H_2O$ without forming $H_2O_2$ (19).

This study has shown that grapefruit juice treated with the enzyme, browned without developing a lag period. The treated juice browned anaerobically, but in an aerobic environment the browning rate was significantly higher (Fig. 7). It was demonstrated that oxygen is involved in browning reactions not only to convert ascorbic acid to dehydroascorbic acid, but also to convert some other reductones generated from dehydroascorbic acid to by-products which form brown pigments.

The brown pigments which were formed through the oxidative pathway are almost twice those formed through the anaerobic pathway. This effect could be derived from the involvement of $H_2O_2$ and oxy--radicals in the aerobic oxidation pathway of ascorbic acid (20,21). The oxy-radicals may generate carbonyls and amino acid free radicals which, by polymerization, could accelerate the formation of more brown pigments.

Although the inhibition of ascorbic acid oxidation in citrus juices (22,23) and other foods by EDTA was reported by several researchers (22-24), the possibility that EDTA could inhibit non-enzymatic browning in grapefruit or other citrus juices has not been explored.

The results illustrated in Figures 8 and 9 showed that EDTA inhibited non-enzymatic browning and ascorbic acid oxidation in grapefruit juice. This demonstrates the involvement of metal ions in the non-enzymatic browning of grapefruit juice and possibly of other citrus products. The involvement of metal ions, oxygen, and oxygen species in non-enzymatic browning of grapefruit juice could be described by the following reactions:



$$\text{(low pH)}\quad AH_2 + Fe^{+3} \xrightarrow{\text{slow}} AH. + Fe^{+2} + H^+ \qquad (1)$$
$$\text{(high pH)}\quad AH^- + Fe^{+3} \xrightarrow{\text{fast}} A^- + Fe^{+2}\ H^+ \qquad (2)$$
$$Fe^{+2} + O_2 \longrightarrow Fe^{+3} + O_2^{\overline{\cdot}} \qquad (3)$$
$$\text{(low pH)}\quad AH\cdot + O_2 \longrightarrow A + HO_2^{\cdot} \qquad (4)$$
$$\text{(high pH)}\quad A^{\overline{\cdot}} + O_2 \longrightarrow A + O_2^{\overline{\cdot}} \qquad (5)$$
$$O_2^{\overline{\cdot}} + HO_2^{\cdot} \xrightarrow{H^+} H_2O_2 + O_2 \qquad (6)$$

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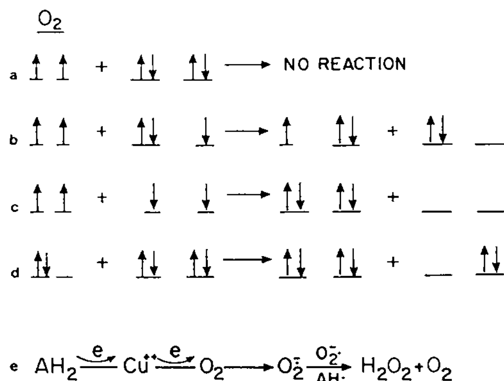



Figure 1. Reactions among oxygen, ascorbic acid and metal ion illustrated by the interaction of electrons from the valence orbitals.

a, Reaction between triplet oxygen and a singlet molecule.
b, Reaction between triplet oxygen and metal ions.
c, Reaction between triplet oxygen and other molecules in the triplet state.
d, Reaction between singlet oxygen and other singlet molecules.
e, Reaction among ascorbic acid, cupric ion and oxygen which generates superoxide ($O_2^-$), ascorbyl radical (AH·) and, by dismutation and further oxidation, produce hydrogen peroxide ($H_2O_2$), oxygen and dehydroascorbic acid.

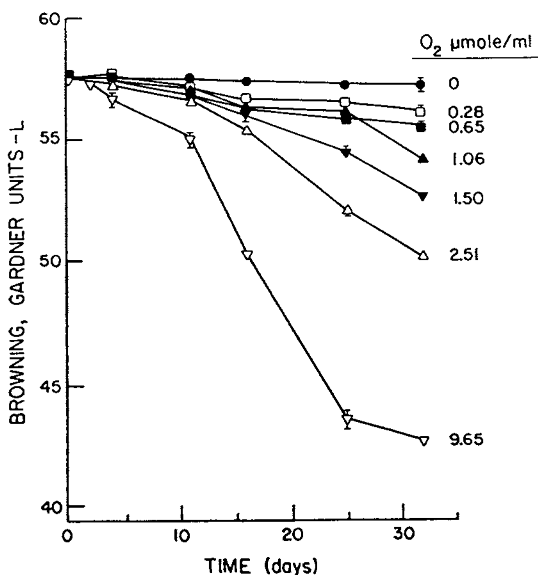



Figure 2. The effect of oxygen concentration on grapefruit juice browning at 23±2°C.

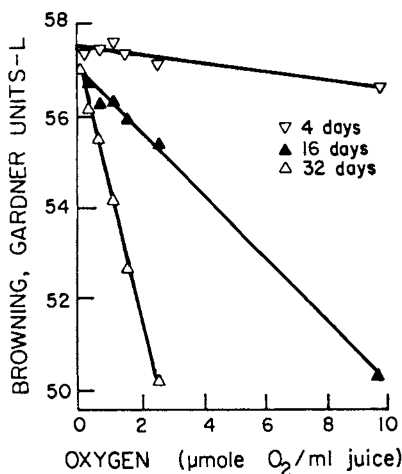



Figure 3. Correlation between oxygen concentration and grapefruit juice browning at 4, 16 and 32 days of storage at 23±2°C.

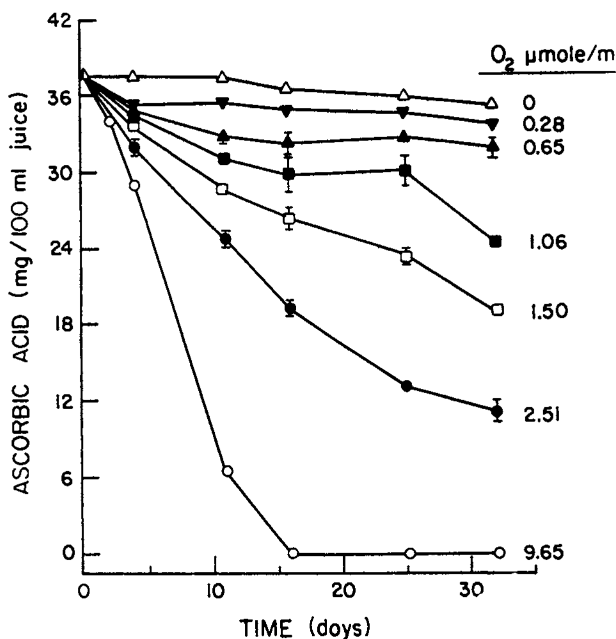



Figure 4. Ascorbic acid oxidation in grapefruit juice as affected by oxygen concentration.

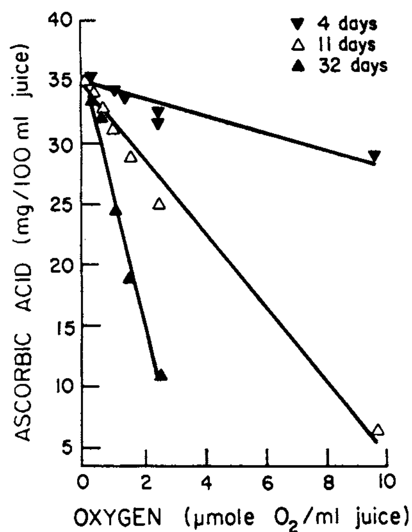



Figure 5. Correlation between oxygen concentration and ascorbic acid oxidation in grapefruit juice during storage at 23±2°C.

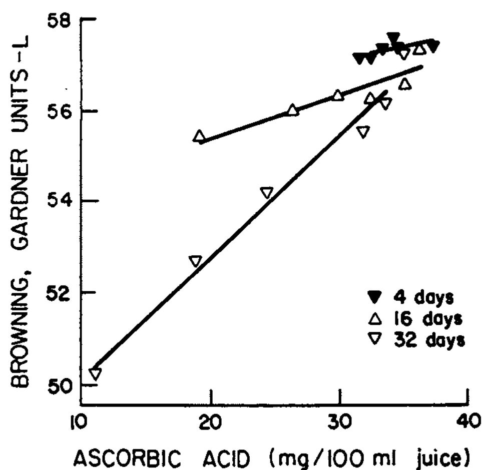



Figure 6. Correlation between ascorbic acid oxidation and browning of grapefruit juice.

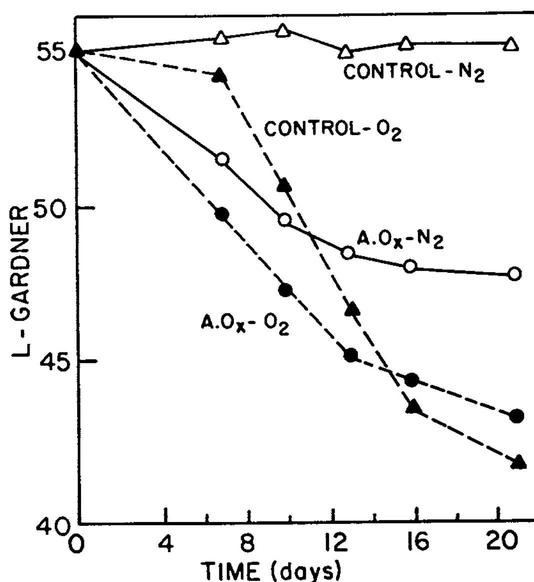



Figure 7. The effect of ascorbic acid oxidation by ascorbic acid oxidase on grapefruit juice browning under different conditions. Δ, the presence of nitrogen; ▲, control in the presence of 9.65 µmole oxygen/ml juice;
O, grapefruit juice treated with ascorbic acid oxidase (1 U/5 ml juice) in the presence of nitrogen; ●, grapefruit juice treated with ascorbic acid oxidase (1 U/5 ml juice) in the presence of oxygen.

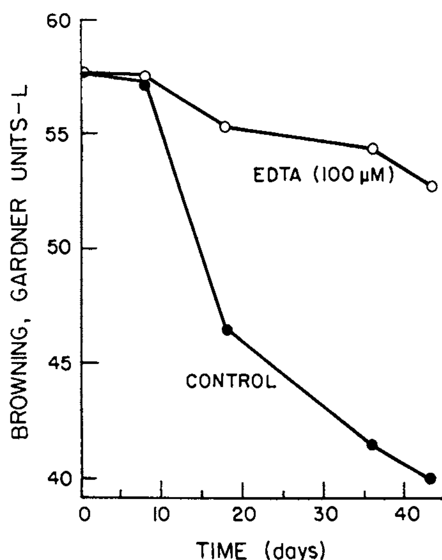



Figure 8. The effect of a chelating agent (EDTA) on non-enzymatic browning of grapefruit juice incubated at 23±2°C.
●, control juice in the presence of 9.65 μmole $O_2$/ml; O, as control, but with added 100 μM EDTA.

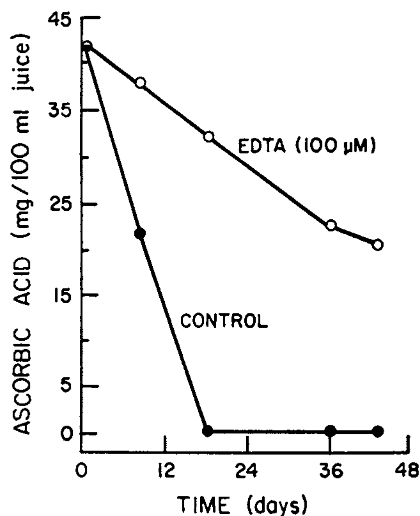
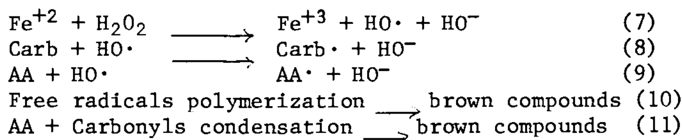



Figure 9. The effect of a chelating agent (EDTA) on ascorbic acid oxidation in grapefruit juice.
●, control juice in the presence of 9.65 μmole $O_2$/ml; O, as control, but with added 100 μM EDTA.



$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + HO\cdot + HO^-$ (7)
$Carb + HO\cdot \longrightarrow Carb\cdot + HO^-$ (8)
$AA + HO\cdot \longrightarrow AA\cdot + HO^-$ (9)
Free radicals polymerization $\longrightarrow$ brown compounds (10)
AA + Carbonyls condensation $\longrightarrow$ brown compounds (11)

$AH_2$ = ascorbic acid; $A^-$ = semiquinone of ascorbic acid; $O_2$ = superoxide radical; $HO_2$ = perhydroxyl radical; Carb· = carbohydrate radical; A = dehydroascorbic acid; AA = amino acids.

The effect of EDTA in oxidation reaction is complicated. EDTA is known to accelerate ascorbic acid oxidation by ferric ions at neutral pH (21,25,26). In a medium at the same pH, EDTA also enhanced the formation of hydroxyl radicals by ferric ion in the presence of ascorbic acid (21,25).

It is known that the redox potential of the $Fe^{+3}/Fe^{+2}$ pair can vary by complexing ligands (27). EDTA reduces the redox potential of $Fe^{+2}$ (28) and this increases the rate constant transfer of the electron from $Fe^{+2}$ to $H_2O_2$, which is formed during autooxidation of ascorbic acid (29), and decomposition of the latter to HO·. However, at low pH 3-4, EDTA was found to inhibit ascorbic acid oxidation by ferric ions (29). Thus, the form the metal chelate takes, as a function of pH, plays a key role in its effectiveness as a catalyst. Cupric ions are known to accelerate ascorbic acid oxidation; however, EDTA inhibits its catalytic effect at both neutral and low pH (24).

Citric juices contain iron and copper ions (30,31) at a concentration which could catalyze ascorbic acid oxidation. Contamination of citrus juices by transition metals will affect the rate of oxidative browning, especially in products exposed to air, such as citrus juices stored in plastic metallized laminate bags which are permeable to oxygen.

## Acknowledgment

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# Chapter 6

# Chemistry of Bioregulatory Agents

## Impact on Food Color

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Bioregulatory agents such as MPTA and DCPTA possess two distinct properties; stimulation of the carotenoid biosynthetic pathway and inhibition of the cyclization of the acyclic to the cyclic carotenoids.The stimulation appear to be an indirect effect whereas the inhibitory effect is a direct one. By application of the compounds at lower concentrations at the early stages of its developmental phase, the inhibitory effect can be minmized resulting in the enhancement of desirable color normally associated with the crop.

Color is an important quality attribute of many food products. And color formation can be affected by bioregulatory agents like MPTA [2-diethylaminoethyl-4-methylphenylether] and DCPTA [2-diethylaminoethyl-3,4-dichlorophenylether] which regulate biosynthesis of carotenoid pigments in a wide array of plants and microorganisms [1,2]. These bioregulatory agents possess two distinct properties; 1) stimulation of the synthesis of carotenoids and 2) inhibition of the transformation of acyclic to cyclic carotenoids. Thus, when citrus fruits are treated postharvest with MPTA, for example, the usual response observed is a rapid formation of red coloration due to a large net synthesis of acyclic lycopene in the flavedo of the peel [3]. The cyclization of the acyclic lycopene to the monocyclic *gamma*-carotene and the bicyclic *beta*-carotene is inhibited [4]. That the stimulation of the carotenoid biosynthetic pathway is regulated by action at the gene expression level is strongly suggested by antibiotic studies [5]. Antibiotics like *alpha*-amanitin, cordycepin and anisomycin which are known transcriptional inhibitors inhibit the biological activity of MPTA. Additionally, cycloheximide, a known translational inhibitor, severely inhibits the bioregulatory action of MPTA in carotenoid biosynthesis. Studies also have shown that DCPTA increases the activities of key enzymes, such as mevalonic acid kinase and isopentenylpyrophosphate isomerase, involved in the isoprenoid pathway, indicating an increase in protein synthesis [6].

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COLOR RESPONSE PATTERN IN CITRUS

The relative effectiveness of a bioregulatory agent on inhibition of cyclization and on stimulation of net carotenoid synthesis leads to variations in the carotenoid response pattern. When fully mature navel oranges are treated postharvest by vacuum infiltration for ten minutes with 2,000 ppm of MPTA, the carotenoid pigment pattern in the flavedo of the peel is quite similar to that observed in the endocarp with the acyclic lycopene as the main pigment as shown in Table I. The control fruits were vacuum infiltrated with water.

The formation of the normally present cyclic carotenoids is inhibited. The transformation of the acyclic lycopene to the monocyclic *gamma*-carotene is partially inhibited and further cyclization to the bicyclic *beta*-carotene is totally inhibited. However, when the treatment of the entire tree which is sprayed with 2,000 ppm MPTA is conducted preharvest at the fully mature stage of fruit development [7], the carotenoid composition of the endocarp reflects a net synthesis of the cyclic pigments and differs from the pattern seen in the peel as shown in Table II.

The usually present cyclic pigments violaxanthin and cryptoxanthin are the main pigments instead of the acyclic lycopene. The inhibition of the cyclization reaction is no longer manifested in the endocarp on preharvest treatment of the tree bearing the fruits, whereas the peel still shows the effect of inhibition. These observations strongly suggest a direct effect of the compound MPTA on inhibition of the cyclization reaction but an indirect effect on stimulation of carotenoid synthesis. Similar results were observed with Valencia oranges with another bioregulatory agent 2-diethylaminoethyl-4-ethylphenylether which possess properties similar to MPTA [7].

Table I. Effect of Vacuum Infiltrated 2000 ppm MPTA on Carotenoid Composition of Postharvest Treated Navel Orange

| | Content [ug/g dry wt] | | | |
|---|---|---|---|---|
| | Endocarp | | Flavedo | |
| Carotenoid | Control | Treated | Control | Treated |
| zeta-carotene | 23.4 | 72.1 | 26.2 | 76.2 |
| neurosporene | | 50.1 | | 61.2 |
| lycopene | | 191 | | 272 |
| gamma-carotene | | 16.1 | | 18.2 |
| beta-carotene | 0.2 | | 0.8 | |
| carotenols incl. epoxides | 201 | 205 | 280 | 310 |

Table II. Effect of 2000 ppm MPTA on Carotenoid Composition of Preharvest Treated Navel Orange

| | Content [ug/g dry wt] | | | |
|---|---|---|---|---|
| | Endocarp | | Flavedo | |
| Carotenoid | Control | Treated | Control | Treated |
| zeta-carotene | 23.2 | 74.2 | 25.1 | 71.2 |
| neurosporene | | 32.3 | | 42.3 |
| lycopene | | 72.6 | | 272 |
| gamma-carotene | | 92.1 | | 19.2 |
| beta-carotene | 3.2 | 112 | 2.2 | 2.6 |
| cryptoxanthin | 18.1 | 70.2 | 8.9 | 10.2 |
| lutein | 2.3 | 6.4 | 1.7 | 2.4 |
| zeaxanthin | 3.1 | 82 | 2.1 | 2.3 |
| violaxanthin | 82.1 | 224 | 75.5 | 89.7 |
| antheraxanthin | 20.1 | 57.2 | 8.9 | 10.5 |
| luteoxanthin | 4.5 | 12.3 | 61.5 | 73.1 |

## COLOR RESPONSE PATTERN IN MOLD

Further demonstration of these two distinct and separate properties is evident in the carotene response pattern observed in further studies of the bioregulation of carotene synthesis in the carotenogenic mold *Phycomyces blakesleeanus*. Mycelia of *P. blakesleeanus* cultured on media containing 10 ppm DCPTA show the acyclic lycopene as the main pigment constituent as shown in Table III.

A 5 mm diameter circular mycelial mat from the 10 ppm DCPTA media [1st transfer] was transferred to media containing 0 ppm DCPTA and allowed to further develop for 3 days to about 35 cm in diameter. Examination of the latter mycelial mat clearly show no evidence of any carryover of DCPTA from the initial media. The carotenoid composition of the first transfer show the usually present bicyclic *beta*-carotene, not the acyclic lycopene, as the main pigment, similar to the pattern normally found in the mycelia of the mold. However, there was a ten-fold increase in the amount of *beta*- and

Table III. Effect of DCPTA on Carotenoid Composition of Mycelia of *Phycomyces blakesleeanus*

| | Content [ug/g dry wt] | | | |
|---|---|---|---|---|
| | DCPTA | | | |
| Carotenoid | 0 ppm | 10 ppm | 1st Transfer* | 2nd Transfer** |
| zeta-carotene | 10 | 52 | 40 | 36 |
| neurosporene | 20 | 60 | 52 | 56 |
| lycopene | 40 | 810 | 84 | 72 |
| gamma-carotene | 12 | 31 | 43 | 39 |
| beta-carotene | 96 | 10 | 920 | 856 |
| alpha-carotene | 12 | 10 | 105 | 93 |

* Mycelial square [5 mm] transferred from 10 ppm DCPTA media to media containing 0 ppm DCPTA.
** Mycelial square [5 mm] transferred from 0 ppm DCPTA media to media containing 0 ppm DCPTA

alpha-carotene over the control, indicating a large stimulation of synthesis of the pigments and absence of the inhibitory effect of DCPTA on cyclization. Similar responses are observed in the second transfer which was accomplished by again transferring a 5 mm square to 0 ppm DCPTA media. Clearly DCPTA in itself is not directly involved in stimulating the net synthesis of the bicyclic carotenes. This is supported by the absence of inhibition of the cyclization reaction in the transfer mycelia. The stimulation or the regulation of gene expression appear to be mediated by the bioinduction of an effector molecule which triggers the derepression of the genetic material, as shown in Figure 1. Similar results, as shown in Table IV, were observed in mature tomato fruits harvested from greenhouse-grown plants whose seeds were treated with DCPTA. The fruits were harvested 68 days after seed germination.

Table IV. Effect of DCPTA seed-treatment upon the carotene content of mature tomato fruits

| DCPTA uM | Lycopene ug/g fresh wt.* | beta-Carotene ug/g fresh wt.* |
|---|---|---|
| 0 | 48.46 [c] | 1.90 [c] |
| 3 | 81.21 [b] | 3.18 [b] |
| 15 | 98.18 [ab] | 4.33 [ab] |
| 30 | 111.83 [a] | 5.24 [a] |
| 150 | 110.10 [a] | 5.67 [a] |

* Carotene contents were determined from five random samples of mature fruits. Means [N=5] followed by the same letter are not significantly different [$p<0.05$] according to Duncan's Multiple Range Test.

There is observed an increased production of the normal pigment constituents and of both the acyclic lycopene and the bicyclic beta-carotene in the fruits from treated plants with no indication of cyclic inhibition. At low concentrations DCPTA has been shown also to affect biomass development in tomato and other crops [8].

## CONCLUSION

It is quite clear from the above observations that MPTA and DCPTA can enhance food color by increasing the formation of specific carotenoids normally associated with the individual food crop. When the bioregulatory agents are applied at the beginning of developmental phase of the crop at low concentrations, the stimulatory effect on carotenoids is manifested with no cyclase inhibition resulting in enhancement of desirable color quality.

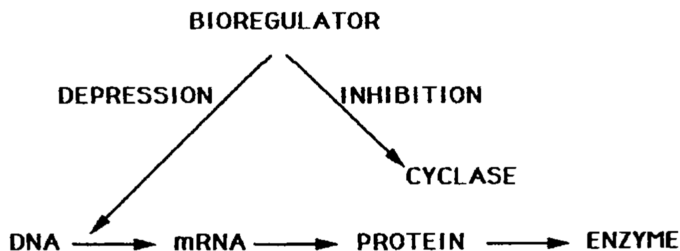


Figure 1. Scheme for the mode of action of bioregulator

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Chapter 7

# Enzymes Involved in Off-Aroma Formation in Broccoli

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The enzyme(s) responsible for aroma deterioration of broccoli was determined by adding back peroxidase, lipase and cystine lyase purified from broccoli to homogenized blanched broccoli samples at the activity levels present in unblanched broccoli. Following incubation for 3 h at 30°C, the samples were cooked at 93°C for 20 min and presented to a panel of judges trained in descriptive analyses. The aroma intensities were compared with those of blanched and unblanched homogenates of broccoli treated in the same way and with standard aroma descriptors. Lyase added alone reproduced the descriptors 'overall', 'sour' and 'sulfhydryl compound' of the unblanched samples. Addition of lipase and lyase together reproduced, in addition, the "ammonia" descriptor of the unblanched samples. None of the enzymes isolated reproduced the 'unripe banana' aroma of the unblanched broccoli.

Enzymes are responsible for most of the hundreds of reactions that occur simultaneously in plants, animals and microorganisms. These enzymes are highly efficient and capable of converting $10^2$ - $10^7$ moles of substrate to product per mole of enzyme each minute at ambient temperature. They are highly specific, permitting reactions to be channeled in appropriate directions at appropriate times. They are responsible for the growth and maturation of the raw materials that provide our food. They are also responsible for the color, flavor, aroma, texture and nutrient quality of the raw materials. Enzyme activities do not cease when the ideal quality characteristics of food materials have been achieved. After harvest, many of the enzymes continue to act on remaining substrates; the rates of the reactions are sometimes accelerated by the general senescence of the tissue and by damage during harvesting and storage.

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What enzyme is responsible for quality deterioration in mature fresh fruits and vegetables? Unfortunately, there is no single key enzyme. The quality factors to be maintained are color, flavor, aroma, texture and nutrition. Deterioration of all these quality factors begins after the raw material reaches maturity and is harvested and stored. But one of these factors is often more important than others. In tomatoes, it is softening caused by the pectic enzymes. In damaged fruit, such as peaches, apples and avocadoes, it is browning due to polyphenol oxidase. In green leafy vegetables it is flavor and aroma deterioration, but may include discoloration (loss of green color and/or browning).

Kohman, in 1928 (1), was among the first to note that a short heat treatment could prolong refrigerated and frozen storage of plant materials. Shortly thereafter, it was shown that the increased storage time resulted from inactivation of the enzymes in plant materials, as well as in associated microorganisms.

Complete inactivation of all enzymes by heat treatment is easily achieved. But heating can cause undesirable losses in color, texture, flavor, aroma and nutritive quality. For maximum quality retention, clearly the need is for sufficient heat treatment to stabilize the product against enzymatic deterioration but at the same time to minimize quality losses due to heating. This need was met by selection of an endogenous enzyme as an indicator of adequate heat treatment. The criteria applied were: loss of enzyme activity should correlate with quality retention on frozen storage; activity of the enzyme should be easily assayed in the processing plant; and the enzyme should be ubiquitous to a number of plant materials.

As early as 1932, catalase was used to monitor the adequacy of heat treatment (2-4), especially in English green peas. In 1949, Joslyn (5) concluded that loss of peroxidase activity paralleled quality deterioration more closely than did the loss of catalase activity. For the next 20-30 years, catalase served as the indicator enzyme for English green peas and a few other vegetables (6) while peroxidase served as the indicator enzyme for the other vegetables. Catalase in most plant materials is about 50-70% as stable to heat as peroxidase and it is also less stable than peroxidase during frozen storage, presenting a dilemma for monitoring adequate heat treatment prior to freezing and storage. In 1975, the U.S. Department of Agriculture (7) recommended that, for the majority of vegetables, catalase inactivation was not a satisfactory predictor of storage stability and that inactivation of peroxidase was necessary to minimize deterioration of quality during frozen storage. Use of peroxidase as the indicator is not without problems as it can recover its activity under certain conditions (8, 9). With the exception of asparagus, there is no evidence that peroxidase is directly associated with quality deterioration in most plant materials.

Enzymes other than peroxidase and catalase have been used less frequently to monitor adequacy of heat treatment. Some enzymes that have been used include polyphenol oxidase for off-color development in fruits, polygalacturonase for loss of consistency in tomatoes, potatoes and eggplants and lipoxygenase and lipase for

off-flavor development in soybean and cereal products, respectively.

Correlation of quality retention during storage with the residual activity of a particular enzyme generally involves storage studies in which the indicator enzyme is inactivated to various degrees. In many studies the enzyme is completely inactivated before storage. Both approaches have shortcomings, including the prolonged storage times needed, even under accelerated storage conditions. Other enzymes may also retain some activity when incomplete inactivation of the indicator enzyme is used, thus preventing clear assessment of the role of the indicator enzyme. Storage studies based on complete inactivation of the indicator enzyme do not establish that this enzyme is directly responsible for the quality deterioration, unless it is the most heat stable enzyme present.

Recently we reported another method of assessing the importance of enzymes in quality deterioration (10). Enzymes thought to be responsible for quality deterioration, such as off-flavor and off-aroma, were isolated from the vegetable of interest by traditional enzyme purification techniques. These isolated enzymes, singly and in all combinations, were added (at the same activity level as found in the unblanched vegetable) to a purée of the same vegetable in which all the enzymes were previously inactivated by heat treatment. After incubation with enzyme(s), the purée was heated to inactivate the enzyme(s), and a trained sensory panel assessed the type and intensity of aroma descriptors detected. Samples of blanched and unblanched vegetables were included, as well as standards for the aroma descriptors. This approach led to determination that lipoxygenase is the most important enzyme in aroma deterioration in English green peas and in green beans (10).

In the present paper, we report on studies to determine the enzymes responsible for off-aroma deterioration in broccoli.

## MATERIALS AND METHODS

**Materials.** About 100 lbs of fresh broccoli (*Brassica oleracea* var. pompejana, cultivar Valiant 501) from Patterson Frozen Foods was picked early in the morning, placed on dry ice and transported to Davis within two hours. A portion of the sample was frozen in a blast freezer immediately; while the other portion was steamed-blanched at 99°C (210°F) for 3.5 min, cooled at room temperature, bagged and frozen. Both samples were stored in lock-top plastic bags at -20°C. All of the chemicals used were of reagent grade and deionized water was used.

**Enzyme Purification.** Broccoli contained sufficient levels of peroxidase, lipase and cystine lyase to permit their isolation in the amounts needed. Only traces of lipoxygenase and catalase were present. Activities (units/g vegetable; see assay methods below) were: peroxidase, 220; lipase, 12; lyase, 0.26. Catalase was $\sim$0 units/g in broccoli compared to 19 in English green peas; lipoxygenase was $\sim$2 units/g in broccoli compared to 110 in English green peas. Peroxidase, lipase and cystine lyase were purified by

In Quality Factors of Fruits and Vegetables; Jen, J.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

the method shown in Figure 1. The method for cystine lyase was modified from the method of Hamamoto and Mazelis (11). The method used was designed so as not to separate isoenzymes. The enzyme preparations were free of the other two enzymes. All steps were performed at 0 to 4°C, either in an ice bucket or in a cold room.

**Enzyme Assays.** Catalase activity was determined by the rate of decrease in absorbance at 240 nm at 25°C, using 0.05 M $H_2O_2$ in 0.05 M sodium phosphate buffer, pH 7.0 (12). Peroxidase activity was determined by the rate of increase in absorbance at 420 nm at 25°C using 0.05 M guaiacol and 0.02 M $H_2O_2$ in 0.2 M sodium phosphate buffer, pH 6.0 (13). Lipoxygenase activity was measured by the rate of increase in absorbance at 234 nm at 25°C, using $2.5 \times 10^{-3}$ M linoleic acid in 0.2 M sodium phosphate buffer, pH 7.0 (14). Lipase activity was determined titrimetrically using a pH stat at 35°C. The substrate was either 0.015% olive oil or tributyrin in 0.025 M Tris buffer, pH 8.0, containing 0.2% sodium taurocholate. One milliequivalent of acid produced per minute is one unit of activity. Cystine lyase activity was determined by the method of Hamamoto and Mazelis (11), using 12 mM cystine and 2.5 μM pyridoxal-5'-phosphate (PALP) in 0.15 M Bicine buffer, pH 8.4. Reaction was for 10 min at 30°C. The reaction was stopped by adding 1 ml 10% TCA to the 1 ml reaction, the mixture was centrifuged and the amount of pyruvate formed measured by 2,4-dinitrophenylhydrazine. One unit of activity for catalase, peroxidase, lipoxygenase and cystine lyase is defined as an increase in absorbance of 1.0 in one minute.

**Protein Assay.** Quantitative protein assays were performed by the Lowry method (15). Protein in column eluates was monitored at 280 nm.

**Aroma Analysis.** Isolated peroxidase, lipase and cystine lyase were added singly and in various combinations to homogenates of blanched broccoli (free of enzyme activities) at the same activity level they were present in the unblanched broccoli. The samples were incubated at 30°C for three hours in a water bath and then cooked on hot plates for 20 min at 93°C. A teaspoon of sample was put into each of several wine glasses, the glasses covered and the samples incubated at room temperature (23±1°C) for at least 15 min before evaluation by the panel.

Judges consisted of 16 panelists experienced in descriptive analysis. Tests were performed in groups of five judges each. Each sample was given in duplicate and a reference (w/o enzyme) was used with each set of samples. A control (w/o enzyme) and an unblanched sample were also included.

The descriptors used and standards provided were 'cooked corn', cooked corn; 'straw', dry straw; 'honey (sweet)', honey; 'grassy', freshly cut grass; 'cabbage', freshly cut cabbage; 'unripe banana', green banana; 'sour', dilute acetic acid; 'ammonia', dilute ammonium hydroxide; 'sulfhydryl compound', very dilute mercaptoethanol. Judges scored intensity of each attribute on a 10 cm line labeled at the ends "Less than" and "Greater than control" and "Same as" in the center. Table 1 shows that the aroma

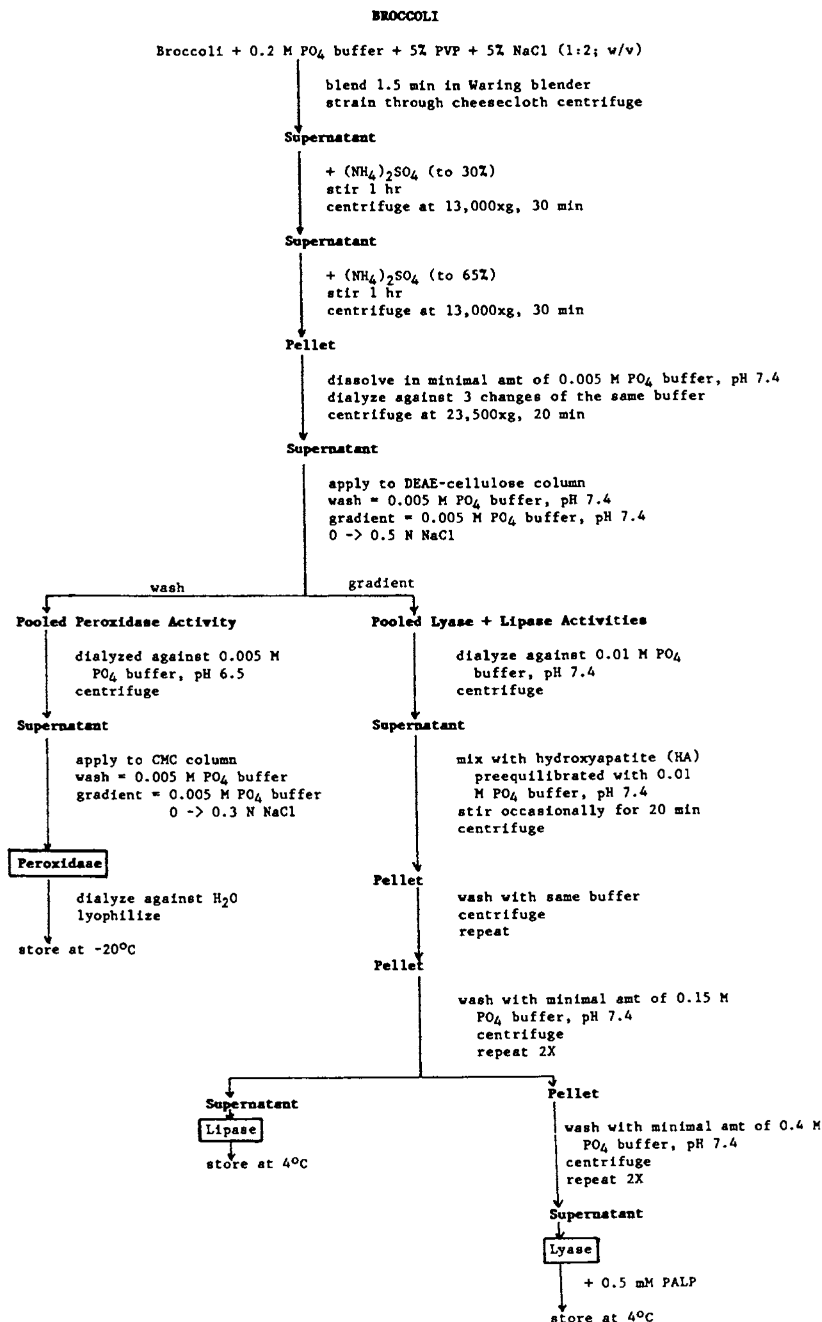


Figure 1. Schematic diagram of the purification of peroxidase, cystine lyase and lipase from broccoli. PVP, polyvinylpyrrolidone; PALP, pyridoxal-5'-phosphate.

Table 1. F Ratios From Analysis of Variance (AOV) for the Aroma Descriptors for Broccoli (n = 16 x 2R)

| Sources of variation | df | Overall | Cooked Corn | Straw | Honey | Grassy | Cabbage | Unripe Banana | Sour | Ammonia | Sulfhydryl Compound |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | 9 | 23.45*** | 2.75** | 0.815ns | 0.79ns | 1.29ns | 3.78*** | 4.17*** | 5.26*** | 3.38*** | 7.92*** |
| Replicate | 1 | 7.46** | 1.125ns | 0.12ns | 2.51ns | 0.11ns | 4.58* | 0.36ns | 0.28ns | 0.07ns | 6.79ns |
| Judge | 15 | 5.07*** | 7.64*** | 8.19*** | 4.92*** | 9.86*** | 5.76*** | 5.99*** | 9.81*** | 3.74*** | 6.54*** |

*, **, ***: Significant at $p<0.05$, 0.01 and 0.001, respectively
ns: Not significant

descriptors 'overall', 'cooked corn', 'cabbage', 'unripe banana', 'sour', 'ammonia' and 'sulfhydryl compound' had significant sources of variation across treatments. By the LSD test (Table 2), the unblanched sample was statistically higher in intensity from the control in descriptors 'overall', 'unripe banana', 'sour', 'ammonia' and 'sulfhydryl compound'. In general, differences in replications were not significant, attesting to the panel's reproducibility (Table 1). As anticipated, judge variation was significant.

The average aroma intensity differences from the blanched reference are illustrated in Figures 2a,b,c. When the average aroma intensity was not different from the reference, the value on the y-axis would be 50, as shown by the horizontal line. The length of the vertical bar represents the degree of lesser or greater intensity difference from the reference. The cross-hatched bars indicate descriptors that were statistically different from the blanched reference.

The following conclusions are drawn from the data:

1. Two blanched controls, one used as the reference, were not statistically different (Fig. 2a).
2. Peroxidase added alone produced aroma intensities not statistically different from the blanched reference (Fig. 2a).
3. Lipase added alone caused a significant increase in the aroma descriptors 'overall' and 'sour' (Fig. 2a).
4. Lyase added alone caused a significant increase in the aroma descriptors 'overall', 'sour' and 'sulfhydryl compound' (Fig. 2a).
5. Peroxidase added together with lyase increased the descriptors 'grassy' and 'sour' to statistically significant levels (Fig. 2b).
6. Peroxidase added together with lipase significantly increased the descriptors 'cooked corn', 'cabbage' and 'sulfhydryl compound' (in one of the two experiments for 'cooked corn' and 'cabbage') (Fig. 2b).
7. In the unblanched sample, the aroma descriptors 'overall', 'unripe banana', 'sour', 'ammonia' and 'sulfhydryl compound' were statistically higher than in the blanched reference (Fig. 2c).
8. Addition of all three enzymes, peroxidase, lipase and lyase, gave results qualitatively similar to the unblanched sample. However, the combined enzymes increased the descriptors 'grassy' and 'cabbage' to a statistically significant level compared to the blanched reference, while at the same time the descriptor 'unripe banana' was not statistically significant (Fig. 2c).
9. Lipase added with lyase gave an aroma descriptor pattern similar to that of the unblanched sample. However, the aroma descriptor 'grassy' increased to a statistically significant level, while the descriptor 'unripe banana' was statistically not significant (Fig. 2c).

In summary, lyase added to blanched broccoli reproduced three of the five aroma descriptor characteristics of unblanched broccoli, namely 'overall', 'sour' and 'sulfhydryl compound'.

Table 2. Fisher's Least Standard Deviation (LSD) Test for Significance Difference Among Mean Intensities for Individual Aromas[a]

| Aroma | LSD | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. Overall | 5.82 | P 45.04 | L' 47.20 | P+L' 51.68 | Con 52.79 | P+L+L' 64.80 | L+L' 66.00 | L 66.30 | P+L 66.68 | Unb 71.96 |
| 2. Corn | 6.03 | P+L 46.82 | P+L' 47.45 | P+L+L' 48.56 | L' 48.93 | P 49.15 | L+L' 49.74 | Unb 51.86 | L 52.02 | Con 54.38 |
| 3. Straw | 5.16 | P 49.64 | P+L 51.12 | Unb 51.45 | L' 52.53 | L 52.64 | L+L' 52.82 | P+L' 53.59 | Con 53.62 | P+L+L' 53.83 |
| 4. Honey | 5.40 | P+L 45.47 | L 45.59 | L+L' 48.24 | P+L+L' 48.85 | L' 49.43 | Unb 49.66 | P+L' 50.87 | P 57.09 | Con 53.73 |
| 5. Grassy | 7.36 | Unb 46.57 | P+L 48.75 | Con 49.21 | L 49.35 | P 49.71 | L+L' 51.20 | P+L+L' 51.33 | L' 51.62 | P+L' 52.09 |
| 6. Cabbage | 5.65 | P 50.02 | L' 52.83 | Con 55.04 | P+L' 57.70 | L+L' 61.93 | P+L+L' 62.14 | P+L 63.42 | Unb 66.25 | L 67.22 |
| 7. Unripe Banana | 4.99 | L 49.33 | P+L 52.46 | P 52.58 | L+L' 52.82 | Con 54.61 | P+L+L' 54.66 | L' 55.03 | P+L' 55.27 | Unb 55.32 |
| 8. Sour | 4.93 | P 50.32 | Con 52.64 | L' 52.77 | P+L' 54.25 | P+L+L' 56.79 | L 59.06 | P+L 59.06 | L+L' 62.30 | Unb 66.20 |
| 9. Ammonia | 4.70 | P 50.98 | Con 52.35 | P+L' 55.01 | L' 55.47 | P+L 57.06 | Unb 57.57 | L 58.13 | L+L' 58.88 | P+L+L' 60.74 |
| 10. Sulfhydryl compound | 5.86 | P 50.44 | Con 54.57 | L' 55.01 | P+L' 57.09 | P+L+L' 61.41 | P+L 62.45 | L+L' 63.00 | Unb 63.08 | L 63.52 |

[a]Symbols used: P = peroxidase; L = lyase; L' = lipase; Unb = unblanched; Con = control. The values with common lines are not different statistically, by LSD at $p<0.05$.

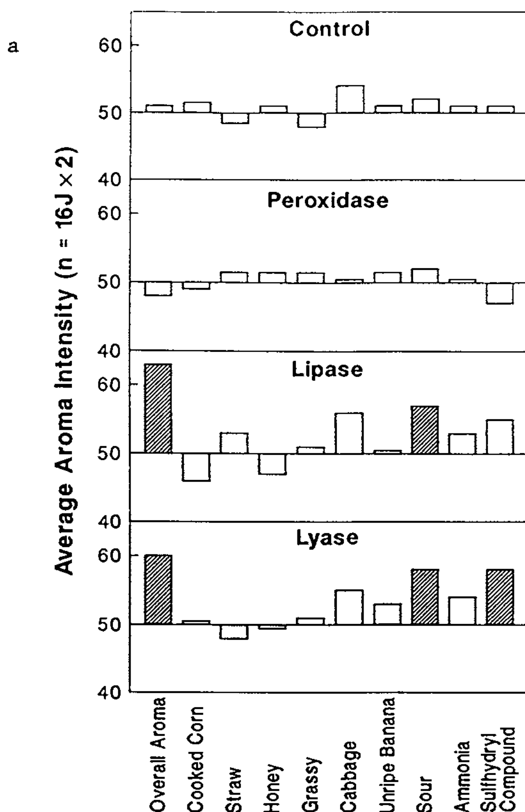


Figure 2. Average aroma intensity differences from the blanched reference of broccoli puree. a, Samples are control, added peroxidase, added lipase, and added lyase. Aroma descriptors designated with shaded bars are statistically different from the blanched reference sample at $p < 0.05$.
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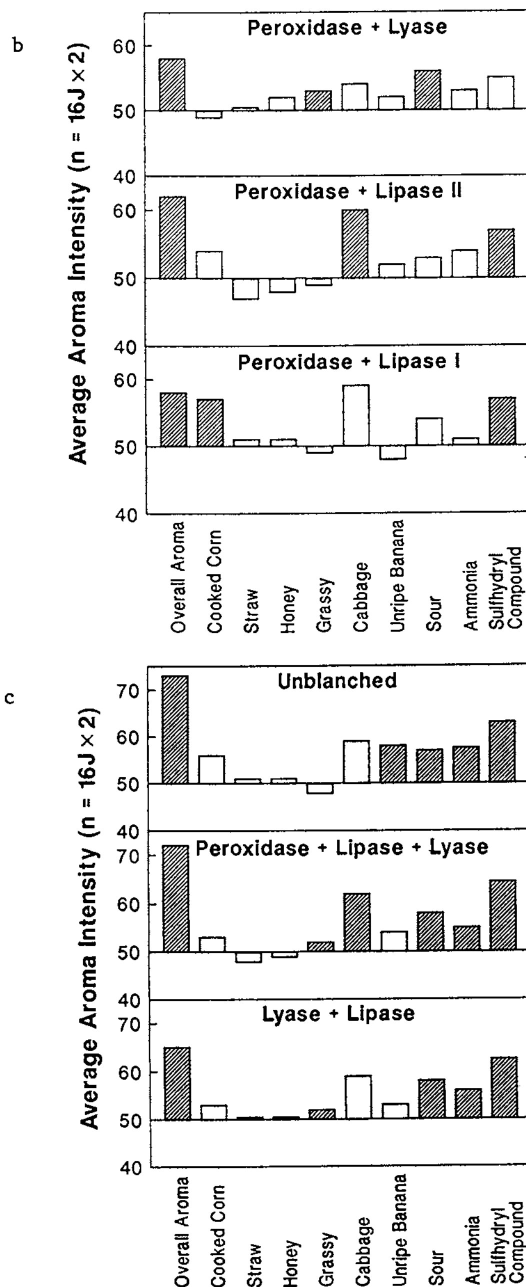
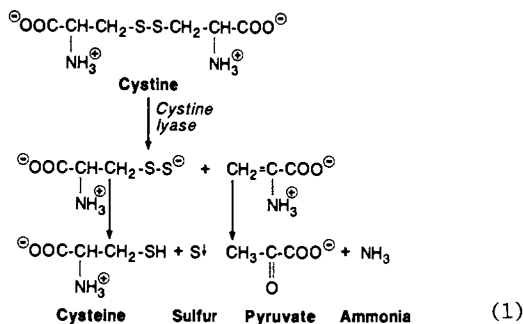



Figure 2. Continued. Average aroma intensity differences from the blanched reference of broccoli puree. b, Samples are added peroxidase + lyase, added peroxidase + lipase II, and added peroxidase + lipase I. c, Samples are unblanched, added peroxidase + lipase + lyase, and added lyase + lipase.

Addition of lyase also increased the aroma descriptor 'ammonia'; the synergistic action of lyase and lipase increased this descriptor to a statistically significant level. None of the three enzymes alone, in pairs, or all together increased the descriptor 'unripe banana' to a significant level as in the unblanched sample. Lyase and lipase added together increased the descriptor 'cabbage' to the level found in the unblanched sample.

As shown in Equations 1 and 2, cystine lyase produces cysteine, pyruvate, ammonia and sulfur from cystine. The cysteine is further acted upon to give pyruvate, hydrogen sulfide and ammonia. Hydrogen sulfide and ammonia formation probably account for the 'sulfhydryl compound' and 'ammonia' aromas detected in the unblanched sample. The pyruvic acid (at the pH of the samples) may account for the 'sour' aroma detected in the unblanched sample, as well as samples treated with lyase and lipase separately.

From the data presented, we conclude that most of the aroma descriptors detected in homogenized unblanched broccoli are due to the action of lyase. However, it appears that lipase may also contribute. None of the three enzymes, alone or in combination, gave the 'unripe banana' aroma detected only in the unblanched sample.

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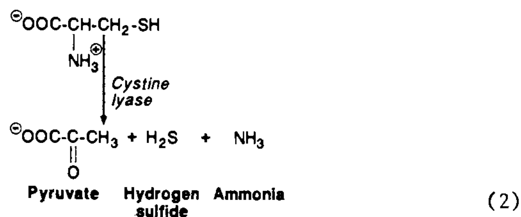


(2)

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# Chapter 8

# Biochemistry and Biological Removal of Limonoid Bitterness in Citrus Juices

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This review paper summarizes the biochemical and biological aspects of limonoids in citrus and presents possible preharvest approaches to reduce bitter limonoids in citrus fruits.

Bitterness due to limonoids in a variety of citrus juices is a major problem of the citrus industry worldwide and has significant negative economic impact. Recently, Maier, et al. (1) published a comprehensive review of limonoid chemistry. More recently, Dekker (2) reviewed the impact of the limonoid bitterness on juice quality and postharvest approaches to the problem. Hasegawa (3) reviewed the metabolism of limonoids in microorganisms and the development of a reactor, that uses immobilized bacterial cells for reduction of bitter limonoids in citrus juices.

Limonoids are a group of chemically related triterpene derivatives found in the plant families, Rutaceae and Meliaceae. Among 37 limonoids reported to occur in citrus and hybrids, four are known to be bitter. They are limonin, nomilin, ichangin and nomilinic acid. Limonin is the major cause of citrus juice bitterness and is widely distributed. Nomilin is also known to contribute to limonoid bitterness in grapefruit juice (4).

Recently, limonoids were shown to occur as glucoside derivatives (Hasegawa, S., et al. Phytochemistry in press). Ten limonoid glucosides have been isolated from grapefruit seeds and orange juices. Each consists of a limonoid linked with one glucose molecule at C-17 by a β-glucosidic linkage. Limonin 17-O-β-D-glucopyranoside appears to have an astrigent and slightly bitter taste (Koski, P., Tropicana, FL. personal communication) as compared to the intense bitterness of its aglycone. Since citrus juices contain relatively high concentrations of limonoid glucosides, these compounds may contribute to the citrus juice flavors.

The monolactones such as limonoate A-ring lactone are the

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predominant limonoid aglycones present naturally in citrus leaves and fruit tisues, whereas the dilactones such as limonin are the predominant limonoid aglycones present in citrus seeds. In this article the author will not make a distinction between monolactones and dilactones.

## Delayed Bitterness

Limonin and nomilin are two bitter limonoids present in citrus juices. Systematic organoleptic tests showed that the bitterness threshold is 6 ppm (5) for limonin, and 6 ppm (6) or 3 ppm (7) for nomilin. The bitterness due to limonin develops gradually in juices after extraction from certain varieties of oranges, grapefruit, lemon, Natsudaidai, mandarin and some other minor citrus such as Iyokan and Ponkan. This phenomenon is generally referred to as "delayed bitterness".

The intact fruits do not contain bitter limonin, but rather a nonbitter precursor, limonoate A-ring lactone (8). When juice is extracted, this nonbitter precursor is gradually converted to limonin under acidic conditions and the conversion is accelerated by the action of limonin D-ring lactone hydrolase, which has been isolated from citrus (9). The bitterness due to nomilin in juices most likely develops in a manner similar to that of limonin bitterness, but this has not yet been directly proven. However, the contribution of nomilin to juices is minor. It occurs mainly in grapefruit juices (4).

## Biosynthesis of Limonoids in Citrus

During the past several years, significant progress has been made in studies on the biosynthesis of citrus limonoids. Based on recent radioactive tracer work, the biosynthetic pathways of the major limonoids in common citrus are well established (Fig. 1).

**Preparations of Radioactive Substrates.** Young citrus seedlings are excellent tools for the preparation of 14-C nomilin from labeled acetate. When fed to stems of lemon seedlings, up to 5% of the labeled acetate was incorporated into nomilin (10). Incorporation of acetate was the best followed by mevalonate and farnesyl pyrophosphate. This high value of incorporation supports the fact that nomilin is one of the major compounds which accumulate in seedlings.

Labeled obacunone was prepared enzymatically from labeled nomilin with nomilin acetyl-lyase which was isolated from *Corynebacterium fascians* (11). Labeled obacunoate was prepared enzymatically from labeled obacunone with obacunone A-ring lactone hydrolase obtained from cell-free extracts of *C. fascians* (12). Citrus seedlings were not capable of accumulating deacetylnomilin or deacetylnomilinate. Therefore, labeled deacetylnomilin was synthesized from labeled acetate in young seedlings of calamondin (*Citrus reticulata* cv. *Austera* x *Fortunella* sp.). Labeled deacetylnomilin was chemically converted to labeled deacetylnomilinate (13). Labeled 6-keto-7β-nomilol was biosynthesized from labeled acetate in calamondin seedlings (14).

Biosynthesis of Major Limonoids. Radioactive tracer work showed that 14-C labeled acetate, mevalonate and farnesyl pyrophosphate are incorporated into nomilin in young lemon seedlings (10). Nomilin did not appear to be further metbolized in the seedlings. On the other hand, in older citrus, nomilin was further converted to obacunone, obacunoate and limonin (11, 15). The conversion of nomilin to obacunone appears to be catalyzed by the action of nomilin acetyl-lyase and the conversion of obacunone to obacunoate by obacunone A-ring lactone hydrolase. Both enzymes have been isolated from cell-free extracts of *C. fascians* (16), but they have not yet been isolated from citrus.

The major difference in the limonoid biosynthetic enzymes of young seedlings as compared with older trees is that nomilin acetyl-lyase activity is missing in young seedlings and, therefore, nomilin accumulates as the predominant limonoid (10). However, when labeled obacunone was fed to a young shoot of either young seedlings or older lemon trees, both tissues converted obacunone to obacunoate and to limonin (11). In young seedlings, although there is no detectable nomilin acetyl-lyase activity, the enzyme systems leading from obacunone to limonin are present just as in older citrus. Thus, nomilin acetyl-lyase appears to play a key role in the regulatory system which controls the biosynthesis and accumulation of limonoids in citrus.

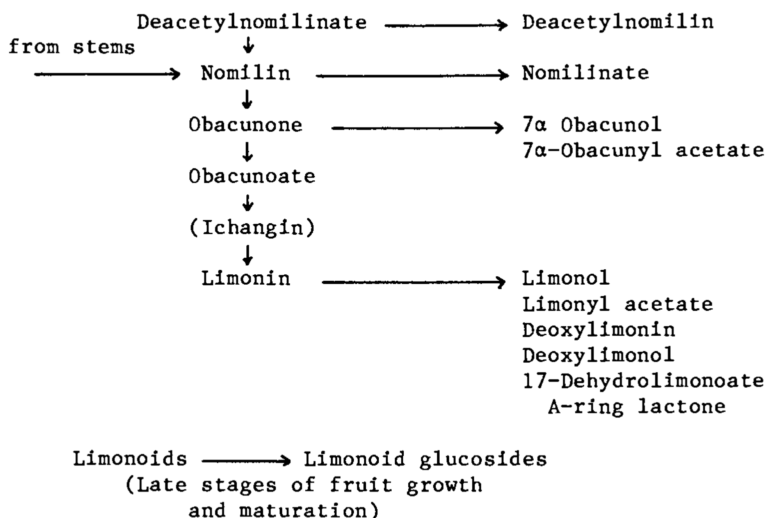


Figure 1. Biosynthetic pathways of limonoids in citrus.

Limonin appears to be the last major limonoid to be biosynthesized. There are several possible precursors present between obacunoate and limonin, including isoobacunoate and

ichangin, all of which have been isolated from citrus. Since labeled isoobacunoate was not converted to limonin in tissues of either seedlings or mature trees in several trials (Hasegawa, S., USDA, ARS, Pasadena, CA, unpublished data), it is most likely that ichangin is the direct precursor of limonin. We have not been able to biosynthesize labeled ichangin for use as a substrate.

Recently, deacetylnomilinate was found to be the initial precursor of all the limonoids known to be present in citrus (13). When 14-C labeled deacetylnomilinate was fed to the stem of a lemon seedling, it was converted mainly to nomilin. According to the previously proposed pathways (17), nomilin and obacunone were thought to be biosynthesized from deacetylnomilinate via nomilinate and/or deacetylnomilin. However, recent work has clearly demonstrated that deacetylnomilinate is the initial precursor of all the known citrus limonoids. This finding well established the biosynthetic pathways of the major limonoids in citrus.

Biosynthesis of Minor Citrus Limonoids The conversion of limonin to 17-dehydrolimonoate A-ring lactone (18) and to deoxylimonin (19) have been shown in citrus. Deoxylimonate is most likely formed from deoxylimonin as seen in bacteria. We recently observed the conversion of labeled nomilin to nomilinate in young lemon seedlings (15), showing that nomilinate is a metabolite of nomilin. Most likely, deacetylnomilin is biosynthesized from deacetylnomilinate (11). There is no evidence of the direct conversion of nomilin to deacetylnomilin in common citrus. The other minor limonoids such as deoxylimonol, limonol and limonyl acetate are considered to be metabolites of limonin. 7α-Obacunol and 7α-obacunyl acetate are most likely metabolites of obaccunone

Biosynthesis of Limonoids in *Citrus ichangensis*

*Citrus ichangensis* possesses an unusual distribution of limonoids (20). Unlike other species of citrus, fruit tissues and seeds of *C. ichangensis* contain very high concentrations of nonbitter ichangensin and very low concentrations of bitter limonin. It also contains relatively high concentration of nonbitter deacetylnomilin. Quantitative analysis showed that a ratio of ichangensin to limonin in the fruit tissue is approximately 50 to 1.

When labeled nomilin was fed to *C. ichangensis* fruit tissues, it was metabolized to deacetylnomilin and ichangensin (Hasegawa, S. et al., USDA, ARS, Pasadena, CA, unpublished data). Therefore, ichangensin is most likely biosynthesized from nomilin via deacetylnomin as shown in Fig 2. The conversion of nomilin to deacetylnomilin is catalyzed by nomilin acetyl esterase. The presence of this enzyme activity in *C. ichangensis* is unique to this species. In other citrus or microorganisms, nomilin is mainly converted to obacunone by nomilin acetyl-lyase (15). The formation of ichangensin is the predominant biosynthetic pathway of limonoids in *C. ichangensis*.

Biosynthesis of Calamin Group Limonoids in Calamondin

In addition to the above, there is another group of limonoids present in a citrus hybrid, calamondin (*Citrus reticulata* cv.

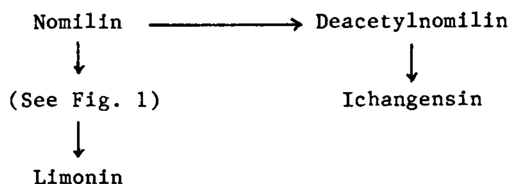


Figure 2. Biosynthetic pathways of limonoids in Citrus ichangensis

Austera x Fortunella sp.) and kumquat (Fortunella magarita). This group includes calamin, retrocalamin, cyclocalamin, isocyclocalamin, methyl isoobacunoate diosphenol, 6-keto-7β-nomilol, 6-keto-7β-deacetylnomilol (21-4). Based on radioactive tracer work, we have proposed the biosynthetic pathways of this group of limonoids as shown in Fig. 3.

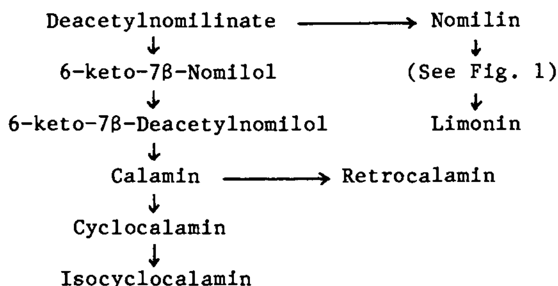


Figure 3. Possible biosynthetic pathways of limonoids in calamondin.

## Biosynthesis of Limonoid Glucosides

Limonoids are also present in citrus as glucoside derivatives. Recently, we reported the findidng of 17-O-β-D-glucopyranosides of the major limonoids such as limonin, nomilin, deacetylnomilin, obacunone, nomilinic acid, deacetylnomilinic acid, isoobacunoic acid, epiisoobacunoic acid, obacunoic acid and *trans*-obacunoic acid in grapefruit seeds (Hasegawa, S. et al., *Phytochemistry* in press). These glucosides are present in high concentrations relative to the aglycones. For example, commercial orange, grapefruit and lemon juices contain an average 320 ppm, 200 ppm and 90 ppm of total glucosides, respectively (25). They may be of significance in the area of taste of citrus products, limonoid debittering and human nutrition.

In further research on the limonoid glucosides, we found that they appear to be present only in fruit tissues and seeds. Radioactive tracer work showed that the fruit tissue itself is

capable of biosynthesizing the glucosides. They are biosynthesized during late stages of fruit growth and maturation in navel and Valencia oranges, and lemon (Fig. 1). In navel oranges, they appeared in the edible portion of fruit before they appeared in the peel, and their concentrations increased as maturity of the fruit progressed.

## Sites of Limonoid Biosynthesis in Citrus

Stems are the major site of nomilin biosynthesis from acetate in citrus (26). Analysis of the phloem, the cortex and the inner core regions of the stem showed that the phloem region is the site of nomilin biosynthesis from acetate (27). Root tissues also have this capacity. Leaves, fruits and seeds are either incapable of biosynthesizing limonoids from acetate or have a very low capacity. However, these tissues are capable of biosynthesizing limonoids from nomilin. Nomilin is translocating from the stem to other locations, where it is further biosynthesized to other limonoids (26).

## Biological Activity of Limonoids

Certain citrus limonoids induce glutathione S-transferase, a detoxifying enzyme, in the liver and small intestinal mucosa of mice, and inhibit a benzo(a)pyrene-induced neoplasia in the forestomch (28). This work is at a preliminary stage and further work is needed to fully determine the anti-cancer activity of limonoids, and, in particular, their glucosides. Since we have found a number of species of bacteria capable of hydrolyzing limonoid glucosides, these compounds may be hydrolyzed by the intestinal flora to liberate limonoid aglycones. Therefore, citrus fruit and juices, as a source of these natural products, may provide chemopreventative benefits against cancer. Citrus seeds are also an excellent source of these compounds.

Certain limonoids also possess an antifeedant activity against insect pests such as fall armyworm, cotton bollworm and spruce budworm (29, 30). Limonoids do not directly kill the pests, but gradually lower their growth rates.

## Preharvest Approaches to the Limonoid Bitterness Problem

Three possible preharvest approaches have been proposed and research on these subjects is underway at the Pasadena laboratory.

Inhibition of Limonoid Biosynthesis by Auxins. Auxins are potent inhibitors of nomilin biosynthesis in citrus seedlings (31). For instance, up to 91% inhibition was observed when 10 ppm of indoleacetic acid was fed to the stem of a lemeon seedling two days prior to and two days following feeding of 25 µCi of 14-C acetate (Table 1). Other auxins tested include 1-naphthaleneacetic acid (NAA), indolepropionic acid, indolebutyric acid, 3-indole acetonitrile, ethyl indole-3-acetate, 3-indoleacrylic acid, 3-(2-hydroxyethyl)indole, indole-2-carboxylic acid and 2,3,4-trichlorophenoxyacetic acid. They were all very effective.

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Radioactive tracer work also demonstrated that NAA inhibits the accumulation of limonoids in fruit tissues of lemon trees (32). When 3-cm-long stems with small lemon fruits attached were fed with 14-C labeled acetate after having already been treated with 20 ppm

Table 1. Inhibition of Nomilin Biosynthesis by Indoleacetic Acid in Young Lemon Seedlings

| IA (ppm) | Nomilin (cpm) | Inhibition (%) |
|---|---|---|
| 0 | 1,127.900 | 0 |
| 10 | 100,400 | 91 |
| 25 | 125,000 | 89 |
| 50 | 95,000 | 92 |

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of NAA for three days, there was an 80-100% inhibition of nomilin accumulation in the fruit when compared to the controls. These results show that auxins may have potential as bioregulators for reduction of bitter limonoids in commercially grown citrus fruits. Field experiments with NAA on orchard navel orange trees are underway.

Alteration of Biosynthetic Pathways of Limonoids. As pointed out previously, unlike other citrus, *Citrus ichangensis* accumulates nonbitter ichangensin in the fruit tissue as the predominant limonoid instead of bitter limonin (20). Ichangensin is biosynthesized from nomilin via deacetylnomilin, which is the major biosynthetic pathway of limonoids in this species. If the genes for the pathway from nomilin to ichangensin or even from nomilin to deacetynomilin (nomilin acetyl esterase) could be transferred by genetic engineering or breeding techniques to other citrus species which have the limonoid bitterness problem, nonbitter ichangensin or nonbitter deacetylnomilin might accumulate as the major limonoid in the fruit tissue rather than bitter limonin. This presents another approach to the limonoid bitterness problem.

Stimulation of Limonoid Glucoside Formation. Limonoids are converted to their glucoside derivatives during late stages of fruit growth and maturation. The conversion appears to be catalyzed by UDP-D-G glucosyltransferase. Since the glucosides appear to be practically nonbitter as compared to the intense bitterness of the parent limonoids, the formation of the glucosides represents a debittering pathway that can be explored in future research dealing with pathway regulation.

## Postharvest Approaches to the limonoid Bitterness Problem

Since this subject was reviewed in 1988 by Johnson and Chandler (33), Dekker (2), and Hasegawa (3), no significant progress has

been made in this field. Following is a brief description of the approaches.

Biological Removal Six species of bacteria, each capable of metabolizing limonoids, have been isolated from soil by enrichment using limonoids as carbon sources. They are *Arthrobacter globiformis* (34), *Pseudomonas* 321-18 (35), *Arthrobacter globiformis* II (36), Bacterium 342-152-1, *Corynebacterium fascians* (37) and *Acinetobacter* sp. (38). Based on the metabolites and enzymes produced by these species of bacteria, five metabolic pathways of limonoids have been established in bacteria (3)(Fig. 4).

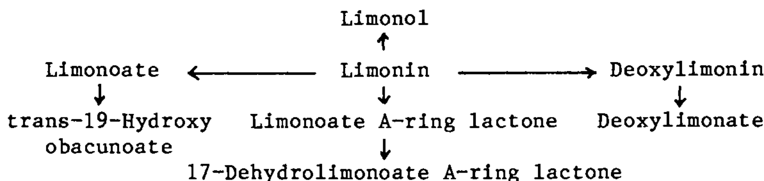



Figure 4. Metabolic pathways of limonin in bacteria.

In addition, nomilin was also metabolized via obacunone in immobilized cells of *C. fascians* (39). The 17-dehydrolimonoid pathway is th major metabolic pathway of limonoids in bacteria, and four limonoate dehydrogenases, which catalyze the conversion of limonoate A-ring lactone to 17-dehydrolimonoate A-ring lactone, have been isolated from different species of bacteria (34, 35, 37). Several other enzymes including limonin D-ring lactone hydrolase, nomilin acetyl-lyase, deoxylimonin hydrolase and obacunone A-ring lactone hydrolase have been isolated and characterized (16, 40, 41). Among limonoid-metabolizing enzymes, limonoate dehydrogenase isolated from *A. globiformis* was the first enzyme used to demonstrate the prevention of the limonin bitterness in navel orange juices (42).

A bioreactor, which uses immobilized bacterial cells, has been developed at the Pasadena laboratory for removal of bitter limonoids in citrus juices (3). When the juice was passed through a column packed with immobilized bacterial cells, limonin in the juice was converted to either 17-dehydrolimonoate A-ring lactone by *A. globiformis* or limonol by *A. globiformis* II and *C. fascians*. Nomilin in grapefruit juice was also metabolized similarly, except that it was also metabolized to obacunone in immobilized cells of *C. fascians*. Debittering of citrus juice by bacterial cells entrapped in a dialysis sac was also demonstrated by Vaks and Lifshitz (38).

The technical feasibility of an immobilized cell system was demonstrated on a laboratory scale for use in reducing limonoid bitterness of citrus juices. The treatment caused no significant adverse effect on the other juice constituents as judged by analysis of ascorbic acid, citric acid, malic acid, sucrose, glucose and fructone.

Physical Removal During the past several years, significant progress has been made in the development of selective adsorption processes for removal of two bitter principles, limonin and naringin, from citrus juices. Adsorbents such as cellulose esters (43), cross-linked polystyrenes (44) and β-cyclodextrins (45, 46) have been shown to reduce effectively the limonin bitterness in the juice.

Cellulose acetate jels and beads were used to effectively remove limonin below the bitterness threshold from citrus juies (43). The resins can be regenerated readily by washing with a small volume of warm water. The process has been used commercially in Australia in debittering bitter orange juices (47).

Polystyrene-divinylbenzene cross-linked copolymer adsorbent resins removed effectively both limonin and naringin from citrus juices (44). Over 85% of limonin was adsorbed from about 50 bed volume juices of grapefruit, navel orange, lemon and tangerine. Up to 70% of naringin was also removed from grapefruit juice.

β-Cyclodextrins form inclusion compounds with limonin and naringin. Reduction of limonin and naringin in citrus juices has been demonstrated using β-cylodextrin monomers (45) and polymers (46). A continuous flow process with polymers, for example, reduced both limonin and naringin contents an average of 50% or more in 16 bed volumes of clarified grapefruit juice (48). The column can be readily regenerated with 2% NaOH solution and the same column can be used many times without losing its effectiveness.

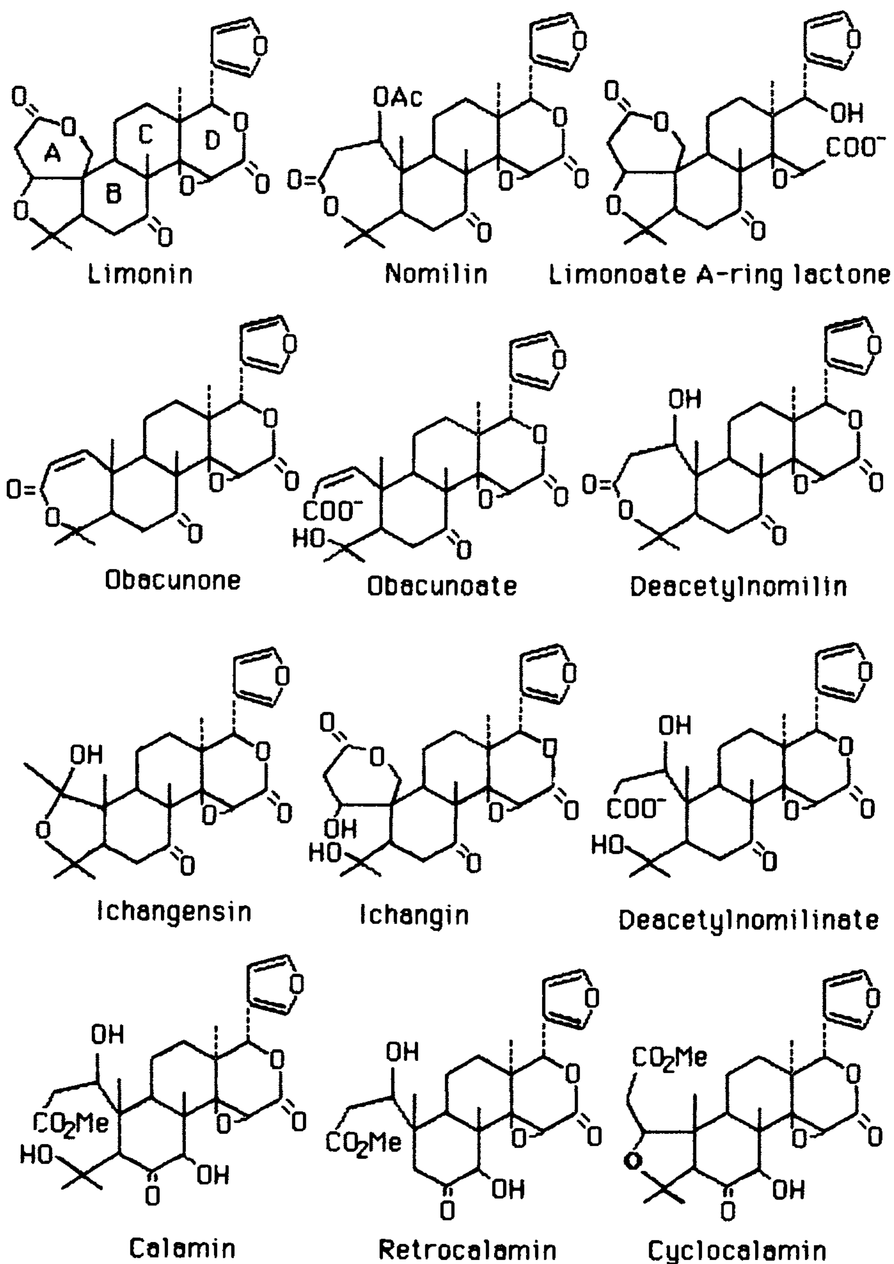
## General Remarks

Biochemical studies carried out at the Pasadena laboratory have shown how, where and when limonoids are biosynthesized and accumulate in citrus. The recent discovery of limonoid glucosides has cleared an unanswered question of how limonoid aglycones disappear at late stages of fruit growth and maturation.

Certain limonoids have been shown to possess antifeedant effects on some insects and anticarcinogenesis activity in mice. Although further research is needed, limonoids appear to have potential as insecticides or chemopreventative agents against cancer. Citrus fruit and seeds are an excellent source of these compounds.

At present there are no totally satisfactory methods of commercially removing limonoid bitterness. Several new approaches are being developed which may lead to a more complete solution to the problem. These include specific adsorbents of limonoids, a biological reactor that uses immobilized bacterial cells, and the preharvest approaches which are presented in the text.

To help visualize the compounds in this chapter, I have included two pages of structures (pp 93 and 94).

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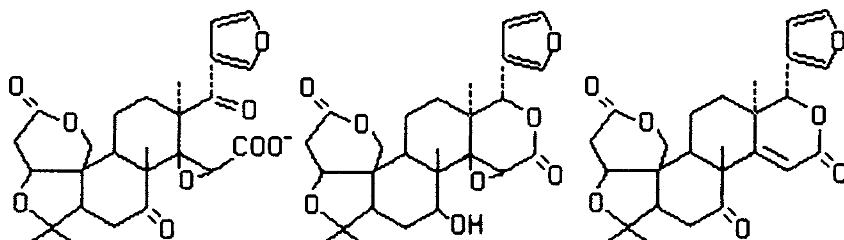
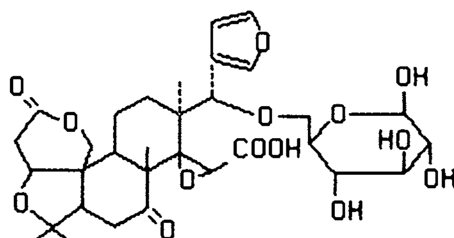
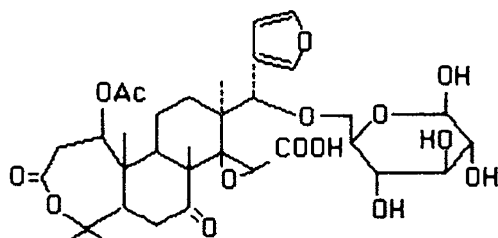



17-Dehydrolimonoate A-ring lactone    Limonol    Deoxylimonin




Limonin 17-O-β-D-glucopyranoside




Nomilin 17-O-β-D-glucopyranoside

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Chapter 9

# Applications of Chemical Kinetic Theory to the Rate of Thermal Softening of Vegetable Tissue

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The rate of thermal softening of vegetable tissue is consistent with two simultaneous pseudo first order kinetic mechanisms. From 80-97% of the firmness is lost by a rapid softening mechanism. The other 3-20% is lost by a distinctly different kinetic mechanism that occurs at 1/30th to 1/170th the rate of the first mechanism. It is well known that a low temperature blanch activates the pectin methylesterase system in vegetables and gives a firmer texture. Kinetic studies show that this increase is caused primarily by an increase in the amount of the slow softening substrate and not by changes in the apparent first order rate constants. We are using the two substrate, two kinetic mechanism theory to define the optimal thermal regime for producing the firmest texture in canned vegetables.

The edible tissue of fruits and vegetables is composed predominantly of parenchyma cells. Mature parenchyma cells are generally 50 to 500μ across, although, in some cases, they may be considerably larger (1, 2). The cell walls are comprised largely of pectin, hemicellulose, cellulose, and sometimes, lignin and protein (3). The individual cells are cemented together by an amorphous layer external to the primary cell wall, called the middle lamella, which consists primarily of the calcium salts of polymers of galacturonic acid that have been partially esterified with methyl alcohol, and is known as the pectic material (4).

The texture of fruit and vegetable tissue derives primarily from the cell wall and the middle lamella. Most of the volume of the interior of the cell is comprised of an aqueous solution of sugars and salts that does not impart structural strength to the tissue. The hydrostatic pressure generated by the physiological processes within the living cell (turgor pressure) imparts rigidity, turgidity and crispness to fresh produce. Processing techniques such as heating and freezing kill the cells with the result that turgor is absent in processed products. Therefore, in processed products, the chemical composition and amount of cell wall and middle lamella material controls the texture.

Changes in texture that occur during processing result from changes in the chemistry of this hydrophilic polymeric material that affect the physical properties (5).

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The chemistry of these changes is complex. For example, pectin can be depolymerized and deesterified by heat or enzymes; it can be crosslinked by means of divalent cation salt bridge formation between the free carboxylic acid groups on the polygalacturonic acid chains and cations (primarily $Ca^{++}$) naturally present in the tissue or added during processing. Depending on which of these reactions occur, vegetable tissue may become softer or firmer during processing.

Softening processes usually predominate during processing. Processed fruits and vegetables are softer, often very much softer than the raw material. Because of the consumer's desire for the firm, crisp and succulent textures of raw vegetables, there is great interest in developing new processing technology that will retain more of the textural character of the raw produce.

The primary textural property of fruits and vegetables is firmness (6). Three principles are used to measure firmness. 1) The puncture test measures the force required to push a probe into the product. 2) The extrusion test measures the force required to make the product flow through one or more slots or holes. 3) The deformation test measures the distance the product compresses under a small force. All three test principles are used on fresh produce, but only the first two (puncture and extrusion) are used on processed material (7).

## Kinetics

The study of the rates at which chemical reactions occur has been a powerful tool for understanding the nature of these reactions ever since the pioneering work of Wilhelmy in 1850. Most of this work has been performed in homogeneous systems with pure reactants. However, the chemical reactions that occur when vegetable tissue is heated are complex, and a number of different reactions can be occurring simultaneously. In addition, the cell wall and middle lamella are not homogeneous. Further, the linkage between these chemical reactions and firmness is not clear, and is probably non linear. Nevertheless, it has been found that applying the theories of chemical kinetics to the rate of thermal softening of vegetable tissue can provide useful insights into the softening mechanisms, and point the way to developing technologies that produce firmer textured processed products even though the progress of the reaction is measured by a physical test (firmness) instead of a chemical test. The physical test is usually either an extrusion test or a puncture test.

A first-order reaction is one in which the rate of the reaction at any time is directly proportional to the concentration of the reactant present at that time. It is described by the well known equation:

$$-dX/dt = K.X \quad (1)$$

where X is the concentration of the reactant, t is the time, and K is the rate constant. Integrating and transposing gives:

$$\ln X = \ln Xo - K.t \quad (2)$$

where Xo is the concentration of the reactant at zero time. A plot of the lnX versus time will be linear for a first order reaction. The slope of the line gives the rate constant K, and the intercept on the ordinate at $t_0$ gives ln Xo.

A number of processes that have been studied show a linear relationship between the log of the amount of substance present versus the time. Many of these processes do not arise from simple first-order chemical reactions, or the chemistry of the process may be unclear. Nevertheless, since they empirically fit the model for a first-order chemical reaction, they are commonly called pseudo first order processes. The slope of the log (concentration) versus time plot is called the apparent first order

rate constant. The modifying adjective "apparent" is used to signify that the process is not truly first order in the chemical sense, but that the experimental data give a good fit to the first order chemistry model.

The temperature dependence of a first order rate constant is given by the well-known Arrhenius equation:

$$\ln K = \ln A - Ea/RT \qquad (3)$$

where K is the rate constant, A is a constant, Ea is the activation energy, R is the gas constant and T is the absolute temperature. A plot of lnK versus 1/T is rectilinear. The value of Ea can be calculated since the slope of the line is Ea/R, and the value of R is known.

Many pseudo first order processes also follow the Arrhenius relationship. In these cases the apparent activation energy can be calculated because of the excellent fit of the data to the Arrhenius equation.

A number of researchers who studied the rate of thermal softening of vegetable tissue have found a good fit of their empirical data to the first order kinetic model because a plot of log firmness versus time is rectilinear. Probably the first of these were Nagel and Vaughn (8) who measured changes in firmness of cucumber pickles by means of a puncture test as they were pasteurized at 160F, 180F and 200F. Nicholas and Pflug (9) confirmed that pickles soften by a pseudo first order process.

Many other reports on heating-softening effects show pseudo first order kinetics when the data are plotted on semilog axes, although not many authors presented their results in this form at that time. Paulus and Saguy (10) found pseudo first order softening kinetics for carrots when cooked and they listed the apparent rate constants and activation energies for three carrot cultivars. Loh and Breene (11) also found the first order kinetic model was adequate to describe the changes in firmness of 11 kinds of fruits and vegetables. Other reports of apparent first order kinetics for thermal softening have been published for cowpeas (12), red kidney beans (13) and potatoes (14). Most of the above reports on softening studies used either short cooking times, or low temperatures which gave slow rates of softening. Some of these reports inferred that the softening rate seemed to deviate from the first order kinetic model with longer process times, but this aspect seems not to have been explored any further.

Large quantities of vegetables and fruits are preserved by canning. This technology requires enclosing the product in hermetically sealed containers and heating at a specified temperature for a specified time to destroy all microorganisms inside the container. Products with a pH above 4.5 require a substantial heating regime to obtain commercial sterility. For example, green beans packed in brine in 1 lb cans require 22 minutes at 240F or 13 min at 250F (15). This heavy heat treatment cannot be compromised because microorganisms of public health significance, such as *Clostridium botulinum*, require this degree of heat treatment to be destroyed. Unfortunately, this amount of heat causes great damage to the texture. Most canned vegetables have a softer than desirable texture.

## Long heating time kinetics

Huang and Bourne (16) studied the rate of softening of green beans, dry white beans, carrots and three varieties of beets for times up to 120 minutes using the extrusion principle to measure firmness and found a similar pattern in the softening curves for every product. Figure 1 plots log extrusion force versus process time for diced beets processed at 220F. The solid circles and solid line show the experimental data.

The softening curve is characterized by an initial rapid decrease in firmness that is almost linear. This is consistent with the findings of apparent first order

softening kinetics in earlier reports. But then rate of firmness loss decreases; the firmness curves off into a second straight line with a shallow slope at longer process times. Since a first order process is represented by a rectilinear plot on a semilogarithmic scale, it is quite clear that simple apparent first order kinetics cannot apply to lengthy process times. The general shape of this curve is typical for all vegetables that we have studied.

Fruits show thermal softening curves similar to those for vegetables but the initial rate of softening is more rapid than for vegetables. Nevertheless, we have been able to obtain thermal softening data for several apple cultivars by cooking at temperatures in the range of 80-99C (Lacroix and Bourne, submitted for publication).

The striking features of Figure 1 are the initial linear relationship with a steep slope and the later linear relationship with a shallow slope. Although inconsistent with a simple apparent first order process, this relationship is entirely consistent with two pseudo first order processes with different rate constants occurring simultaneously. One process is rapid and the other process is slow.

From analogy with kinetic theory for two apparent first order processes we can postulate that the firmness of vegetable tissue is composed of two substrates, "a" and "b" and that substrate "a" softens rapidly by mechanism 1 while substrate "b" softens slowly by a different mechanism (mechanism 2). When the linear portion of mechanism 2 is extrapolated back to zero process time (dotted line in Figure 1) and this extrapolated line is subtracted from the solid line above it, a second line is obtained as shown by the open circles and dashed line in Figure 1. The derived dashed line represents mechanism 1 and the slope is its apparent rate constant. The linear portion of the solid line represents mechanism 2 and the slope is its apparent rate constant.

The chemical kinetics theory for two simultaneous first order processes is as follows:

assume there are two substrates, "a" and "b"

$$-da/dt = K_a.a \quad \text{and} \quad -db/dt = K_b.b \tag{4}$$

where "a" is the firmness contributed by the first component and "b" the firmness contributed by the second component; t is the time, $K_a$ is the apparent rate constant for substrate "a" and $K_b$ is for substrate "b". $K_a > K_b$ since substrate "a" decays more rapidly than substrate "b" when the tissue is heated.

Integrating and transposing in the usual manner gives:

$$\ln a = \ln a_o - K_a.t \quad \text{and} \quad \ln b = \ln b_o - K_b.t \tag{5}$$

where $a_o$ and $b_o$ are the amount of substrate "a" and "b" respectively at zero process time. At any process time the total firmness = (a + b) and this value can be calculated when $a_o$, $K_a$, $b_o$ and $K_b$ are known. Since "a" has a high softening rate, its contribution to firmness becomes inconsequential after a long heating time.

Experimentally determined values for the apparent first order rate constants for vegetables processed at 230F (16) are given in Table I below. The values for $K_a$ are 28-fold to 170-fold greater than the values for $K_b$.

Both apparent rate constants, $K_a$ and $K_b$, give an excellent fit to the Arrhenius temperature relationship. Figure 2 plots the apparent rate constant $K_a$ for dry white beans versus 1/T and is typical of the data obtained with the other vegetables. Table II lists the apparent activation energies for the two substrates (16).

Table I. Apparent First Order Rate Constants for Vegetables Processed at 230F

| Vegetable | $K_a$ ($min^{-1}$) | $K_b$ ($min^{-1}$) |
|---|---|---|
| Green beans | 0.149 | 0.0016 |
| Dry white beans | 0.109 | 0.0039 |
| Green peas, size 3 | 0.211 | 0.0043 |
| Green peas, size 5 | 0.337 | 0.0041 |
| Beets, Detroit dark red | 0.171 | 0.0013 |
| Beets, yellow | 0.195 | 0.0013 |
| Beets, Winter keeper | 0.199 | 0.0013 |
| Carrots | 0.139 | 0.00082 |

Table II. Apparent Activation Energy for Thermal Softening ofVegetables

| Vegetable | Substrate "a" Kcal/mole | Substrate "b" Kcal/mole |
|---|---|---|
| Dry white beans | 24.9 | 12.9 |
| Green peas, size 3 | 27.1 | 24.4 |
| Green peas, size 5 | 35.0 | 22.1 |
| Beets, Detroit dark red | 22.6 | 12.9 |
| Beets, Winter keeper | 27.1 | 18.4 |
| Carrots | 15.2 | 5.1 |

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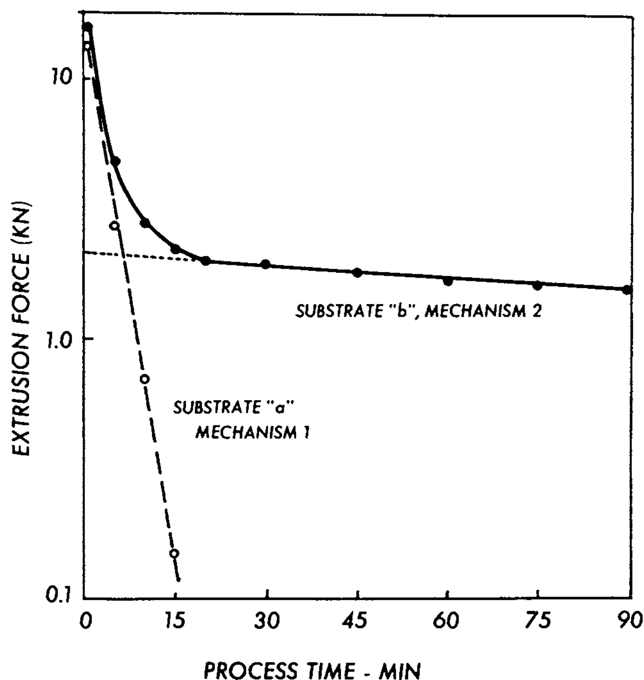


Figure 1. Softening of diced beets cooked at 220F. (Reproduced with permission from Ref. 16. Copyright 1983 Food and Nutrition Press.)

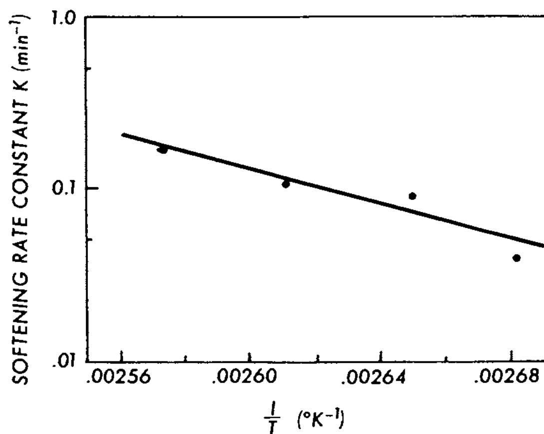


Figure 2. Arrhenius plot for apparent softening rate constant for dry white beans. (Reproduced with permission from Ref. 16. Copyright 1983 Food and Nutrition Press.)

One more valuable piece of information can be extracted from Figure 1. The intercept of the extrapolated line on the ordinate gives the amount of substrate "b" at zero time. The difference between this point and the total firmness gives the amount of substrate "a" at zero time. Table III lists the proportion of total firmness of the raw vegetable supplied by substrate "b" (16).

Table III. Contribution of Substrate "b" to Raw Firmness

| Vegetable | Total Firmness kN | Substrate "b" kN | Substrate "b" / Total Firmness |
|---|---|---|---|
| Green beans | 11.8 | 0.80 | 0.066 |
| Green peas, size 3 | 7.2 | 1.14 | 0.159 |
| Green peas, size 5 | 8.5 | 1.23 | 0.145 |
| Beets, Detroit dark red | 15.3 | 1.53 | 0.100 |
| Beets, yellow | 15.8 | 2.04 | 0.129 |
| Beets, Winter keeper | 17.1 | 1.60 | 0.094 |
| Carrots | 17.0 | 0.50 | 0.029 |

Note that substrate "b" contributes between 3% and 15% of the firmness of the raw vegetable. While this may appear to be a minor contribution to raw firmness, it is the major contributor to the firmness of the canned product. Figure 3 shows the softening curve for cut green beans processed at 115.6C (240F). It is a typical thermal softening curve (17). The National Food Processors Association recommended process for this product packed in #303 cans is 22 min at 115.6C. The vertical line in Figure 3 shows this time. It is evident that substrate "a" has practically disappeared by the time a commercial process has been completed.

Substrate "a" provides more than 90% of the firmness in the blanched bean before canning but less than 5% of the firmness after canning. Hence, the amount of substrate "a" has a minor effect in determining the firmness of the canned product. Since its rate constant is so high, even substantial changes in the apparent rate constant for substrate "a" would have a minor effect on firmness after canning. What really determines the firmness after canning is the amount of substrate "b" present. Therefore, we have named the intercept of the extrapolated line on the ordinate, (which measures the amount of substrate "b" at zero time) the "thermal firmness value" because this is the firmness component that can withstand a heavy heating regime with little degradation (17).

It is well known that a low temperature blanch at 170-175F before canning gives a firmer texture in vegetables than a conventional blanch at 200-212F (5). The chemistry underlying this process is clear: The low temperature blanch activates the enzyme pectinmethylesterase, which demethylates the esterified carboxylic acid groups on the polygalacturonic acid polymer and allows salt bridge formation with divalent cations such as $Ca^{++}$ that are naturally present in the tissue (18).

The two substrate theory of thermal softening provides a kinetic explanation of this phenomenon (17). Figure 4 shows the softening curves for diced carrots blanched at 74C and 100C and then cooked at 100C. Both curves show the typical rapid initial softening followed by a much slower rate of softening. The slopes for substrate "a" derived by subtraction of the extrapolated substrate "b" line from the experimental curve and the intercepts on the ordinate are shown in the figure. The apparent rate constants and thermal firmness values are given in Table IV below.

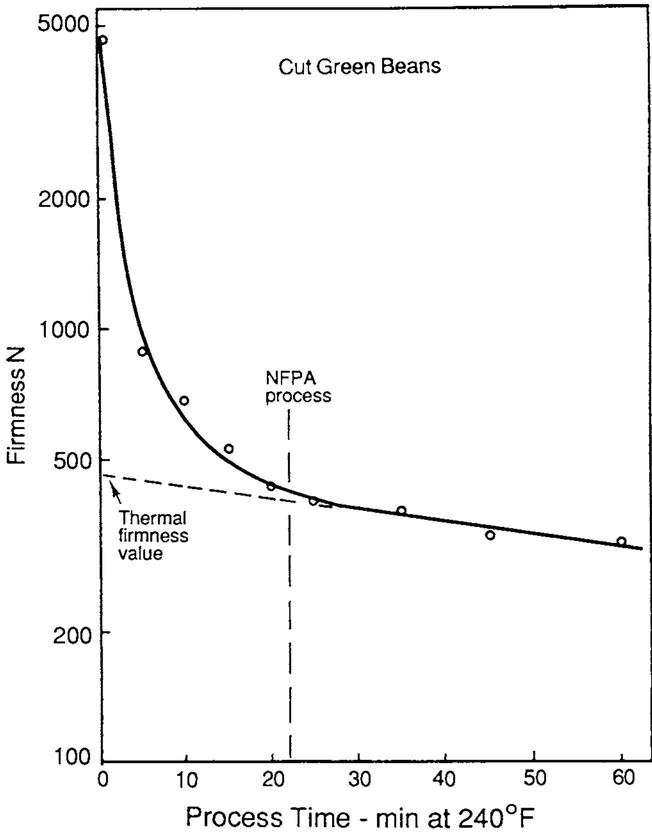


Figure 3. Softening curve for cut green beans cooked at 240F. (Reproduced with permission from Ref. 17. Copyright 1987 Institute of Food Technologists.)

Table IV. Kinetic Parameters for Thermal Softening of Carrot

| Blanch Temp °C | $K_a$ ($min^{-1}$) | $K_b$ ($min^{-1}$) | Thermal Firmness (N) |
|---|---|---|---|
| 100 | 0.052 | 0.00051 | 201 |
| 74 | 0.030 | 0.00096 | 505 |

A comparison of Table IV and Figure 4 shows that the low temperature blanch reduces $K_a$ by 57%. However, since substrate "a" has practically all disappeared by the time a commercial process has been completed, the change in $K_a$ is of little consequence. $K_b$ for the 74C blanch is 88% higher than for the 100C blanch. Since both these rates are very slow, they too have little effect on the firmness after canning. The dominant factor is the change in thermal firmness, the amount of substrate "b". It is 201 for the 100C blanch and 505 for the 74C blanch—an increase of over 150%. The kinetic approach clearly shows why the low temperature blanch gives a firmer-textured canned product.

Future directions

The two substrate, two pseudo first order softening mechanisms described above point the direction our research should be taking if we are to produce canned vegetables with significantly firmer texture.

Shortening the process time by increasing the rate of heat transfer can only promise a moderate increase in firmness because reducing the process time by a few minutes is not sufficient to retain any appreciable amount of substrate "a" (see Figure 3).

Searching for a way to slow the apparent rate constant $K_a$ can also provide only small gains because $K_a$ is so fast that even reducing it by 50% will have little net effect on firmness after a commercial process.

The real gains in firmness in commercially canned vegetables will be found by studying the factors that affect the thermal firmness value (see Figure 4). Future research should concentrate on studying genetic factors and processing variables that increase the thermal firmness value because these increases can withstand the heat treatment of a commercial process and deliver firmer textured vegetables to the consumer.

An example of this approach is shown with carrots (19). Seventeen cultivars of carrots were sliced transversely and blanched 4 min at 212F or 165F, then canned and processed 23, 30, 45, 60, 75 min at 250F. The firmness of the phloem tissue and xylem tissue (core) were separately measured by a puncture test. Figure 5 shows log firmness versus process time for the cultivar Dominator Sunseed together with the equations for the lines of best fit and the correlation coefficients R. Similar plots were obtained for the other sixteen cultivars. Sufficient data was obtained to measure substrate "b" and the thermal firmness value. No data were collected to measure substrate "a" because it is not needed. However, the firmness at zero process time was measured and these points are plotted on the ordinate. The correlation coefficients range from $r = 0.98$ to $r = 1.00$ which shows the excellent fit of the empirical data to the kinetic model.

The thermal firmness values for the two tissues and two blanch temperatures for the seventeen cultivars are shown in Table V. The data for the phloem and xylem tissues for every cultivar and both blanch temperatures are consistent with the two

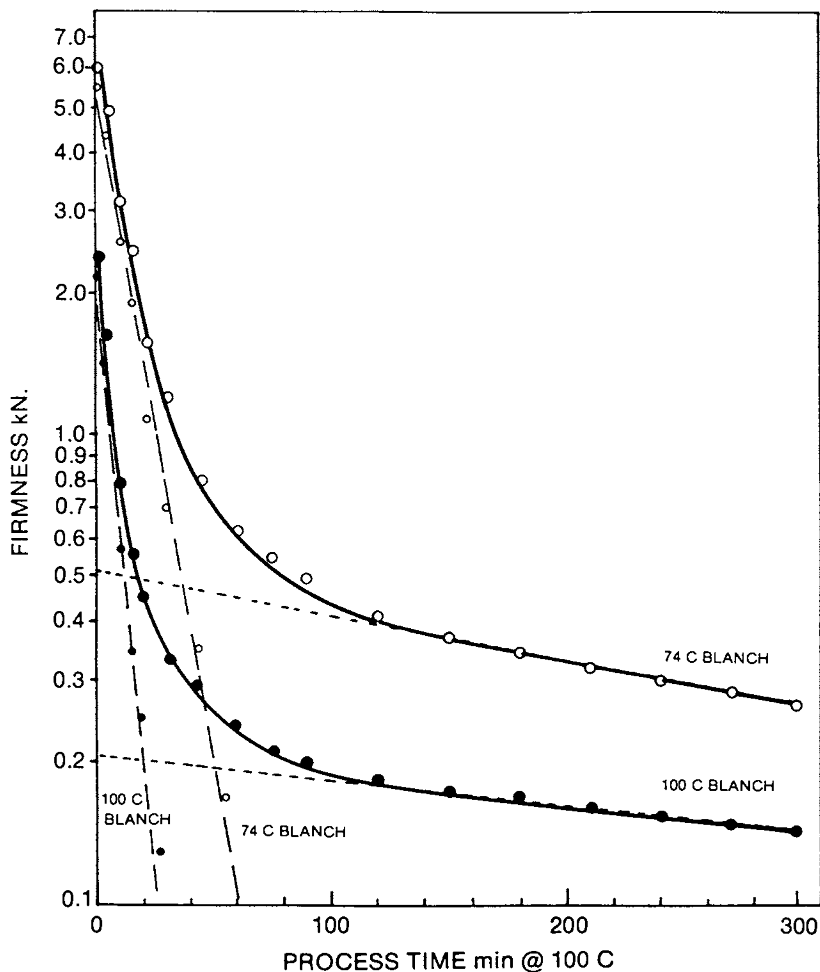


Figure 4. Softening curve for diced carrot blanched at 74C and 100C before cooking at 100C. (Reproduced with permission from Ref. 17. Copyright 1987 Institute of Food Technologists.)

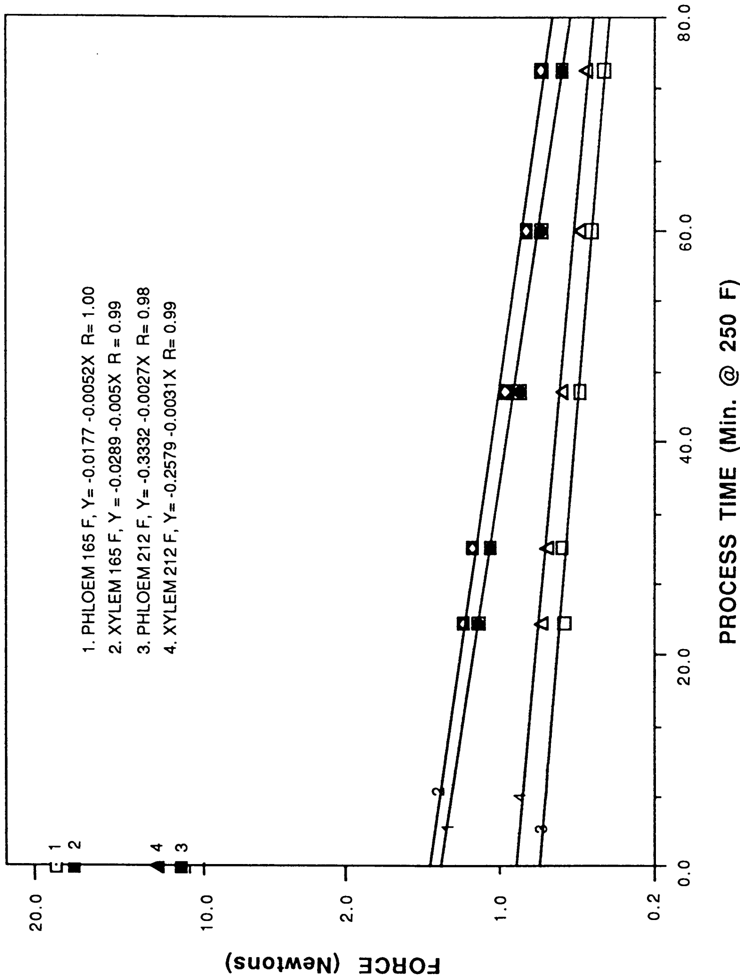


Figure 5. Substrate "b" softening for sliced carrots, cultivar Dominator Sunseeds.

substrate theory of thermal softening. For every cultivar, the thermal firmness of both phloem and xylem tissue is higher for the 165F blanch than for the 212F blanch. The mean increase in thermal firmness is 85% for phloem tissue and 149% for xylem tissue. This example shows how the kinetic approach can identify processes that provide a firmer textured product.

Table V. Thermal Firmness of Canned Carrot Slices (newtons force)

| Cultivar | 165F Blanch phloem | 165F Blanch xylem | 212F Blanch phloem | 212F Blanch xylem |
|---|---|---|---|---|
| Goldwine Sunseeds | 0.703 | 0.864 | 0.442 | 0.529 |
| Cellobunch, Asgrow | 0.381 | 0.736 | 0.365 | 0.423 |
| Dominator Sunseeds | 0.960 | 0.936 | 0.464 | 0.552 |
| PY60 Peter Edwards | 0.778 | 0.914 | 0.403 | 0.363 |
| XPH 5120 Asgrow | 0.891 | 1.105 | 0.411 | 0.536 |
| XPH 985 Asgrow | 0.658 | 0.913 | 0.435 | 0.446 |
| 3011 Sunseeds | 0.682 | 1.331 | 0.350 | 0.421 |
| Oranza Bejo | 0.830 | 0.995 | 0.353 | 0.300 |
| XPH 875 Asgrow | 0.735 | 0.992 | 0.433 | 0.504 |
| Norman Bejo | 0.528 | 0.645 | 0.305 | 0.219 |
| Desdan Arco | 0.851 | 1.133 | 0.416 | 0.463 |
| Larando Bejo | 0.852 | 1.255 | 0.482 | 0.473 |
| W243 Crookham | 0.656 | 0.946 | 0.357 | 0.401 |
| Aux 8160 Sunseeds | 0.526 | 0.823 | 0.291 | 0.384 |
| XPH 5119 Asgrow | 0.749 | 1.116 | 0.357 | 0.436 |
| Arco 154 | 0.849 | 1.529 | 0.423 | 0.444 |
| Barnum Bejo | 0.844 | 1.934 | 0.457 | 0.405 |
| mean value | 0.734 | 1.069 | 0.397 | 0.429 |

There is considerable variation in thermal firmness among cultivars. For xylem tissue blanched at 165F it ranges from a low of 0.381 for the Asgrow Cellobunch cultivar to 0.960 for Dominator Sunseeds cultivar, and for the phloem tissue blanched at 165F it ranges from 0.645 for Norman Bejo cultivar to 1.934 for the Barnum Bejo cultivar. It is likely that a large part of the variation is genetically controlled. If so, the kinetic analysis will provide plant breeders with a test procedure that will enable them to select breeding lines that will give a firmer textured product after canning.

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# Chapter 10

# Structural and Compositional Changes During Processing of Dry Beans (*Phaseolus vulgaris*)

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Improved utilization of dry beans can be maximized through an understanding of how physical and chemical components function and react under given process conditions. Variability in the physico-chemical composition of dry beans occurs among cultivars, geographic locations, crop seasons, and growing & harvesting conditions (1 and 2). Undesirable chemical changes also occur during adverse storage condition resulting in poor quality such as bin burn, hard-shell and hard-to-cook defects. In general, dry beans are cooked, fried, or baked to be used in soups, eaten as a vegetable, or combined with other protein foods to make a main dish. Commercially, they are typically processed in cans to yield a number of bean-based foods. Research and quality control programs are directed to provide a consistent product possessing characteristics of good flavor, bright color, attractive appearance, firm texture and high nutritional quality.

The physical and chemical properties of dry beans are primary factors in determining subsequent final product quality. Dry bean seed structure is comprised of a seed coat and an embryonic cotyledon. Structurally, seed coat, cell walls, middle lamella and other cellular membranes greatly influence performance. Further, chemical components (carbohydrates, proteins, phytate, polyphenols and lignin) directly influence quality.

The seed coat is the outermost tissue layer which protects the embryonic structure and consists of approximately 7-8 % of the total dry weight in the mature bean with a protein content of 5% (db) (3 and 4). Two external anatomical features include the hilum and micropyle which each have a role in water absorption. The major components in the seed coat structure include a waxy cuticle layer, palisade cell layer, hourglass cells and thick cell-walled parenchyma cells. The cuticle is the outermost portion of the seed coat and its

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hydrophobic property retards water penetration though does allow permeation of some polar and non-polar compounds. Sefa-Dedeh and Stanley (5), demonstrated seed coat thickness, seed volume, and hilum size along with protein content were all factors in regulating water uptake.

The cotyledon (approx. 92 % w/w) contributes a valuable component to the functional (appearance, texture, flavor, etc.) and nutritive value of the bean. Parenchyma cells make up the major portion of the cotyledon which are bound by a distinct cell wall and middle lamella with few vascular bundles (Figure 1 a). The cell walls are comprised of an organized phase of cellulose microfibrils surrounded by a continuous matrix of hemicellulose, pectic polysaccharides, hydroxyproline rich glycoprotein, and lignin. These cell walls function to give rigidity to the cotyledon tissue. The middle lamella is composed primarily of pectic substances which provide adhesion to adjacent cells resulting in the integrity of total tissue. In addition, pectic substances also allow divalent cation cross-linking and thus, forming intercellular polyelectrolyte gels which significantly contribute to the textural quality (6,7 and 8). The cell wall polysaccharides contribute an important source of crude fiber (3.4-7.2%); however, the significant proportion of the crude fiber (80-93%) is localized in the seed coat (9 and 10).

***Bean Preparation and Process Conditions***

Preparation of dry beans involves preliminary hydration followed by various heat treatments to obtain a tender, palatable product. Water and heat play an important role in chemical reactions, heat transfer and chemical transformations, such as protein denaturation and starch gelatinization. Inadequate water uptake may result in insufficient heat transfer to inactivate antinutritional factors and result in reduced cookability. In general, beans with an initial moisture content between 12 and 18%, are soaked to hydrate the seed to a moisture content of 53 to 57% and subsequently blanched, cooked or canned. This cooking step, if done for an optimal time, renders the seed nontoxic, improves digestibility, develops acceptable flavor and softens the seed coat and cotyledon.

Processing environmental factors, such as temperature, pH, ionic strength, and the presence of selected food constituents (product sauce formulations) influence the predominate reactions which affect bean quality and performance. Process media pH plays an important role in determining ionic charge on major constituents, particularly starch and protein. Changing pH will result in changing ionic properties of these constituents and ultimately influence their functionalities. Starch granule hydration and gelation

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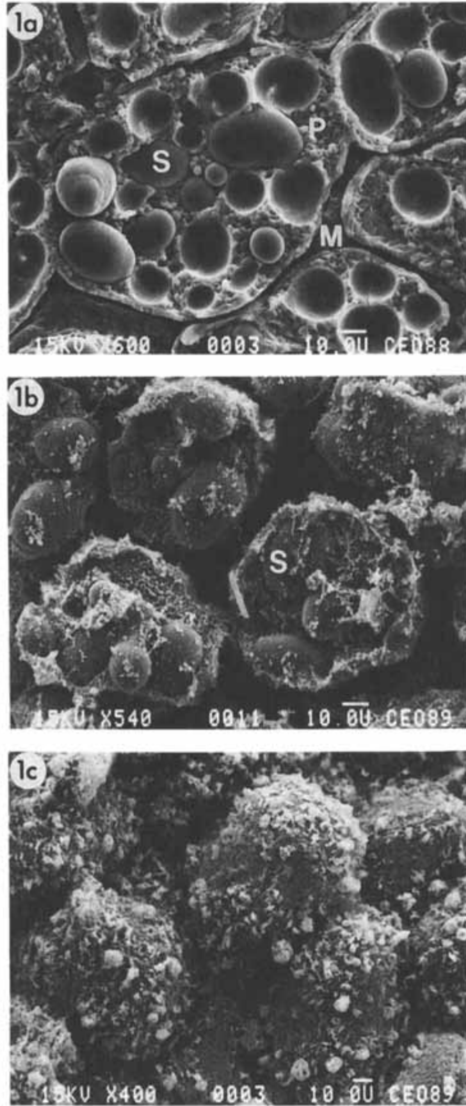


Figure 1. Structural changes of navy beans at various stages of processing: 1a, dry bean; 1b, soaked/blanched bean; 1c, canned bean (scanning electron photographs: S = starch granule; P = protein bodies; M = middle lamella)

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properties are pH dependent due to changes in intragranular bonding forces and the variable degree of hydrolysis of the native starch molecule. Similarly, functional properties of proteins vary with changes in pH due to the differential ionization. Thus, the water absorption and the water holding properties of cooked beans are very much influenced by pH of the soaking and final canning or cooking media.

Selected results of an experiment using citrate buffer as a soak and process media ranging from pH 3 to 7 are presented in Figure 2. The final equilibrated pH of the canned beans demonstrate a native buffering capacity effect of the bean. Soaking and blanching weights increased with increase pH; however, the final drained weight decreased with increased pH. These results were attributed to increased bean clumping and splitting resulting a greater solid loss to the canning brine with increased pH. Within this range of the pH, bean texture (compression and shear) was softest at pH range 4-5. This curvilinear textural relationship is complex due to competitive interactions and ionization states of bean macromolecular constituents (starch, protein and structural polysaccharides) under the tested conditions. Further, increased pH produced a darker color product (decreased Hunter L, $a_L$ and $b_L$ values) and though untested, these data are generally consistent with pH influence attributed to Maillard Browning reaction products (Uebersax, M.A. and Ruengsakulrach, S., Michigan State University, unpublished data).

***Structural Changes***

Thermal processing induces the largest alteration in structure and the initiation of diverse chemical reactions among bean constituents. The scanning electron microscope (SEM) photograph of soaked/blanched beans (Figure 1b) illustrates the increase in solubility of protein (loss of indigenous spherical structure) and the relatively unchanged starch granules. During the soak/blanch treatment, native protopectin may also form pectin which will rapidly polymerize. Soluble protein and pectin may leach causing increased viscosity of the cooking media. It has been proposed that the differences in pectin composition could be a major factor determining cookability of dry beans (11). Soaked/blanched beans are subjected to further heating under pressure during retort processing (Figure 1 c). The absorbed water and heating initiate thermal degradation or inter/intra-cellular and cohesive materials (middle lamella) and thus allows cells to separate and soften (12 and 13). Results demonstrated that in dry and soaked/blanched (30 minutes at 21°C + 30 minutes at 88°C) beans, fracture occurs across the cell wall; however, in the canned bean fracture occurs in the middle lamella, leaving the cell intact (Figure 1 a, b

and c). This cell separation may account for the notable texture differences exhibited. Various significant chemical changes have been induced within the cell inclusions during heating. Protein bodies lose their normal spherical structure due to swelling and denaturation (14). Starch granules demonstrate the deformation, expansion and loss of birefringence associated with gelatinization, although the presence of intact cell walls impede conformational changes (15). Hahn et al. (15) reported that the range of intracellular starch gelatinization in soaked beans to be from 76°C to over 95°C. Intracellular starch gelatinization and protein denaturation occurs during moist heating which develops a uniform smooth texture. Characteristic cooked bean flavor develops through the degradation or interaction of native tissue constituents mediated by Maillard reaction.

***Compositional Changes***

**Carbohydrates** Starch functional properties influencing process yield and product texture include swelling, solubility, gelatinization temperature and pasting characteristics. In-vitro, several changes occur upon heating a starch-water system, including extensive swelling, increase in viscosity, translucency, solubility, and loss of birefringence. Reddy et al.(16) suggested that the swelling ability and solubility depend on starch source, temperature and pH. Most legume starches exhibit a restricted swelling and bean starches have a high initial gelatinization temperature. Brabender viscosity patterns show restricted swelling characteristics similar to those shown by chemically cross-linked starches (17 and 18). According to Schoch and Maywald (19), these restricted swelling pastes are classified as Type C starches which show no pasting peak, with high viscosity which remains constant or increases during cooking. Elbert and Witt (20) studied the course of starch gelatinization from *Phaseolus vulgaris* before and after cooking in water. They found that an initial lower moisture content was found to be associated with a lower tendency for the starch to gelatinize in situ. Factors which influence this property may include the size and shape of the starch granules, the ionic charge on the starch, the kind and degree of crystallinity within the granules, the presence or absence of fat and protein, and perhaps, the molecular size and degree of branching of the starch fractions (19). Varriano-Marston and de Omana (21) observed that many granules in black bean starch exhibit some birefringence, even after prolonged cooking, indicating incomplete gelatinization. Such incomplete gelatinization of starch granules in seed legumes may be due to the barrier imposed by cellular structures such as cell walls and protein and/or the

inherent structural characteristics of the starch granules. Most bean starches show some tendency to retrograde during cooling and have relatively constant cold-paste viscosity during a holding period at 50°C. Retrograded starch present in final canned bean products may contribute to their relatively low digestibility.

Although, total sugars (monosaccharides and oligosaccharides) represent only a small percentage of the total carbohydrate, these reducing sugars can participate in non-enzymatic browning reactions and contribute to flavor formation (22). Among the sugars, oligosaccharides of the raffinose family (raffinose, stachyose, verbascose, and ajugose) predominate in most legumes and account for 31,1% to 76.0% of the total sugars (23-29). The sugar content of soaked beans is a function of soaking time (30), but not the bean-to-water ratio. The sucrose, raffinose, and stachyose content of dry beans decreased approximately 20%, 35%, and 45%, respectively after soaking. Sugar losses during soaking are not proportional to the solubility of the respective sugars, however, heat treatments increase sugar solubility and enhances leaching from the tissue. The physical and chemical changes occurring during soaking and blanching treatments were studied by Tittiranonda (31). Two soaking methods (1:4, bean: water ratio) included room temperature soaking (21°C) and hot soaking (88°C, 30 mins). After each soaking treatment, soaked beans were cooked (88°C) for 30, 60 and 90 minutes. The content of available carbohydrates, total soluble sugars, reducing sugars, and non-reducing sugars in legumes decreases during soaking and cooking are presented in Table 1.

Substantial amounts of flatus-producing components in beans can be reduced by various common processes (soaking, cooking and discarding the cook water, germination, or fermentation). Since the sugars of raffinose family are water-soluble, discarding the soak and cook waters will remove most of these sugars; however, substantial losses in total solids, vitamins and minerals are also sustained.

**Proteins** Generally, proteins react non-covalently with substances in their environment primarily through hydrophobic forces and ionic bonding. The macromolecular structure of proteins and the large differences in the intrinsic reactivities of their side chains dramatically influence water interaction and functional properties. Elias et al. (32) have studied the effects of processing on the protein content of five cultivars of dry beans. On a dry weight basis, the cooked beans had a protein content which was 70 to 86% of the raw beans. Four to ten percent of the protein of the raw beans was leached into the cooking brine. These results are in good agreement with those of Koehler and Burke (33) and

Table 1. Changes of Sugar Content During Preparation of Navy Beans[a]

| Soaking and Cooking Condition | Glucose | Sucrose | Raffinose | Stachyose |
|---|---|---|---|---|
| Raw beans | 0.12 a | 2.50 c | 0.27 a | 2.32 b |
| **COLD SOAKING (21°C)** | | | | |
| **Soak time** | | | | |
| 8 hours | 0.10 a | 2.05 b | 0.44 a | 1.35 a |
| 16 hours | ND | 1.44 a | 0.42 a | 1.15 a |
| **Cook time** (88°C) | | | | |
| 30 minutes | ND | 1.03 b | 0.27 a | 0.88 b |
| 60 minutes | ND | 0.76 a | 0.23 a | 0.87 b |
| 90 minutes | ND | 0.62 a | ND | 0.73 a |
| **HOT SOAKING (88°C/30 min)** | | | | |
| **Hot-soaked beans** | ND | 1.42 a | 0.22 a | 1.38 a |
| **Cook time** (88°C) | | | | |
| 30 minutes | ND | 0.63 c | ND | 0.76 b |
| 60 minutes | ND | 0.52 b | ND | 0.60 a |
| 90 minutes | ND | 0.40 a | ND | 0.53 a |

[a] Means within each column and treatment followed by like letters are not significantly different (p,0.05). All values are expressed in percent dry basis.

Haytowitz and Matthews (34) who reported 93% to 100% retention of protein after cooking. However, they did not agree with those of Meiners et al. (35) who reported 50% to 60% loss of protein after cooking. The loss in protein is attributed to the extraction of soluble proteins, hydrolysis of protein to free amino acids, and non-enzymatic browning reactions. Phytohaemagglutenin (PHA) is a tetrameric heat labile glycoprotein (subunit, ca. MW 30,000) exhibiting antinutritional responses due to disruption of intestinal microvilli. Proper and thorough heating is essential for inactivation of this factor (36). Almas and Bender (37) attributed the reduction in the available lysine content and protein quality of legumes during heating to non-enzymatic browning reactions. The observed losses were related to the length and severity of the heat treatment and losses in the reducing sugar content.

**Minor Constituents** Losses of total bean solids, N compounds, total sugars, oligosaccharides, Ca, Mg, and three water-soluble vitamins (thiamin, riboflavin and niacin) were measured and found to be very small at soaking temperatures up to 50°C; however, a three to four fold increase was found when the soaking temperature was raised above 60°C (38). There have been few published reports on the effects of processing on the lipid composition of legumes. Although lipids constitute only a small percentage of the dry bean (2-3%), the fatty acids are highly unsaturated (39). Lipid oxidation, catalyzed by heat, enzymes, light or metals, leads to the formation of hydroperoxides which further decompose to produce off-flavors (40). These decomposition products (carbonyl compounds) can chemically interact with peptides to yield crosslinked end products. Thus, the storage of legumes can result in a loss of quality (off flavors and odors), nutritional value and functionality. Dry beans contain 3.9% to 4.8% total ash (33,35,41) and considerable losses of these mineral constituents leach during both soaking and cooking procedures. Greatest losses occur as a result of increased solubility and tissue breakdown as preparation temperature increases.

Phenolic acids have increasingly been recognized to influence quality of dry bean during storage and subsequent thermal processing due particularly to reaction and cross linking with proteins. The predominant phenolic acids found in dry navy bean are p-coumaric, ferulic and sinapic acids (Figure 3). Storage of dry beans at high temperature and humidity conditions will result in "hard-to-cook" (HTC) phenomenon. This defect, characterized by extended cooking time required for adequate cotyledon softening, is distinguished from a defect termed "Hard Shell". HTC beans present: 1) an energy problem during preparation, 2) inferior nutritional qualities, and 3) poor acceptance by

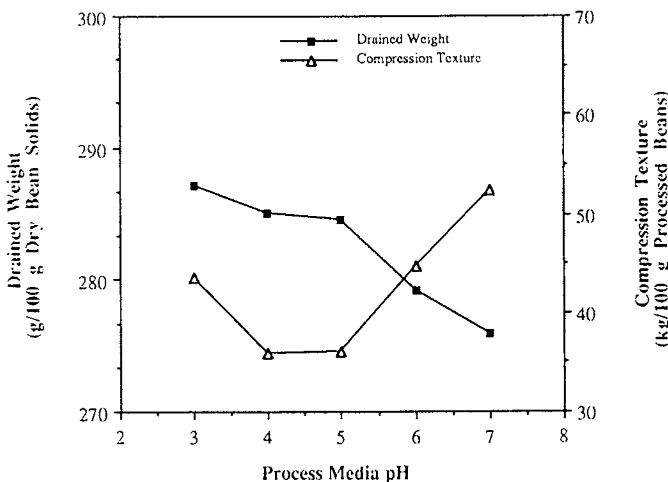


Figure 2. Quality characteristics of navy beans (var. Seafarer) canned in different pH process media

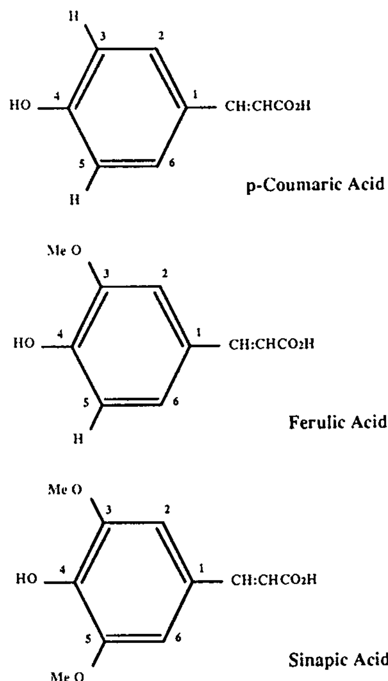


Figure 3. Structures of predominant phenolic acids in dry navy beans

consumers (42). HTC contributes to lower nutritive value through longer cooking times resulting in the loss of labile amino acids and impaired protein digestibility (43). The extent of the nutritional damage produced is determined by the time, temperature and relative humidity of storage. Several hypotheses have been proposed to explain HTC including: 1) lipid oxidation and/or polymerization (39,44 and 45); 2) phytin catabolism, and pectin demethylation with subsequent formation of insoluble pectate (46-48); 3) autolysis of cytoplasmic organelles, weakening plasmalemma integrity and lignification of middle lamella (14); and 4) interactions of proteins and polyphenols and polymerization of polyphenolic compounds (49). Exposure to high temperature and humidity storage may potentiate phytase which hydrolyzes phytate, thus reducing chelation of $Ca^{++}$ and $Mg^{++}$ ions within the middle lamella. Recently, Hincks and Stanley (50) proposed a multiple mechanism of bean hardening which included phytate loss as a minor contributor during initial storage and phenol metabolism as a major contributor during extended storage. Phenolic acids and their derivatives are widely distributed in plants (51) and can be present in either free and/or bound forms. Analysis of changes in total phenolic content of plant tissues must involve determining the content of all phenolic forms.

Changes in phenolic acid content related to HTC defect were studied by Srisuma et al.(52). Navy beans were stored for 9 months under three conditions (5$^{o}$C/40% RH, 20$^{o}$C/37% RH, and 35$^{o}$C/80% RH) to produce different degrees of HTC. Canned product quality characteristics demonstrate undesirable darker color (decreased Hunter L, a and b values) and increased firmness with adverse storage (Table 2). Several researchers attributed this darker color to polymerization of phenolic compounds (53-54). Changes in free hydroxycinnamic acids, hexane soluble and methanol soluble hydroxycinnamic acids, cell wall bound hydroxycinnamic acids, and lignin content were determined. Storage induced HTC beans contained higher levels of hydroxycinnamic acids (especially ferulic acid) than the control beans in all fractions prepared from seed coats and cotyledons except for the methanol soluble and cell wall bound phenolic acid fractions from cotyledons. No significant changes in lignin content were detected among the treatments. Large increases in free hydroxycinnamic acid content were associated with the degree of hardening (e.g. ferulic acid levels, mg/g in seed coat: control, not detectable; moderately hard, 23.1; hard, 86.3; and in cotyledon: control, 8.4; moderately hard, 46.6; hard, 64.8) , thus suggesting a relationship between the two phenomena.

Table 2. Canned Bean Quality Attributes of Navy Beans Stored Under Selected Conditions for 9 Months[a]

| Storage Condition (°C/% RH) | Subjective Classification | Hunter Color | | | Drained Weight (g)[b] | Shear Force (kg/100g) |
|---|---|---|---|---|---|---|
| | | L | aL | bL | | |
| 5/40 | Normal | 52.4c | -4.9a | 15.2b | 301.0c | 51.0a |
| 20/73 | Partially Hard | 51.8b | -4.8a | 15.1b | 285.4b | 58.5b |
| 35/80 | Hard | 34.0a | 0.1b | 13.4a | 234.2a | 237.9c |

[a] Means within each column followed by like letters are not significantly different (p,0.05).
[b] expressed as g/100 g of initial dry bean solids

**Summary**

Numerous chemical and physical changes occur during production, handling and processing of dry edible beans. Many of the chemical changes are associated with the macro-constituents of the seed. Interaction of water, heat and pH directly influence the functional properties of starch and proteins and significantly alter the cellular structure and its disruption. In order to adequately control or modify processed bean quality, a greater understanding of the chemical interaction occurring during processing must be achieved.

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## Chapter 11

# Function of Metal Cations in Regulating the Texture of Acidified Vegetables

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The use of metal ions to modify the texture of fruit and vegetables has been investigated and used for nearly 50 years. In general, monovalent ions have been found to cause softening while divalent ions, usually calcium, either inhibit softening or in some cases actually increase firmness. However, there have been few instances in which the effect of metal ions on tissue softening rates during processing or storage have been measured. The development of a rapid procedure to measure softening rates in cucumber tissue has made it possible to investigate quantitative effects of metal ions on texture. A salt softening effect in blanched, acidified cucumber tissue was observed when NaCl or other alkali metal chlorides were added. Low concentrations of calcium, strontium, barium, and lanthanide ions inhibited the rate of tissue softening in the presence of a high concentration of NaCl. The effects of multivalent ions on inhibition of tissue softening has led to the conclusion that the egg box model, which was developed to explain gel formation by calcium ions in dilute polypectate solutions, is not a suitable model to explain metal ion effects on cucumber tissue texture. Hopefully, development of this experimental approach will lead both to improved understanding of the mechanisms of plant tissue softening and to better means to retain firmness in cucumber pickle products.

The ability of metal ions to modify the texture of fruit and vegetable tissue has been studied since 1939, when Kertesz (1) found that calcium ions improved the firmness of tomatoes. A very simplified generalization of the effects of metal ions on fruit and vegetable texture is that monovalent cations, usually $Na^+$ and $K^+$, cause tissue softening. Examples of this softening effect include results with peas (2,3), dried peas (4), carrots (5), potatoes (6) and green beans (7). On the other hand calcium, a divalent cation,

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either causes tissue firming or prevents softening of plant tissues. Calcium effects appear to be general in plant tissues. All of the commodities mentioned above respond to calcium addition. In addition, the texture of apples (8) and apricots (9) is affected by calcium.

The USDA Food Fermentation Laboratory has been concerned over a long period of time with the factors which affect the textural quality of acidified vegetables, particularly cucumbers. As is the case with other processed fruit and vegetable products, metal ions have been found to have important textural effects on cucumbers. In contrast to observations with other plant tissues, NaCl has been found to improve firmness retention in fermented cucumbers, in addition to its ability to select for lactic acid bacteria in fermentations and its use as a flavoring (10-13). Table I shows the effect that NaCl has in preventing softening during fermentation and storage of cucumber slices. The probable reason that softening inhibition occurs is that NaCl can inhibit to some degree the action of polygalacturonases which soften tissue. This is suggested in Table I by the fact that NaCl was not required to maintain firmness in blanched cucumber slices during fermentation. In addition, earlier results of Bell and Etchells (14) show that NaCl reduces the rate of softening in pasteurized cucumber tissue to which a fixed amount of polygalacturonase activity is added. Calcium is currently added to most commercial cucumber pickle products due to its ability to prevent texture loss in both fermented (10, 11, 15-17) and non-fermented pickles (18, 19).

Table I. Effect of heat and salt on cucumber slice firmness

| Heat Treatment | NaCl, % | pH | Firmness score |
|---|---|---|---|
| None | 0.0 | 3.5 | 3.1C[1] |
| | 1.4 | 3.3 | 4.6B |
| | 3.9 | 3.2 | 5.2B |
| | 6.5 | 3.2 | 8.3A |
| 77°C, 3.5 min | 0.0 | 3.6 | 7.9A |
| | 1.4 | 3.4 | 8.3A |
| | 3.9 | 3.3 | 8.6A |
| | 6.5 | 3.2 | 8.9A |

[1]Means followed by different letters indicate treatments were different at the 0.05 significance level.

Source: Data are from ref. 10.

It is somewhat surprising, considering the amount of work done and the variety of different plant tissues studied, that there have not been multiple mechanisms proposed to explain metal ion effects. There has been nearly complete agreement that these effects occur due to the interaction of metal ions with the demethylated galacturonic acid residues in the pectic substances of the cell wall. Monovalent ions, i.e. NaCl, are thought to act by displacing calcium ions from pectate binding sites and thereby disrupting the

pectin gel structure. This is illustrated by some data of Van Buren (20) on green beans (Fig. 1). A direct effect of NaCl on degradation of cell wall polymers has not been reported. For divalent cations, i.e. calcium, Rees and his group in England proposed an "egg box" model (21) to explain the binding of calcium ions to polypectate in aqueous solution (Fig. 2). The key elements of this model are that calcium cations provide ionic cross-links as negatively charged galacturonic acid polymeric chains line up with each other and provide pockets into which the metal ions can fit. At low calcium levels there will initially be dimerization of pectate molecules. As more ions are bound, the dimers will aggregate into a large gel structure.

I emphasize that the egg box model was developed to explain the binding of divalent ions in polypectate solutions, not the effect of calcium ions on the physical properties of plant tissues. A rather large volume of data on the interactions of metal ions with pectin, particularly the work of Rees and his collaborators (21-26) and Kohn and coworkers (27, 29), all seem to be explainable in terms of the egg box model. With plant tissues there is much less quantitative data on ion binding in the tissues or in isolated cell walls. However, the data which have been obtained seem to be generally consistent with the egg box model (30-33). As a result, the egg box model has often been used to attempt to explain calcium ion effects on the physical properties of both living and processed plant tissues (34-40). However, clear demonstrations that physical changes in plant tissues, e.g. firming, inhibition of softening, or inhibition of cell elongation during growth, are caused by egg box binding of metal ions are lacking. In a few instances data have been published which are at least difficult to reconcile with the egg box model. Stoddard et al. (41) were unable to correlate the degree of calcium cross-linking to the rate of cell wall elongation. Tepfer and Taylor (42) found a lack of correlation between the ability of divalent ions to cause pectate gelation and their ability to inhibit the acid-induced elongation of bean hypocotyls. McFeeters et al. (19) found that changing pectin methylation in the presence of calcium ions had little or no effect on the ability of cucumber tissue to maintain firmness during storage. At low methylation there should have been more and stronger calcium cross-linkages with pectin and an inhibition of tissue softening.

## Effect of Monovalent Ions on Cucumber Softening Rates

Now let us consider recent results from this laboratory concerning the effects of metal ions on the rates of texture changes in acidified cucumber tissue texture. To investigate the effect of NaCl on cucumber texture in the absence of enzymatic degradation, the firmness changes in blanched tissue under acid conditions, pH 3.2-3.8, were measured (43). The procedure was to blanch cucumber slices or mesocarp tissue pieces in boiling water for 3 min to inactivate pectinesterase (19). After cooling, the tissue samples were covered with a brine which contained acetic acid and $SO_2$, to equilibrate at 0.6% and 200 ppm, respectively, and the appropriate amount of NaCl. The tissue equilibrated with the brine in the cold and then samples were incubated at 44C so they would soften reasonably rapidly. Firmness of the tissue was evaluated at the

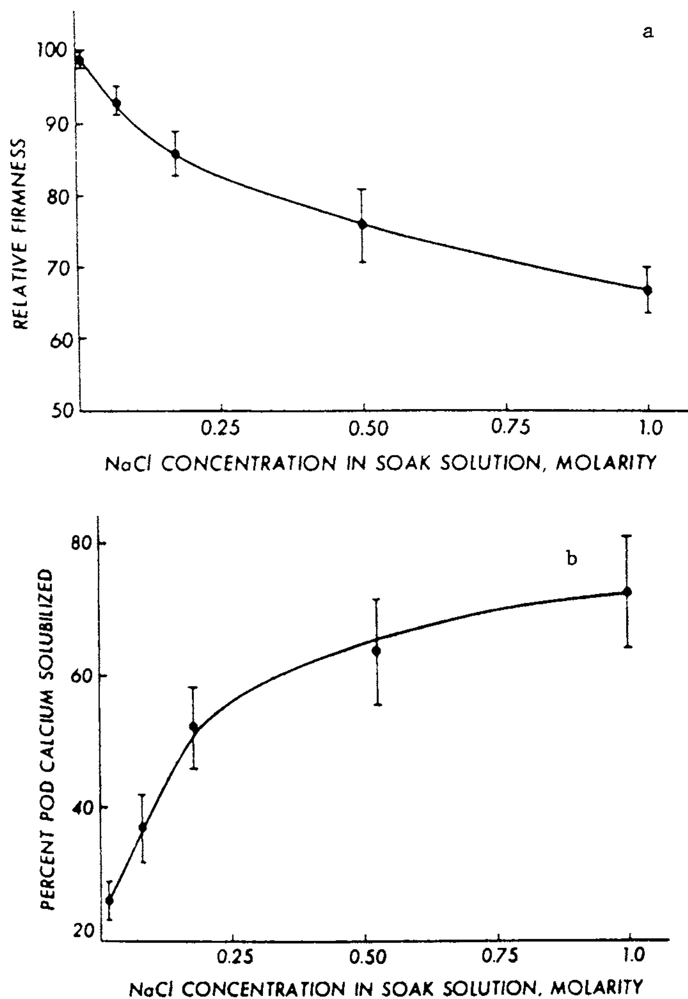


Fig. 1. Effect of NaCl on the texture and solubilization of calcium ions from green bean pods. a) Firmness of pods using a Kramer shear press. b) Solubilization of calcium ions from green bean tissue. Reprinted from reference 20.

beginning of the 44C storage and at several subsequent times by a punch test on 15 tissue pieces from duplicate jars of sample using the Instron Universal Testing Machine (44). The first order rate of the softening reaction was calculated from the slope of the softening curves (Fig. 3).

The rate of softening of cucumber mesocarp tissue increased as the equilibrated salt concentration increased for both whole cucumber slices and cucumber mesocarp tissue pieces (Fig. 4). Therefore, if the enzymatic degradation of cell walls is prevented, cucumber tissue does soften in response to NaCl, as has been observed in other fruits and vegetables. There is a question about the mechanism of the degradative process that leads to softening in acid conditions. The salt softening described for other vegetables has been observed under low acid conditions. It has been proposed that softening in those commodities is caused by $\beta$-elimination degradation of pectin, though direct evidence for $\beta$-elimination reactions in vegetable tissues has not been obtained (7). However, with the equilibrated pH of the tissue in these experiments in the range of 3.2-3.8, the pH within the cucumber cell wall is almost certainly too low for $\beta$-elimination to occur (45). Therefore, there may be a different salt softening mechanism in acidified plant tissues as compared to low acid vegetables. Fig 4b also shows the degree of pectin methylation in the cell walls of the cucumber mesocarp tissue used in this experiment. Two points should be remembered. First, the degree of methylation is relatively constant in blanched tissue and, secondly, it is quite high. Over 60% of the galacturonic acid residues have methyl esters and are, therefore, uncharged. This becomes an important consideration when we consider the effect of calcium ions on the softening rate in light of the requirement of ionized carboxyl groups for cross-linking of pectin molecules according to the egg box model. The salt softening effect in cucumbers was also similar to results in green beans (46) in that sodium and potassium ions had similar softening effects. In fact, at 0.25 M all of the alkali metal ions had a similar effect on the rate of cucumber tissue softening (Table II). Thus, the size of the ions do not appear to affect their ability to increase softening rates.

Table II. Effect of 0.25 M alkali metal ions on the rate of softening of blanched cucumber mesocarp tissue held at 44°C

| Metal Ion | Softening rate, $day^{-1}$ |
|---|---|
| None | 0.055A[1] |
| Lithium | 0.175B |
| Sodium | 0.194B |
| Potassium | 0.159B |
| Rubidium | 0.169B |
| Cesium | 0.166B |

[1]Means followed by different letters indicate treatments were different at the 0.05 significance level.
Source: Data are from ref. 43.

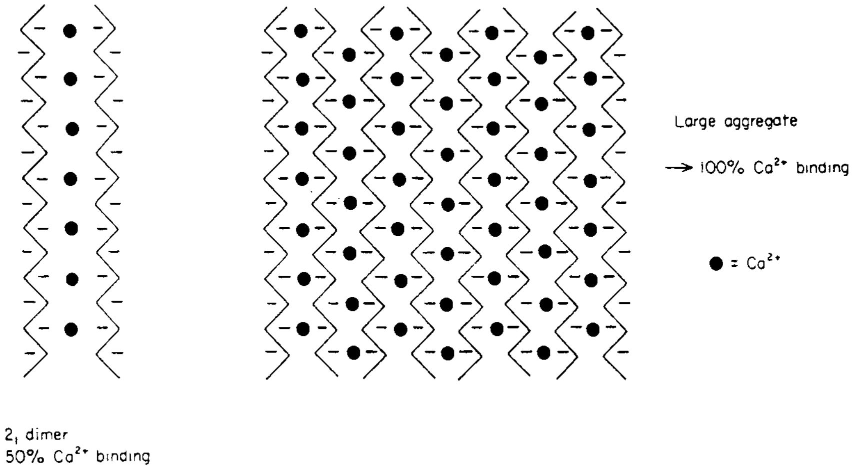


Fig. 2. Egg box model for cross-linking of polypectate chains by calcium ions. Reprinted from reference 25.

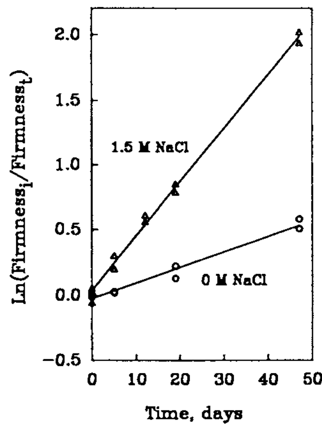


Fig. 3. First order plot of cucumber tissue softening in acid brine with and without added NaCl. The subscript i is the initial firmness, and the subscript t indicates firmness at any subsequent time. From reference 43.

Inhibition of Softening Rates by Calcium

Once it had been demonstrated that NaCl enhanced the rate of cucumber tissue softening, the next question was whether and in what way calcium ions would inhibit the rate of softening. To answer this question a combination of 1.5 M NaCl and 0 to 80 mM calcium ions were added first to blanched cucumber slices and then to cucumber mesocarp tissue (47) (Fig. 5). In both experiments there was an excellent fit of a hyperbolic curve to the softening rates as a function of calcium added, since the hyperbolic model accounted for over 99% of the experimental variation. For the cucumber slices (Fig. 5a), half of the observed inhibition of softening rate occurred at 6.3 mM calcium. For the mesocarp pieces (Fig. 5b), half maximal inhibition occurred at 1.5 mM calcium ion. These results indicated that even in the presence of high NaCl concentrations low calcium ion concentrations could saturate some binding site that resulted in inhibition of texture loss.

A problem with the results from these experiments was that the absolute rates of softening were highly variable from one lot of cucumbers to another even though the pattern of responses to metal ions was very consistent. Notice, for example, that the softening rates without added calcium differ by a factor of about 15 (Fig. 5) even though the rates at high calcium concentrations were similar. In view of the inhibitory effect of calcium on softening rates, the natural calcium levels in lots of cucumbers obtained over a two year period were compared to the softening rates in 1.5 M NaCl, but in the absence of added calcium. Fig. 6 shows a reasonable hyperbolic relationship between calcium level and softening rates with the model accounting for 97% of the experimental variation. This suggested that a major reason for lot to lot variation in softening of cucumber tissue was the natural variation in the calcium content of the fruit.

The "Egg Box" Model as an Explanation for Softening Inhibition

Now let us turn our attention to the question of whether it is reasonable to attribute the inhibition of softening by calcium ions to cross-linking of pectin molecules in the cell wall according to the egg box model(21). The conditions of the calcium inhibition experiment shown in Fig. 5b were such that it would be expected that calcium cross-links would be relatively infrequent. If we consider the calcium concentration which gives half maximal inhibition of softening, 1.5 mM, there was only a small proportion of calcium ions present relative to 1500 mM sodium ions. The pK of galacturonic acid residues in pectate solutions is about 3.6 (48) while the pH in the experiment was 3.2 so that a large proportion of the carboxyl groups would be protonated and, therefore, uncharged. The degree of pectin methylation was 62%, which means that only 38% of the galacturonic acid residues in the cell wall would be non-esterified even if the pH were high enough for the carboxyl groups to be ionized. If it is assumed that the methoxyl groups in the tissue were randomly distributed, as has been reported by Anger and Dongowski (49), the selectivity coefficient between sodium and calcium ions binding to pectin will be relatively small (27). Taking all of these factors into account it was estimated that there would be only one calcium

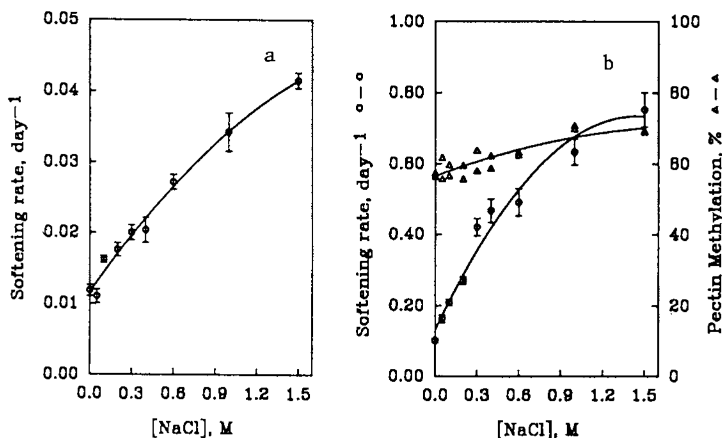


Fig. 4. Effect of NaCl concentration on the rate of cucumber tissue softening in acidic conditions. a) Softening rates of cucumber slices. b) Softening rates and pectin methylation changes in cucumber mesocarp tissue pieces. From reference 43.

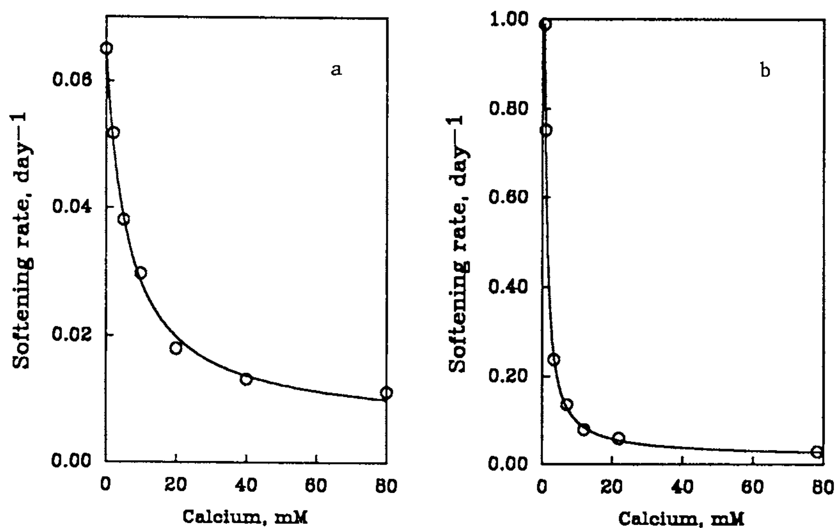


Fig. 5. Effect of calcium chloride concentration on the rate of cucumber tissue softening in 0.6% acetic acid and 1.5 M NaCl. a) Softening rates of cucumber slices. b) Softening rates of cucumber mesocarp tissue pieces. From reference 47.

cross-link per 2700 galacturonic acid residues. It seems difficult to explain such a large calcium effect on texture upon such infrequent cross-linking events in the cell wall. Nevertheless, such an estimate contains many assumptions so that it could easily be off by a considerable magnitude. Also, it is at least possible that in plant cell walls even rare calcium cross-links could have a large effect on texture, though Deuel et al. (50) and Doesburg (45) suggested that isolated calcium cross-linkages would not give a strong pectin gel structure. Therefore, the effects of other ions on softening rates were evaluated to assess in another way whether egg box binding is a reasonable mechanism to explain the effect on calcium on cucumber tissue texture under acid conditions.

The results of the addition of 10 mM levels of various ions in place of calcium are shown in Fig. 7. The ions are shown in the order of increasing binding affinity to pectate based upon the data of Kohn (28, 29), except for barium and aluminum for which binding data are not available. Calcium, strontium and barium were good softening inhibitors. However, zinc, cobalt and cadmium ions, which have affinities equal to or greater than calcium, showed little or no inhibition of softening. Copper, which binds much more strongly to pectin than calcium, has considerably less of an inhibitory effect than calcium. The conclusion from these results was that little or no correlation existed between the affinity of metal ions for pectin and their inhibition of softening. Like the estimate of calcium cross-link frequency, this result also appeared to be inconsistent with the egg box model. In this experiment only the alkaline earth ions, calcium, strontium and barium were good inhibitors of softening so it appeared that the binding sites for calcium, whatever their chemical structure, were relatively specific for calcium. To test this idea another group of ions, the lanthanides, were tested for their ability to inhibit softening. Several lanthanide ions have been found to specifically bind with high affinity to calcium sites in calcium binding proteins such as calmodulin (51). Thus, in their size and electronic structure they seem to be reasonably good analogs of calcium. The lanthanide ions were added to blanched cucumber mesocarp tissue in the presence of 1.5 M NaCl and their ability to inhibit softening was calculated in terms of the equivalent amount of calcium required to give the same degree of inhibition of softening rates. Since all of the lanthanides had a calcium equivalence greater than 0 mM, they all inhibited cucumber tissue softening to some degree (Fig. 8). Since 10 mM concentrations of each ion were used, any calcium equivalence value greater than 10 mM meant that the ion was a better inhibitor of softening than calcium. The six lanthanides with ionic radii greater than gadolinium exceeded the 10 mM equivalence level. The conclusion from these experiments was that the binding sites which affected texture were quite specific for calcium ions or calcium ion analogs rather than for ions which bound to pectic substances with high affinity.

One final experiment addressed to the question of the egg box model and textural effects was to determine the ability of cadmium ions to reverse the effect of calcium ions on softening. Cadmium ion did not inhibit softening (Fig. 7) even though Kohn (29) has demonstrated that it binds to pectate with greater affinity than calcium. It also has an ionic radius nearly the same as calcium

In Quality Factors of Fruits and Vegetables; Jen, J.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

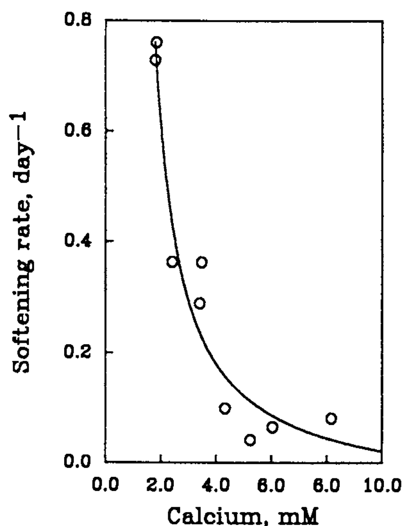


Fig. 6. Relationship between the natural concentration of calcium ions in cucumbers and softening rates of cucumber tissue in 0.6% acetic acid and 1.5 M NaCl. From reference 47.

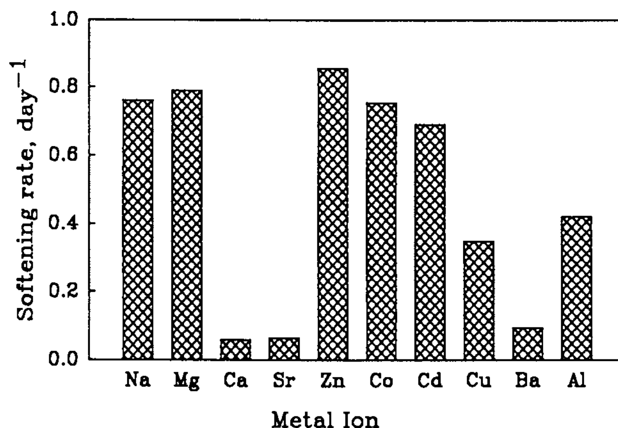


Fig. 7. Effect of 10 mM concentrations of divalent and trivalent cations on the rate of cucumber mesocarp tissue softening in 0.6% acetic acid and 1.5 M NaCl. From reference 47.

(52) and, of course, the same charge as calcium. It was possible that cadmium did not inhibit softening because it did not form cross-linkages with pectin in a way which was effective in inhibiting degradation of the cell wall structure. To test this possibility it was reasoned that if cadmium and calcium ions were added together to cucumber tissue along with 1.5 M NaCl, the cadmium ion should inhibit the calcium effect on softening if the egg box model was operative. Softening rates with 36 combinations of calcium and cadmium ion concentrations were measured. The data were analyzed by doing reciprocal plots similar to those which are used to evaluate enzyme inhibition by competitive, non-competitive and uncompetitive inhibitors. In the reciprocal plot (Fig. 9), high softening rates are toward the x-axis and high cadmium concentrations are toward the y-axis. The line without calcium added to the cucumber tissue shows that the same softening rate occurred with 2 to 80 mM cadmium added. This confirmed the result found for 10 mM cadmium in Fig. 7 over a range of concentrations. The expectation was that if calcium was having an effect due to egg box binding, cadmium ions should competitively displace calcium ions and reverse the calcium effect. This would result in a group of lines at different concentrations of added calcium all intersecting at the y-axis at the zero added calcium intercept. The fact that the reciprocal plots were parallel to the x-axis at all calcium concentrations indicated that cadmium was not competing with the site through which calcium had its textural effect. Since both ions presumably would bind to pectin, the simplest conclusion was that calcium had its effect on softening rates by binding at some site other than pectin carboxyl groups.

## Summary and Conclusions

To briefly summarize the results as they relate to the egg box model, estimates of a very low frequency of calcium cross-links expected under the experimental conditions used, the lack of correlation between the affinity of metal ions for pectin and the ability of those ions to inhibit softening, the apparent specificity of softening inhibition by calcium or calcium analogs, and the lack of competition between calcium and cadmium ions, all appeared to be inconsistent with the hypothesis that egg box binding was responsible for the inhibition of softening of cucumber tissue by multivalent ions. We need to begin looking for other mechanisms by which cucumber tissue softening can be inhibited by calcium and certain other ions. These results do not in any way suggest that the egg box mechanism is not a valid mechanism to explain metal ion binding in pectin solutions. It appears, however, that the egg box model cannot be extended to explain the physical effects of calcium ion addition to cucumber mesocarp tissue in this situation. It also means that we must be careful in trying to explain the textural effects of metal ions in terms of the egg box model as we study other processing situations. The egg box model may be operative in some situations, but not in all.

There does not exist a known alternative mechanism to the egg box model which can explain the metal ion effects which have been observed. While the experimental results obtained to date argue rather strongly against the egg box idea, they do not suggest an

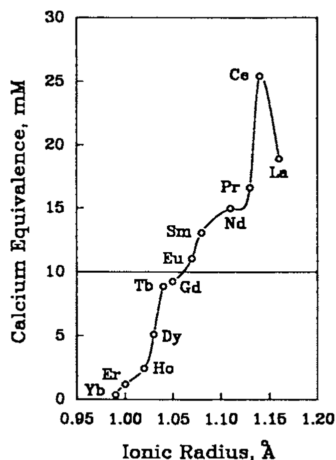


Fig. 8. Comparison of the inhibitory effect of 10 mM lanthanide ions on cucumber tissue softening rates relative to the inhibitory effect of calcium ion. From reference 47.

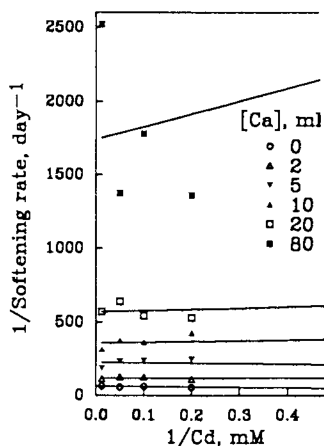


Fig. 9. Evaluation of possible competition between calcium and cadmium ion for inhibition of cucumber mesocarp softening. From reference 47.

obvious alternative. Some possible mechanisms which can be imagined include: 1. Non-enzymatic degradation of one or more cell wall polysaccharides which can be specifically inhibited by calcium and calcium analogs; 2. A heat stable enzyme which degrades a cell wall component and which is inhibited by calcium; or 3. A calcium mediated structural protein, similar to the cadherins found in animal cells (53), which is involved in plant cell interactions. The fact that it is now possible to experimentally affect the rates of softening processes in cucumbers by a variety of metal ions provides us new tools to investigate the chemistry of plant tissue texture. The ability to carry out experiments with gram to kilogram quantities of a single tissue type should aid efforts to isolate and characterize degraded saccharides from softened mesocarp. Several of the lanthanide ions, which proved to be excellent inhibitors of softening, have useful spectroscopic properties, especially their ability to perturb NMR spectra (51). This could be a way to probe the interaction of metal ions with cell wall components. Questions about the generality of these texture effects in other fruits and vegetables and the interaction between metal ions and other processing variables, such as pH, are experimentally accessible with the techniques now available. Hopefully, as these experimental possibilities are exploited new ideas concerning the mechanisms of tissue softening and the effects of metal ions on softening will emerge.

Beyond the interesting scientific questions concerning the chemistry of texture, the purpose for doing research in this area is to have an impact on the processing practices of the industry and ultimately an impact on the quality of products available to people. This sometimes seems to be a distant goal. However, it should be possible to identify processing operations in which changes can be made to improve texture retention as we look at interactions between acidification practices, the addition of sodium and potassium salts, and calcium levels in the tissue, both the natural calcium and that added during processing.

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Chapter 12

# Quality and Stability of Enzymically Peeled and Sectioned Citrus Fruit

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Peeling and sectioning of citrus by vacuum infusion of pectinases resulted in cleaner sections with less adhering albedo and better quality than those from conventional machine-peeling followed by a lye bath. Enzyme separated segments were larger, waste was eliminated, and segments had adequate structure to allow marketing as a dry pack. When commercial pectinases were adjusted to equivalent peeling activity, segments differed in vesicle turgidity, membrane strength, and fluid loss during storage. Fluid loss from dry-pack segments was found to be correlated with loss of membrane strength. In stored, dry-pack unpasteurized grapefruit segments, naringin crystals formed in albedo cells still adhering to section membranes after treatment. Crystal formation was minimized by water sprays or lye solution dips. This process provides a new approach for commercial sectioning of citrus.

As currently marketed, citrus sections (primarily grapefruit and orange) consist of segment juice sacs excised from the carpellary membranes. Before sections can be cut from fruit, all vestiges of inner peel or albedo must be removed. To accomplish this, present technology requires steaming the fruit to loosen the peel, machine peeling, and lye treatment of the peeled fruit to remove any remaining albedo. Section production is labor intensive in that each section must be cut by hand from the peeled fruit. Cut sections are small and friable, and because of damage incurred during cutting, must be packed in liquid. Only 60% of the edible portion of fruit is recovered as cut sections (1). These inefficiencies have kept section costs high and driven much of the production capacity to cheaper labor markets.

An alternate technology with the potential to minimize labor input and increase product yield was described by Bruemmer et al. (1,2). Vacuum infusion was utilized to permeate the albedo of grapefruit with any of several commercial pectinases.

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Depolymerization of middle lamellar pectins by these preparations diminished albedo and segment membrane integrity, facilitating peel removal and separation of sections. Sections thus produced, while larger and less damaged than cut sections, shared their fragility. Coating with gelatin was suggested as a means to increase structural integrity of enzyme separated sections.

Recent work has shown that section firmness is enhanced by modifying the enzyme sectioning process to leave segment membranes intact (3,4). Recovery of segments with membrane cover intact also permits utilization of 100% of the edible portion of fruit. Firm dry segments thus produced are amenable to packing without liquid cover, as suggested by Bruemmer et al (1). Unpasteurized, dry pack segments could provide the consumer with a product having the quality of fresh fruit, if flavor, texture, and microbial deterioration could be controlled. The purpose of this study was to optimize initial segment quality and to develop means to extend shelf life of dry pack citrus segments.

## Materials and Methods

Vacuum Infusion. Fresh washed grapefruit (*Citrus paradisi* Mac F.) or oranges (*C. sinensis* L.) were warmed to 30°C core temperature by immersion in warm water or holding overnight at 32°C. To minimize contamination of segments by surface microflora, fruit were immersed in boiling water for 90 sec. Fruit were then scored radially three times from stem to blossom end, dividing the peel into six equal sections. This scoring, which penetrated the flavedo but not the albedo, facilitated infusion of liquid. After scoring, fruit were submerged in a 30°C bath of commercial pectinase in deionized water, placed in a vacuum chamber, and evacuated to approximately 75 torr. After holding at this pressure for two minutes, the vacuum was released slowly. Infused fruit were stored for one hour at 30°C before peeling and segmenting. Ease of peel removal was evaluated on a scale of 1-5, with one being similar to uninfused fruit, and five connoting easy, complete slippage of peel with no adhering threads or core. Segmenting was similarly evaluated, with one equivalent to untreated fruit, and five indicating that segments separated easily without carpellary membranes.

Initially, pectinases were evaluated for peeling and segmenting activity at equivalent protein levels, using the Lowry procedure as modified by Potty (5) for protein determination. In later work, pectinases were tested at a number of concentrations to determine the amount required to yield a specific peel rating using regression analysis.

Enzyme Activity. Seven commercial pectinases were obtained from Miles Laboratories, Elkhart, IN 46514, Novo Laboratories, Wilton, CN 06897, and Rohm Tech Inc., Malden, MA 02148. All preparations were assayed for polygalacturonase (PG), cellulase, and pectinesterase (PE) activities by the methods of Vas, et al. (6) as modified by Bruemmer et al. (1). PG and cellulase activities were determined by reduction of specific viscosity in an Ostwald-type viscosimeter held in a 50°C water bath. PG activity was measured on both 1.5% polygalacturonic acid (U.S. Biochemical Corp.), titrated

to pH 5.0 with NaOH, and on 0.45% rapid-set lemon pectin (Sunkist Growers, Inc.). Carboxymethylcellulose (2%) was used as the substrate for cellulase activity. Decrease in specific activity was graphed against the log of inoculation time, and the time required for 25% reduction of initial viscosity determined. A unit of activity was defined as the amount of enzyme necessary to effect a 25% reduction in initial viscosity of 6 ml of substrate in 10 min. PE activity was determined with 15 ml of 1% pectin solution adjusted to pH 4.0 prior to addition of enzyme. Consumption of 0.1 N NaOH at 50°C was graphed, the slope determined by least squares, and PE units calculated by the procedure of Rouse (7).

Microbial Stability. Enzymically peeled and segmented grapefruit were used to determine susceptibility of unpasteurized segments to microbial colonization. Although reasonable sanitary procedures were followed during segment preparation, no attempt was made to maintain an aseptic environment; segments were therefore presumed to have an endogenous microflora. Segments were sealed in polyethylene bags with and without prior dipping in 0.2% potassium sorbate, and stored at 2°C. A similar group of segments was challenged with an inoculation of common citrus fruit decay microorganisms before being bagged with or without the sorbate dips. Inoculum contained the yeast *Saccharomyces cerevisiae* at $10^4$ colony forming units (CFU) per g of tissue and the bacteria *Lactobacillus plantarum* and *Leuconostoc mesenteroides* at levels providing a total of $10^6$ CFU's/g of fruit.

At weekly intervals bags were withdrawn from storage; five segments of each treatment were weighed, combined with twice their volume of 0.1% peptone, and blended at high speed for 10 sec. After dilution, samples were plated in duplicate on acidified potato dextrose agar (PDA) for enumeration of yeasts and orange serum agar (OSA) for detection of bacteria. Plates were incubated for 72 hr at 25°C (PDA) or 35°C (OSA) before reading.

Fluid Loss and Texture. Liquid loss from dry pack segments during 2°C storage was measured by weighing segments in a reclosable bag, draining free liquid from the bag weekly, and reweighing. Loss was expressed as % of original weight.

Two aspects of texture were considered: firmness of the intact segment and toughness of the carpellary membrane. Both were measured on stored segments with an Instron model 1011 Universal Tester (Instron Corp., Canton MA 02021) set on compression mode. Firmness was determined by crushing a pair of segments in an Ottowa Texture Measurement System to 3 mm final thickness. Crushed segments were then transferred to a Kramer Shear Cell and the force necessary to shear the membranes determined.

Flavor Comparisons. Relative flavor quality of segments stored in sealed plastic bags of varying oxygen permeabilities was measured with a rank sum test (8). Fresh segments were bagged in highly permeable strip sealed polyethylene bags, composite heat sealed bags of intermediate $O_2$ permeability (3000 cc/$m^2$ day), and heat sealed bags of low $O_2$ permeability (4-6 cc/$m^2$ day). Bags were stored at 2°C and sampled at weekly intervals. To eliminate segment variability, 24 segments from each storage regime were blended for 10

sec, filtered through cheesecloth, and tested as the juice. An experienced panel of 20 judges was presented the juices in red glasses in a red illuminated positive pressure room, and asked to rank them in order of preference. Rank sums were computed and significance of results determined from tables (9).

Calcium Firming. Soft segments from late season fruit were coated with an edible calcium alginate film to improve firmness. Segments were dipped in alginate solution, drained briefly, then immersed in $CaCl_2$ hardener solution. Additional calcium was provided for some segments by supplementation of the hardener solution with 5% Ca as $CaCl_2$.

Surface Naringin Reduction. Crystal aggregates occurring on surfaces of stored segments were collected, purified by recrystallization and TLC, and confirmed as naringin by melting point, IR, and TLC in several solvent systems (Baker, R. A., and Parish, M. E., Jour. Food Sci., in press). Reduction of surface naringin levels was attempted with water mist spray and by a 10 sec immersion in 1% NaOH at 60°C, followed by water misting. Residual surface naringin was removed for analysis by gently scrubbing the segment surface with a soft brush while immersed in deionized water. Naringin contents of wash waters and blended segments were determined by the Davis test (10).

## Results and Discussion

Enzyme Activity and Peeling Efficacy. Excessive treatment with infused pectinases caused segment membranes to separate from juice sacs, yielding soft, friable segments. Termination of activity when a peeling and sectioning rating of 4 was achieved improved segment textural quality by retaining carpellary membranes. Ease of peeling was still acceptable at this activity level.

Peeling and sectioning ratings of grapefruit infused with commercial pectinases at equivalent protein levels varied from 2.5 to 4.0 (Table I).

Table I. Effect of Pectinases Infused at Equivalent Levels on Peeling, Sectioning, and Segment Fluid Loss

| Pectinase | Peel Rating | Section Rating | Fluid Loss % |
|---|---|---|---|
| 1 | 3.0 | 2.5 | 2.9 |
| 2 | 2.5 | 3.0 | 3.0 |
| 3 | 4.0 | 4.0 | 4.3 |
| 4 | 3.8 | 3.8 | 8.2 |
| 5 | 3.8 | 3.4 | 9.4 |
| 6 | 4.0 | 2.8 | 11.3 |
| 7 | 3.0 | 3.0 | 13.0 |
| Cut Sections | - | - | 16.1 |

While the two pectinases with the best peeling and sectioning ratings did have the highest PG activity per unit protein, other pectinases showed no correlation between ratings and PG activity (4). PE and cellulase activity levels were unrelated to peeling or sectioning ability, as was noted by Bruemmer (1). Fluid loss was not correlated

to peeling or sectioning efficacy of pectinases, nor to their specific enzyme activities.

At concentrations providing equivalent peeling ratings, pectinases should exhibit similar activity levels of the responsible enzyme(s). However, when the concentrations necessary for a 4 peel rating were determined for each pectinases by regression analysis, and the individual enzyme activities calculated for those concentrations, no such similarity was evident (Table II.)

Table II. Enzyme Activities of Pectinase Infusions Giving a Peel Rating of 4

| Pectinase | PG pectin | PG PGA | PEU | Cellulase |
|---|---|---|---|---|
| 1 | 94 | 100 | 10 | 37 |
| 2 | 177 | 273 | 11 | 85 |
| 3 | 214 | 1619 | 115 | - |
| 4 | 118 | 581 | 17 | 24 |
| 5 | 343 | 41 | 18 | 27 |
| 6 | 21 | 18 | 17 | 9 |
| 7 | 26 | 10 | 269 | 11 |

Activity ranges were 9-fold for cellulase, 27-fold for PE, and 160-fold for PG. Several factors could account for this lack of correspondence. Pure substrates are used in assays, while mixed substrates are encountered within the peel. The pectinases are crude extracts containing many more activities than those assayed, e.g., pectin lyase. To obtain a more accurate estimate of a pectinase's peeling ability, Ben-Shalom et al. (11) suggested using a substrate derived from alcohol insoluble solids of citrus peel. In addition, pectinases varied considerably in pH and temperature optima and stability. For some pectinases, assayed activities may be substantially greater than activities seen in peel. An assay incorporating substrates and conditions similar to those found in peel may be useful in screening for peeling activity, but standard assays appear to be of limited value as predictors.

Textured Properties of Stored Segments. When grapefruit were peeled and segmented with pectinases at concentrations yielding peel ratings of 4, fluid loss from segments during $2^{o}C$ storage varied from 9.4 to 28% (Table III).

Table III. Textural Properties of Stored Segments

| Pectinase | Liquid Loss % | Vesicle Comp. (lbs) | Membrane Shear (lbs) |
|---|---|---|---|
| Water Con. | 4.0 | 239.0 | 242.0 |
| 1 | 9.4 | 164.9 | 151.3 |
| 6 | 13.1 | 98.7 | 108.8 |
| 4 | 14.5 | 107.1 | 90.9 |
| 7 | 15.9 | 120.5 | 88.5 |
| 5 | 16.7 | 121.9 | 106.7 |
| 3 | 24.1 | 96.9 | 73.0 |
| 2 | 28.0 | 92.5 | 82.9 |

This compared to a 4% fluid loss from segments derived from water infused control fruit. Fluid loss levels were higher than in Table I due to the use of softer, late season fruit in this experiment.

Firmness of segments was substantially reduced by the pectinase infusion process. Vesicle compression values for pectinase derived segments ranged from 38 to 69% of the value for water infused control segments. Of the pectinase derived segments, those produced with Pectinase 1 were the firmest, and also had the least fluid loss during storage.

Segment toughness, as measured by membrane shear, was also reduced by pectinase treatment. Some reduction of membrane toughness is desirable if segments (particularly those of grapefurit) are produced with carpellary membranes intact. Shear values of treated segments varied from 30 to 65% of control segment shear, with Pectinase 1 again giving the highest value. In general, membrane shear was comparable to vesicle compression for pectinases, with Pectinase 1 yielding segments with highest values, and Pectinases 2 and 3, the lowest. An inverse correlation between firmness and fluid loss was seen with Pectinases 1,2, and 3, but not the other pectinases.

Microbial Stability. Bacterial contamination of uninoculated segments was low, and declined during 9 weeks of 2°C storage (Table IV).

Table IV. Bacterial and Yeast Growth in Stored Segments

| | Uninoculated Segments Log CFU/g Tissue | | | |
|---|---|---|---|---|
| | Bacteria | | Yeast | |
| Week | Control | + Sorbate | Control | + Sorbate |
| 0 | 1.5 | 1.5 | 0.5 | 0.5 |
| 1 | 0.6 | 0.8 | 0.5 | 1.2 |
| 3 | 0.5 | 0.5 | 0.5 | 0.5 |
| 5 | 0.8 | 0.5 | 1.8 | 0.5 |
| 7 | 0.5 | 0.5 | 3.4 | 0.5 |
| 9 | 0.5 | 0.5 | 6.1 | 0.5 |
| | Inoculated Segments | | | |
| 0 | 6.2 | 6.2 | 4.2 | 4.2 |
| 1 | 6.0 | 6.3 | 4.0 | 4.0 |
| 3 | 6.0 | 5.8 | 4.4 | 3.7 |
| 5 | 6.2 | 6.0 | 4.7 | 4.2 |
| 7 | 6.0 | 5.6 | 4.8 | 3.8 |
| 9 | 6.4 | 5.6 | 5.3 | 3.9 |

Dipping segments in 0.2% potassium sorbate before storage did not accelerate this decline. Yeast contamination of dry packed segments was also low initially, but began to increase rapidly after three weeks of storage. This increase, which reached $10^6$ CFU/g after 9 weeks, was completely suppressed by the 0.2% sorbate dip. Initial bacterial levels of inoculated segments averaged slightly over $10^6$ CFU/g. There was no increase in CFU during storage; apparently the combination of low temperature and low segment pH inhibited growth of the added Lactobacillus and Leuconostoc. A slight reduction in CFU's was seen when inoculated segments were treated with sorbate. Initial Saccharomyces cerevisiae levels in inoculated segments averaged

In Quality Factors of Fruits and Vegetables; Jen, J.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

slightly above $10^4$ CFU/g, and increased to $10^5$ CFU/g during storage. No growth of yeast occurred when segments were treated with sorbate. These results suggest that, if stored at 2°C, segments would not be subject to appreciable bacterial growth. Yeast growth does occur under these conditions, but can be controlled with potassium sorbate. It was also found that growth of yeast was suppressed by 2°C storage in bags of low $O_2$ permeability.

Flavor Stability. Enzyme peeled unpasteurized segments are viable tissue, and are thus subject to both enzymic and nonenzymic flavor degradation. An attempt was made to control flavor deterioration by packaging freshly prepared segments in sealed plastic bags of limited oxygen permeability. Flavor of segments stored at 2°C in highly permeable polyethylene bags deteriorated rapidly, and after three weeks was disliked by an experienced taste panel at the 1% level of significance (Table V).

Table V. Rank Sums of Segments Stored in Bags of Varying $O_2$ Permeability

| | $O_2$ Permeability | | |
|---|---|---|---|
| Week | High | Medium | Low |
| 1 | 52 | 49 | 43 |
| 2 | 55 | 41 | 48 |
| 3 | 63 | 38 | 43 |

Ranges of insignificance: 5%, 40-56; 1%; 37-59

The panel found no significant difference between the segments in medium and low permeability bags after two weeks storage, but after three weeks storage preferred (at 5% level of significance) segments stored in moderately permeable bags. A restricted (but not anaerobic) oxygen environment would seem to be essential for flavor stability of dry packed unpasteurized segments.

Calcium Firming. Citrus sections tend to become progressively softer as fruit mature, severely limiting the period in which firm, high quality sections can be produced. Attempts have been made to extend the production season by firming canned sections with calcium salts added to the packing syrup (12). With dry pack segments another means must be found to apply calcium salts. Preliminary work revealed that simultaneous infusion of calcium lactate and pectinases for peeling was unsuccessful. At levels adequate to firm segments, calcium interfered with pectinase peeling and segment separation. A secondary infusion of fruit with calcium lactate or chloride after peeling was also unsuccessful, as was dipping in calcium salt solutions.

Coating segments with an edible alginate film utilizing a calcium salt hardener provided sufficient calcium to effectively firm soft, late season segments (Table VI). Calcium supplied by the hardener increased segment firmness 54%, and membrane shear, 50% over controls. An additional 5% calcium in the hardener further increased segment firmness and membrane shear over control values, by 89 and 85%, respectively.

Table VI. Texture and Fluid Loss of Uncoated and Alginate Coated Segments After 5 Weeks Storage

| Treatment | Vesicle Comp. (lbs) | Membrane Shear (lbs) | Fluid Loss % |
|---|---|---|---|
| Uncoated | 77.4 | 102.1 | 24.1 |
| Alginate | 119.3 | 152.8 | 22.6 |
| Alg. + 5% Ca | 142.8 | 189.0 | 20.3 |

Reduction of fluid loss from stored segments by calcium coating was not commensurate with increased segment firmness or membrane toughness. For example, an 89% increase in firmness was accompanied by only a 16% decrease in lost liquids. Increasing firmness due to calcium cross-linking of structural pectins was insufficient to block migration and loss of juice vesicle fluids.

Naringin Crystallization. Grapefruit segments stored at 2°C often developed small white specks, predominently on the outer (peel contact) surface. Microscopic examination of these specks revealed they were stellate or fan-shaped clusters of colorless needle shaped crystals. When purified by TLC and recrystallized, their melting point, IR, and TLC behavior identified these as naringin (Baker, R. A., and Parish, M. E., Jour. Food Sci., in press). In addition to being a visual defect, the intense bitterness of naringin made the presence of these crystals undesirable. When the outer surface of enzyme peeled segments was examined, a thin layer of adhering albedo cells was found. Grapefruit albedo contains from 2-5% naringin (13); crystallization during storage of the sections occurred within this layer of infused albedo cells. Since most naringin of grapefruit occurs in the albedo and segment membranes, and these are removed in conventional processing, crystallization of naringin has not been reported as a problem in cut sections.

In enzyme peeled segments, approximately 18% of the total naringin was localized in the layer of albedo cells on the outer membrane. Attempts to remove these cells prior to storage by water misting of whole peeled fruit reduced surface naringin to 12% of the segment total. Surface naringin could be reduced to 9% of the segment total by a brief immersion of whole peeled fruit in 1% NaOH, followed by water misting. Gentle brushing of whole peeled fruit under a water spray or hot water misting could be other alternatives for reducing surface naringin levels. Segments produced with different pectinases varied in susceptibility to specking, suggesting the presence of naringinase activity in certain pectinases (14). Infusion with naringinase active pectinases could reduce surface crystallization of naringin without further treatment.

A similar surface crystallization of glucoside occurs in freeze damaged oranges, when hesperidin specks appear on carpellary membranes (15). In limited work with pectinase peeled oranges, no crystallization of hesperidin occurred in segments during storage.

Conclusions. Pectinase peeling and segmenting of citrus fruit offers an alternative methodology to current techniques, and may substantially increase yield. Marketing of segments as a dry packed refrigerated product requires that textural, microbial, and flavor deteriorations be controlled. Fluid loss and firmness of stored

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segments varied with pectinase used and maturity of fruit. Coating soft segments from late season fruit with a calcium alginate film with or without an additional 5% Ca in the hardener increased firmness, and to a lesser degree decreased fluid loss. Segments stored at 2°C incurred little bacterial growth, but were subject to contamination with yeast. Yeast growth could be prevented either by dipping segments in 0.2% potassium sorbate or by sealing in bags of low oxygen permeability. Flavor quality of chilled segments deteriorated rapidly in highly permeable packaging; restricted atmosphere bagging retarded flavor changes. Both flavor and appearance were adversely affected by naringin crystallization on segment surfaces during storage. Surface naringin levels could be reduced by water misting, washing with 1% NaOH, or possibly by using pectinases containing naringinase activity.

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## Chapter 13

# Rheological Properties of Plant Food Dispersions

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Plant food dispersions such as tomato concentrates and concentrated orange juice are important items of commerce. The viscosity function and the yield stress are two important rheological properties that have received considerable attention. Corrections for slip, due to the formation of a thin layer of fluid next to solid surfaces, in a concentric cylinder viscometer depended on the magnitudes of applied torque and on the shear-thinning characteristics of the dispersion. Mixer viscometers were used for obtaining shear rate-shear stress and yield stress data, but the latter were higher in magnitude than those obtained by extrapolation of flow data. Finisher screen openings and concentration methods, were found to affect the rheological properties of tomato concentrates. The apparent viscosity of 65 $^{O}$Brix concentrated orange juice was strongly influenced by temperature. The dispersed phase plays a major role in the magnitude of apparent viscosity and yield stress, and functional relationships for predicting the properties were developed.

Many products derived from fruits and vegetables are important items of commerce. These products including, tomato concentrates, concentrated orange juice, apple sauce, and baby foods are suspensions or dispersions. Some of these foods, principally tomato concentrates, are used to make other foods (e.g., sauces). The word suspension denotes that the insoluble solids settle rapidly, but in the aforementioned foods there is little or very slow settling such that they can be called either cohesive suspensions or dispersions. The aforementioned

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foods have the common feature in that they contain a continuous phase made up of an aqueous solution of sugars, pectins, flavor compounds, and other chemical compounds, and a dispersed phase made up of solids that originate from the cell walls and other parts of the fruits and vegetables.

Rheological properties of these foods are useful in applications related to their handling (e.g., pressure drop and pumping power requirements in processing plants), their quality control, and in their sensory analysis. Further, a clear understanding of the role of the components of the plant food dispersions (PFDs) in the rheological behavior can lead to the formulation of products with the desired characteristics. While the present study deals with only the rheology of PFDs, it is important to recognize that there are detailed treatments on the subject of food rheology (1, 2, 3, 4) and on the general subject of rheology (5, 6), and they should be consulted for a deeper appreciation of the subject of rheology.

## Classification of Rheological Behavior of Food Dispersions

For the most part, PFDs exhibit shear-thinning (pseudoplastic) rheological behavior that is either time-independent or time-dependent (thixotropic). In addition, many PFDs also exhibit yield stresses. The time-independent flow curves are illustrated in Figure 1. The shear-thinning behavior appears to be the result of breakdown of relatively weak structures and it may have important relationship to mouthfeel of the dispersions. Because the viscosity of non-Newtonian foods is not constant but depends on the shear rate, one must deal with apparent viscosity defined as:

$$\eta_{a\dot{\gamma}} = (\sigma/\dot{\gamma}) \quad (1)$$

In Equation (1), $\sigma$ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate ($sec^{-1}$), and $\eta_{a\dot{\gamma}}$ is the apparent viscosity (Pa sec) at a specified shear rate. It is clear that in Equation (1) one can use a magnitude of shear rate that is appropriate for a specific application.

## Yield Stress

Yield stress also is an important property of PFDs. For example, its magnitude plays a role in the coating of solid surfaces and in impeding the settling of fine solid particles, such as spices, that may be added to PFDs and that must remain in suspension. From Figure 1, one can define a yield stress $\sigma_0$ that is obtained by extrapolation of shear stress - shear rate data to zero shear rates and

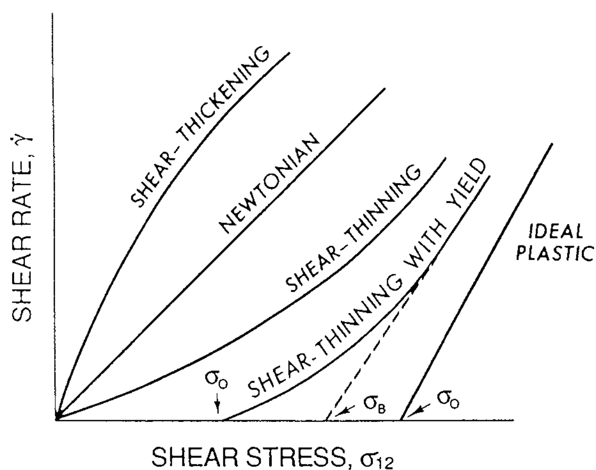


Figure 1. Time-independent flow behavior of fluid foods on a shear rate-shear stress diagram; $\sigma_0$ is yield stress by extrapolation of data to zero shear rate; $\sigma_B$ is yield stress by extrapolation of straight line portion of data.

a yield stress $\sigma_B$ that is obtained by extrapolation of the straight line portion of the data as shown by the dotted line. In general, $\sigma_B$ will be larger than $\sigma_0$. Yield stress has been related to the strength of the coherent network structure, followed by a rupture of the network bonds or linkages connecting the flow units (7).

Rheological Models.

Rheological models can be used to understand better the rheological behavior of a material. There are several rheological models that have been proposed in the literature and they have been reviewed in (8, 9).

The power law model (Equation 2) has found extensive use in the food literature:

$$\sigma = K \dot{\gamma}^n \tag{2}$$

In Equation (2), n is the flow behavior index (-), K is the consistency index (Pa $sec^n$), and the other terms have been defined before. For shear-thinning fluids, the magnitude of $n<1$ for shear-thickening fluids $n>1$, and for Newtonian fluids $n=1$. For PFDs that exhibit yield stresses, models that contain either $\sigma_0$ or a term related to it have been defined. These models include, the Bingham Plastic model (Equation 3), the Herschel-Bulkley model (Equation 4), the Casson model (Equation 5), and the Mizrahi-Berk model (Equation 6).

$$\sigma = \sigma_0 + \eta_B \dot{\gamma} \tag{3}$$

$$\sigma = \sigma_{0H} + K_H \dot{\gamma}^{n_H} \tag{4}$$

$$\sigma^{0.5} = K_{0c} + K_c \dot{\gamma}^{0.5} \tag{5}$$

$$\sigma^{0.5} = K_{0m} + K_m \dot{\gamma}^m \tag{6}$$

In the above equations, $\sigma$ is the shear stress, $\dot{\gamma}$ is the shear rate, $\eta_B$ is plastic viscosity, $K^2{}_{0c}$, $K^2{}_{0m}$, $\sigma_0$, and $\sigma_{0H}$ are the yield stresses according to the different models, and the other terms are constants to be determined from experimental data.

Effect of Temperature on Rheological Behavior.

Because many liquid foods are subjected to a wide range of temperatures during processing, storage,and transportation the effect of temperature on the viscosity function is of interest. The Arrhenius model (Equation 7)

has been used extensively to describe the effect of temperature on the viscosity of many foods:

$$\eta = \eta_{oo} \exp (E_a/RT) \tag{7}$$

In the case of non-Newtonian fluids, one can use either the apparent viscosity, $\eta_a$, at a specified shear rate or the consistency index, K, in the power law model in place of $\eta$.

For foods with high sugar content and at relatively low temperatures, the simple Arrhenius relationship may not be adequate to characterize the temperature dependence (10, 11). For highly concentrated sugar solutions, the WLF equation (Equation 8) was shown to be satisfactory (12) to describe the influence of temperature on the viscosity.

$$\ln (\eta/\eta_g) = -17.44 (T - T_g)/[51.6 + (T - T_g)] \tag{8}$$

where, $T_g$ is the glass transition temperature and $\eta_g$ is the viscosity at that temperature. Studies (13, 14) with concentrated juices suggest that the Fulcher model (Equation 9) also can be used to describe the effect of temperature on viscosity.

$$\ln \eta = A + \frac{B}{T - T_0} \tag{9}$$

where, A, B, and $T_0$ are constants to be determined.

## Measurement of Rheological Properties of Fluid Foods

Following Scott Blair's (15) classification of instruments for the study of texture, the instruments for measuring the flow properties of fluid foods can be classified into the categories: (1) fundamental, (2) empirical, and (3) imitative. Fundamental tests measure well defined properties utilizing geometries that are amenable to analysis of fluid flow. Empirical tests measure parameters that are not clearly defined, but the parameters have been shown to be useful from past experience. Imitative tests measure properties under test conditions similar to those in practice (16).

The fundamental methods can be classified under the specific geometry employed in the measurement. For each geometry, the equations necessary to calculate the shear rate and the shear stress are derived from the equations of motion and continuity, and the measured torque. The applicable equations as well as the instruments have been well described in several texts (17, 18, 19, 20) and they

will not be discussed here. Three requirements are common to all the fundamental methods: (1) laminar flow of the fluid, (2) isothermal operation, and (3) no slip at solid-fluid interfaces. One problem with a number of plant food suspensions is the formation of a thin film of fluid at the solid-liquid interface and the possibility of slip at interface (21, 22, 39).

Empirical methods of viscosity measurement include rotational viscometers with spindle geometries, such as pins and flags, whose flow fields are difficult to analyze mathematically in order to obtain expressions for shear rate and shear stress. The use of these complex geometries has been limited to quality control purposes. Several consistometers have been developed for characterizing purees of fruits and vegetables, including many baby foods. These methods are used for quality control of the products and in studies dealing with the effects of changing processing conditions on the consistency of pureed foods. Description and operation of these instruments can be found, among others, in (23, 24).

In the case of imitative methods, the properties are measured under test conditions that simulate those in practice. There are several instruments that perform imitative tests on solid foods (e.g., butter spreaders, the General Foods Texturometer, and the Brabender Farinograph).

## Refinements to Empirical and Imitative Methods

A few refinements to empirical and imitative methods have been presented and these will be considered here.

### Mixers for Determining Flow Properties.

Quantitative shear stress--shear rate data can be obtained with agitators having complex geometries assuming that the shear rate is directly proportional to the rotational speed of the agitator and if the flow behavior of the fluid can be described by the power law model. The mixer viscometric technique is based on the assumption that for complex geometries one can assume an effective shear rate that is dependent on the RPM and on the design characteristic of the impeller (25, 26, 27, 28, 29, 30). The procedure has been described in detail by Rao (27) and will be considered only in brief here.

It is assumed that the impeller (agitator) exerts an average shear rate that is directly proportional to the rotational speed:

$$\dot{\gamma} = k_s N \qquad (10)$$

where, $\dot{\gamma}$ is the shear rate, N is the rotational speed of the impeller, and $k_s$ is the proportionality constant. For

any impeller, the constant $k_s$ can be determined from a plot of (1-n) vs log ($p/k\ N^{n+1}\ d^3$ ); the slope of the line is equal to -log $k_s$ (28). For a given impeller tests must be conducted such that the following data are obtained; p, the power (Nm/sec); N , the rotational speed ($sec^{-1}$); d, the diameter of the impeller (m); and the power law parameters of several test fluids. The flow behavior index of the power law model can be determined as the slope of a plot of the data: log RPM vs. log torque. The consistency index can be determined from the torque (M) and power law parameters of a standard (x) and the test (y) fluids as described in (27).

The method proposed by Rieger and Novak (28) to determine the magnitude of the proportionality constant $k_s$ is relatively simple. However, a small error in determining the magnitude of the slope will result in a large error in the magnitude of $k_s$. In contrast, the method of Metzner and Otto (25) and Wood (31), involves more work but it provides precise values of the constant $k_s$ (32). Mackey et al (33) pointed out that it is not practical to use a single value of the proportionality constant at low rotational speeds or when using impeller to cup ratios significantly less than 1.0. Steffe and Ford (34) evaluated the storage stability of starch-thickened strained apricots with a mixer viscometer.

## Back Extrusion Technique

A back extrusion technique was described (35, 36) that can be used for calculating the power law parameters for a variety of foods, including food suspensions. The technique also can be used for fluids that follow Hershel-Bulkley model. The back extrusion method requires inexpensive cups and plungers (either solid or hollow), but also expensive instruments capable of providing force-distance data. Rheological data presented in the cited studies on food gels and baby foods seems to indicate that the back extrusion technique can provide reliable magnitudes of rheological parameters. In particular, yield stress can be determined by noting that the equilibrium force after the plunger has stopped is the sum of buoyancy force and the restraining force created by the yield stress.

## Effect of Slip on Rheological Behavior

In many PFDs a thin layer of liquid forms at solid boundaries, and this in turn results in slip at the boundaries (22, 38). Wall slip was also observed for polymer solutions in capillary flow (39) and for molten polymers in capillaries made from different materials of construction (40). Yoshimura and Prud'homme (41)

suggested that the expression "apparent slip" be used to describe the phenomenon in foods and other materials because the slip is due to phase separation while in the case of molten polymers and polymer solutions there is no phase separation at the walls. In order to avoid the errors in measurement due to slip or apparent slip in viscometers, mixers were employed in a few studies on food dispersions (27, 32, 34). However, the assumption of an average and uniform shear rate directly proportional to the rotational speed is an approximation and the error due to the approximation is not known.

Qiu and Rao (Qiu, C. G.; and Rao, M. A. J. Texture Stud., submitted) determined slip coefficients and slip velocities for apple sauce in a concentric cylinder viscometer as well as the effect of insoluble solids content on them. Three concentric cylinder units specified in the theory of Mooney (42) were employed. Rotational speeds were determined with the different concentric cylinder systems at the same magnitude of torque. Figure 2 shows, for one sample of apple sauce, the shear rates uncorrected and corrected for slip plotted against the shear stress. The magnitudes of the flow behavior index of the power law model (Equation 2) did not change significantly due to correction for wall slip; however, the magnitudes of the consistency index increased due to wall slip corrections.

It is well known that insoluble solids content of plant food dispersions plays an important role in the rheological behavior of the dispersions (37, 43, 44). For apple sauce samples modified by the addition of pulp, good linear correlation ($R^2 = 0.979$) was found between ln (weight of pulp/weight of total sample) vs. ln $\beta$.

$$\beta = 4.83 \times 10^{-8} \left( \frac{\text{weight of pulp}}{\text{weight of sample}} \right)^{-6.79} \tag{11}$$

That the magnitude of $\beta$. decreased with increase in pulp content is note worthy because it means that plant food dispersions with high solids content will be highly viscous, but will exhibit little wall slip. In contrast, dispersions with relatively low solids content will exhibit significant wall slip. It must be emphasized that Equation 11 is an empirical equation derived for the food samples employed in this study. While it provides important trends about wall slip, the equation cannot be extrapolated beyond the range of variables employed in this study.

## Yield Stress with a Mixer Viscometer

As stated earlier, yield stress is also an important property of many foods (21). In the relaxation method, it

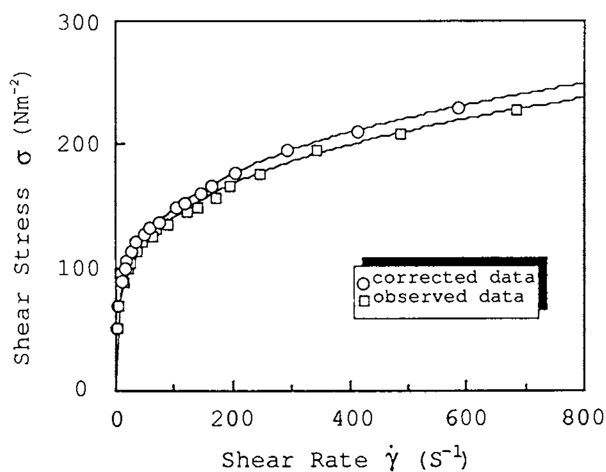


Figure 2. Shear rates corrected and uncorrected for slip against shear stress for apple sauce.

is determined using rotational viscometers by recording at a low rpm the shear stress level at which no stress relaxation occurs on reducing the rpm to zero (11, 45). This method, however, is time consuming and requires great care in order to obtain reliable results. Other experimental methods also have been proposed (46), but for the most part magnitudes of yield stress are determined by extrapolation of shear rate-shear stress data according to several flow models: Casson (Equation 5), Herschel-Bulkley (Equation 4), and Mizrahi-Berk (Equation 6) (47, 48).

Dzuy and Boger (7) employed a mixer viscometer for the measurement of yield stress of a concentrated non-food suspension and called it the "vane method". Yield stress of apple sauce samples was determined by means of the vane method by Qiu and Rao (37) using two vanes. The maximum recorded torque ($T_m$) value, and the diameter ($D_v$) and the height (H) of the vane were used to calculate the yield stress ($\sigma_v$) according to the equation:

$$T_m = \frac{\pi D_v^3}{2}\left(\frac{H}{D_v} + \frac{1}{3}\right)\sigma_v \qquad (12)$$

For the purpose of comparing the magnitudes of yield stress of several food samples determined by the vane method, one must employ not only a specific impeller but also a specific rpm (37).

Comparison of Yield Stresses from Flow Models and Vane Method.

Magnitudes of the yield stresses from the vane method were much higher than those from the flow models; in a few instances the former were more than twice the latter. In an attempt to understand the difference between the two types of yield stresses, we note that in addition to the yield stress ($\sigma_0$) that is obtained by extrapolation of shear rate - shear stress data to zero shear rate, one can obtain a Bingham yield stress ($\sigma_B$) by extrapolation of the straight line portion of the shear rate - shear stress data (49). Magnitudes of $\sigma_B$ determined from plots of shear rate - shear stress were much higher in comparison to the magnitudes of the yield stresses obtained by extrapolation of shear rate - shear stress data to zero shear rates. Further, while they were close, they were still higher than the magnitudes of yield stresses determined by the vane method. Nevertheless, considering the uncertainties involved in obtaining $\sigma_B$ and the range of values that can be obtained with the vane method, it appears that the vane method gives magnitudes that are closer to $\sigma_B$ (37).

Correlation Among the Different Yield Stresses.

Magnitudes of $\sigma_B$ were linearly related with the yield stresses from the vane method at 0.4 rpm ($\sigma_{2v4}$) and those obtained by extrapolation of shear rate - shear stress data according to the M-B model ($\sigma_{0MB}$):

$$\sigma_B = 47.0 + 0.45\ \sigma_{2v4};\ R=0.895 \tag{13}$$

$$\sigma_B = 47.5 + 0.67\ \sigma_{0MB};\ R=0.887 \tag{14}$$

Thus far, recent developments in the measurement of rheological properties were discussed. Next, we consider the rheological properties of important PFDs, such as, tomato concentrates and concentrated orange juice.

Rheological Properties of Tomato Concentrates

The simple power law (Equation 2) described well the shear rate-shear stress data of tomato concentrates (48, 45, 50). Tomato concentrates are very shear-thinning fluids with reported magnitudes of the flow behavior index being in the range of 0.22 to 0.26. The effect of temperature on the apparent viscosity can be described by the Arrhenius model. The magnitude of $E_a$ of twelve out of sixteen concentrates was about 9.63 kJ/mol (48).

Harper and El Sahrigi (50), Rao et al. (48), and Tanglertpaibul and Rao (45) found that the apparent viscosity and concentration were related by a power relationship; the magnitude of the exponent ranged between 2.0 and 2.5. This general relationship is important because it can be used to estimate the viscosity of tomato concentrates at one concentration from the known value at another concentration.

Effect of Finisher Screen Size

Tanglertpaibul and Rao (45) found that in the range of finisher screen openings (FSO) 0.508 to 1.143 mm, smaller FSO yielded lower apparent viscosities evaluated at a shear rate of 100 $sec^{-1}$ ($\eta_{100}$). However, concentrates from 0.686 mm screen had the highest apparent viscosity. Similar results were found for tomato juice also. This phenomena can be explained in that small screens reduced the size of solid particles and at the same time remove some of the large particles. The net result is that one obtains finished products with narrow particle size distribution and a small amount of large particles. Jinescu (51) suggested that small suspended particles may give high viscosity due to their greater surface area.

Large particles also contribute to high viscosity. Therefore, very small screens can affect the gross viscosity of tomato concentrates in two opposite manners: (1) increase gross viscosity due to large surface area of small particles, and (2) reduce gross viscosity due to the exclusion of large solid particles. A screen size of 0.686 mm might have resulted in tomato juice and concentrates with appropriate particle sizes that yielded the highest viscosity.

Effect of Methods of Concentration.

The effect of three different methods of concentration: juice evaporation (JE), serum evaporation (SE), and reverse osmosis concentration of serum (SRO) on $\eta_{100}$ of tomato concentrates was studied by Tanglertpaibul and Rao (45). At low concentrations of total solids, apparent viscosities of SRO and SE concentrates were not significantly different. However, at high concentrations apparent viscosities of SE concentrates were less than those of SRO concentrates. In addition, both SRO and SE concentrates showed higher apparent viscosities than those of JE concentrates at the same concentrations. It appears that concentrating tomato serum by means of either evaporation or reverse osmosis does not have significant effect on apparent viscosity of reconstituted concentrates with unheated pulp. When heat is applied to whole tomato juice, both serum and pulp are subjected to heat; consequently, the structure of pulp may be affected by heat, particle sizes and/or volume of the pulp may be reduced during the heat treatment. Further, because tomato juice has a lower heat transfer coefficient than the serum (52), concentration of tomato juice to the same °Brix requires longer heating time than tomato serum.

When tomato concentrates are diluted to lower concentration, the diluted products have lower viscosity than if they are concentrated straight from the juice. Heat alters the structure of pectic substances by means of hydrolysis. Colloidal properties of serum may be altered by heat resulting in lower apparent viscosity of reconstituted tomato concentrates with unheated pulp. Caradec and Nelson (53) also reported that viscosity of tomato juice serum decreased with heat treatment.

Marsh et al. (54) found that pulp lost bound water as a result of the physical forces that developed as concentration progressed and the loss altered the ability to influence consistency. Labuza (55) suggested that the apparent loss of consistency was most likely due to the failure of the macromolecular polymeric substances, comprising of the water insoluble solids, to adsorb to their maximum extent. In addition, cell wall materials became less rigid and smaller in size when heat was applied, and colloidal properties of pectins were changed.

Yield Stress of Tomato Concentrates.

Yield stresses of tomato concentrates from the juice evaporation process using four FSO were determined (45) by the stress relaxation method (18) over the concentration range of 9 to 14% T.S. The magnitudes of yield stress increased not only with total solids content but also with the FSO. The use of larger finisher screens resulted in concentrates with higher yield stress.

Yield stresses can also be obtained by extrapolation of shear rate-shear stress data to zero shear rate according to one of several flow models. The application of several models was studied by Rao et al. (48) and Rao and Cooley (47). The logarithm of the yield stresses predicted by each model and the total solids (TS) of the concentrates were related by quadratic equations. The equations for the yield stresses predicted by the Herschel-Bulkley model (Equation 4) which described very well the flow data of Nova and New Yorker tomato cultivars were:

Nova cultivar,

$$\ln \sigma_{0H} = -0.6443 + 0.3652\ (TS) - 0.00645\ (TS)^2 \quad (15)$$

New Yorker cultivar,

$$\ln \sigma_{0H} = -0.1642 + 0.2219\ (TS) - 0.00248\ (TS)^2 \quad (16)$$

Rheological Properties of Concentrated Orange Juice

Orange juice is by far the leading juice consumed in the U.S.A. The juice consumed in the U.S.A. contains more than about 10% pulp measured volumetrically by centrifuging a 12 °Brix sample at 360g for ten minutes. Concentrated orange juice (COJ) containing lower amount of pulp (1-8%) is also produced in large quantities. Several studies have been conducted on COJ (56, 57, 58, 59, 60, 11) with viscometers capable of yielding shear stress and shear rate data so that the data can be used for engineering design and for comparison with other studies.

COJ of 65 °Brix is a mildly shear-thinning fluid (60) with magnitudes of flow behavior index of the power law model (n) (Equation 2) of about 0.75 that is mildly temperature dependent. In contrast, the consistency index (K) is very sensitive to temperature; for example, Vitali and Rao (60) found for a COJ sample magnitudes of 1.51 Pa sec$^n$ at 20 °C and 27.63 Pa sec$^n$ at -19 °C. Mizrahi and Firstenberg (58) found that the modified Casson model (Equation 5) described the shear rate-shear stress data better than the Herschel-Bulkley model (Equation 4).

Effect of Temperature on Apparent Viscosity

The Arrhenius model (Equation 7) described passably the

effect of temperature on the apparent viscosity at 100 $sec^{-1}$ ($\eta_{100}$) of COJ and its serum. The experimental data and the predictions of the model deviated at the low and high ends of the temperature range. Magnitudes of $E_a$ of COJ of 65 $^{o}$Brix over the temperature range -19 to 29 $^{o}$C were about 44.8 kJ/mol and those for the serum were about 53.6 kJ/mol (11, 60). The high magnitude of $E_a$ reflects the large changes in viscosity due to small changes in temperature that is characteristic of all concentrated juices (10). As the concentration of sugar in COJ was decreased from 65 $^{o}$Brix to 52 $^{o}$Brix, the magnitude of $E_a$ decreased from 44.8 to 31.4 kJ/mol. Because the Arrhenius model did not describe very well the experimental data of temperature - apparent viscosity of COJ, the Williams-Landel-Ferry and the Fulcher models were employed (14). However, the latter models are not as simple in form as the Arrhenius model and they contain one or more additional constants. The magnitude of $E_a$ was influenced by the pulp content also; for 65 $^{o}$Brix COJ samples, their magnitudes with zero and 11.1% pulp were 51.5 and 42.7 kJ/mol, respectively (60).

Effect of Concentration ($^{o}$Brix) on Apparent Viscosity

Exponential relationships described the relationship between the sugar concentration ($^{o}$Brix) and apparent viscosity ($\eta_{100}$). The slopes of such plots for COJ samples containing 50 to 65 $^{o}$Brix ranged from 0.187 at 9.9 $^{o}$C to 0.127 at 30 $^{o}$C. Mathematical expressions containing temperature and concentration were also developed by Vitali and Rao (60).

Effect of Pulp Content on Apparent Viscosity.

Vitali and Rao (11) found exponential relationships between pulp content and apparent viscosity of 65 $^{o}$Brix COJ samples at a constant temperature, but the slopes of the straight lines for data at different temperatures were not constant. For example, the slope of the plot of pulp content (%) against ln ($\eta_{a,100}$) at 18 6 $^{o}$C was 0.084 and at 29.4 $^{o}$C it was 0.144. The role of pulp content in rheological properties of COJ was discussed in Vitali and Rao (11) and Vitali et al. (14).

Rheological Properties of Apple Sauce and Apricot Puree

In addition to tomato concentrates and COJ, significant studies have been conducted on the factors affecting the rheological properties of apple sauce and apricot puree. Rao et al. (61) found that the consistency index (K) of

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apple sauce samples was affected by the apple cultivar, apple firmness, and the finisher screen opening. The magnitude of sauce consistency was strongly influenced by the amount of pulp and this point will be discussed in a later section. Duran and Costell (62) interpreted the rheological properties of apricot puree in terms of observations on flocculated suspensions that will be discussed later.

## Role of Dispersed and Continuous Phases on Rheological Behavior

Michaels and Bolger (49) suggested that the basic flow units of flocculated suspensions are flocs made up of small clusters of particles and the water enclosed. At very low shear rates, the flocs group into clusters or aggregates that may form network extending to the wall of the container and giving the suspension a finite yield stress. The clusters are large but comparatively weak structures. At high shear rates, the aggregates are broken down into individual flocs and the model predicts a straight line relationship between shear stress and shear rate.

The total energy dissipation rate, $E_{tot}$ (J/ml) is the sum of a network energy term ($E_N$), a creep energy term ($E_{cr}$) to break the structural bonds, and a viscous energy term ($E_v$) to overcome the purely viscous drag. The corresponding shear stresses can be denoted as $\sigma$, $\sigma_N$, $\sigma_{cr}$, and $\sigma_v$, respectively. Therefore, the stress ($\sigma$) necessary to produce deformation at a constant shear rate is divided into three parts:

$$\sigma = \sigma_N + \sigma_{cr} + \sigma_v \qquad (17)$$

At zero shear rate, the network yield stress $\sigma_N$ equals $\sigma_0$ the yield stress obtained by extrapolation of the shear rate-shear stress data to zero shear rate. The Bingham yield stress ($\sigma_B$) can be obtained by extrapolation of only the linear portion of the shear rate-shear stress diagram to zero shear rate.

The principal contribution of the study of Michaels and Bolger (49) was that for $\sigma_0$, $\sigma_B$, and $\eta_{\infty}$, the role of particle concentration was identified.

Metz et al (63) suggested that the morphology of a suspension must be taken into consideration and that $\sigma_N$ is proportional to the square of the concentration of the aggregates, where as $\sigma_{cr}$ and $\sigma_v$ are proportional to the square of the concentration of the particles. However, it would be necessary to know the magnitude of the interaggregate binding force to calculate $\sigma_N$ and the work required to break a bond to calculate $\sigma_{cr}$ and $\sigma_v$.

The continuous medium of many food dispersions, usually called serum, is an aqueous solution of sugars, organic acids, salts, and pectic substances whose rheological behavior is either Newtonian (61, 64) or mildly shear-thinning (11) depending on its composition. The exact chemical composition of the continuous medium depends on the particular commodity, the cultivar, and other factors such as the extent of ripening. The amount and size distribution of the insoluble matter in the dispersions depend to a large extent on the size of the screen employed in the finishing operation during their manufacture (45) and to a limited extent on the speed of the finisher (61). It is clear that food dispersions are complex materials whose characteristics with respect to the nature of the insoluble solids as well as those of the fluid media are determined a priori to experimentation.

The continuous and dispersed media were separated by centrifugation and their characteristics were studied for orange juice (11, 57, 58), apple sauce (61), and tomato concentrates (44). The continuous media of apple sauce (about 16 °Brix) and tomato concentrates having sugar concentrations (6 - 25 °Brix) were Newtonian fluids. In contrast, the serum of concentrated orange juice (COJ) with concentration of about 65 °Brix was a shear-thinning fluids.

In non-food suspensions the volume fraction of solids, $\Phi$, plays an important role (51, 65). Because of the compressible nature of the solids in PFDs, the magnitude of $\Phi$ is difficult to determine as it depends on the centrifugal force employed in the separation of the phases. In the case of COJ, the amount of dispersed solid matter in a 12 °Brix sample is determined at 360 x g so that the pulp content in different samples is determined at a standard centrifugal force and a standard concentration that sets the continuous medium's viscosity (66). This method of determining the pulp content was also found to be suitable for apple sauce (61). The pulp content of COJ, as determined by the standard method, ranges from about 2% to 16%. In contrast, limited data on apple sauce (54 samples) indicate that their pulp content varies between 35% and 50%. Because of the highly viscous nature of tomato concentrates and the large magnitude of pulp content, the technique used for COJ is not suitable so that higher centrifugal forces and ratios such as pulp : serum (44) and pulp : total sample weight have been employed (67).

Because no general theories exist even for concentrated non-food suspensions of well defined

spherical particles (68), approaches to studying the influence of the viscosity of the continuous medium and the pulp content have been empirical. One model that was proposed for relating the apparent viscosity of food suspensions ($\eta_{a,fs}$) was (43):

$$\eta_{a,fs} = \eta_{a,serum} + A\ (\text{Pulp})^{B} \qquad (18)$$

The model predicts the viscosity of the serum when the pulp content is zero. The coefficient A indicates the contribution of a unit amount of pulp content and the coefficient B is the slope of a plot of log pulp vs. log specific viscosity of the dispersion and it reflects the relative intensity of pulp content in different food suspensions or the effect of different processes and/or cultivars that could influence the pulp content. The magnitudes of B for the tomato concentrates, COJ, and apple sauce varied over the limited range of 1.26 - 1.49, suggesting that there was not much difference in the influence of pulp content. For tomato concentrates and apple sauce, the contribution of the dispersed solids was much more than that of the continuous medium (43).

The viscosities of the continuous media can be expected to depend on their composition, viz., sugar and pectin content. Correlations in terms of the relative viscosity, $\eta_r$, can be used. However, the viscosity of the dilute aqueous solutions of organic acids and salts is generally not known. For relatively low magnitudes and narrow ranges of sugar concentrations such as the case of the continuous media of apple sauce and tomato concentrates, good correlations were obtained with just the pectin content as the independent variable (61, 44):

$$\eta_{serum} = A_1 + B_1\ (\text{pectin content})^{C_1} \qquad (19)$$

where, $A_1$, $B_1$, and $C_1$ are constants that need to be determined from experimental data.

Intrinsic Viscosity of Tomato Serum.

Because the continuous phase or serum influences the rheological properties of suspensions, its characteristics, such as the intrinsic viscosity, are of interest. The magnitude of intrinsic viscosity can be determined by extrapolation techniques, such as with Huggins's equation (Equation 20), and from the slopes of straight lines of quantities based on relative viscosity (Equation 21) (69).

$$\frac{\eta_{sp}}{C} = [\eta] + k' [\eta]^2 C \qquad (20)$$

$$\eta_r = 1 + [\eta] C \qquad (21)$$

where, C is concentration (g/dl), $\eta_{sp} = (\eta_r - 1)$, $\eta_r = (\eta$ of solution/$\eta$ of solvent), $[\eta]$ is intrinsic viscosity, and k' is Huggins's constant. The magnitude of intrinsic viscosity varies slightly with the method of determination. It also depends very strongly on the extent of heat treatment, such as during concentration, of the food suspension (69). The latter results are consistent with those of other studies in which the viscosity of serum samples were shown to be affected by heat treatment (53).

Influence of Pulp Content on Yield Stress of Apple Sauce.

Michaels and Bolger (49) predicted that $\sigma_B$ would be proportional to the square of the volume fraction of solids. Qiu and Rao (37) verified the prediction of Michaels-Bolger model with data on pulp adjusted apple sauce samples, where pulp content was expressed as: weight of pulp/total weight of sample. Because of the high correlation coefficient for these data (R=0.999), it appears that pulp content expressed as: weight of pulp/total weight of sample is a good indicator of the insoluble solids content of concentrated suspensions.

Correlation of Yield Stress with Pulp Content and Particle Size.

One can expect the magnitudes of yield stress to be dependent on both the pulp content and on the particle size.Qiu and Rao (37), determined correlations between yield stress determined by the vane method ($\sigma_{2v4}$) on one hand and the pulp content (PC1) and an average particle diameter (WTD) on the other, where PC1 was determined as volume percent of pulp after centrifugation at 360g for 10 minites and WTD was the weight average particle size determined from wet sieving data. One correlation form tested was:

$$\sigma_{2v4} = 11.5\ (PC1)^{1.38} + 43.0\ (WTD)^{-0.16};\ R^2 = 0.823 \qquad (22)$$

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The magnitude of $R^2$ for the correlation was 0.823. The exponent of the particle diameter was negative, indicating that the magnitude of $\sigma_{2v4}$ increased with decrease in particle diameter over the range of experimental variables employed in this study; it also increased with pulp content. It is emphasized that the effects of pulp content and particle size are valid within the range of the experimental variables employed in the study

Engineering Data on Flow of Plant Food Dispersions.

Because of their commercial importance, data related to the handling of foods are very useful. For information related to the practice of chemical engineering, such as pressure drop in pipe flow, the reader is referred to studies by Metzner (70) and others (3, 71). Here studies conducted specifically with foods are considered.

Dervisoglu and Kokini (72) found that the wall stresses for apple sauce, mustard, tomato ketchup, and tomato paste in galvanized steel tubes were much higher than for polyvinylchloride (PVC), teflon, and glass; in order they were: glass < teflon < PVC < galvanized steel. Kokini et al. (73) showed that lubricated flow conditions induced as a result of injecting solutions of sodium carboxymethylcellulose (CMC) reduced friction in the flow of tomato pastes.The volumetric flow rate, Q, under lubricated flow conditions in a tube of radius, R, could be described by the relationship:

$$Q = \frac{3n+1}{R^n}\left(\frac{\Delta P}{2KL}\right)^{1/n} \pi\,(1-r^{0.5}) \qquad (23)$$

where, $r = \left(\frac{R_p^2}{R^2}\right)$, $R_p$ is the radius of suspension plug.

The agreement between Equation (23) and experimental data was good with a correlation coefficient R = 0.98 for inclined plane flow and 0.8 for pipe flow.

Wall effects in capillary flow of suspensions such as apple sauce occur as a result of velocity gradient near the wall that in turn causes the suspended particles to move away from the wall region. The net result is slip of the fluid at the wall (29, 74). The correct shear rate can be calculated from flow rates corrected for slip. The procedure, due to Mooney (42), requires the use of several capillaries of different different length to diameter ratios has been applied to food suspensions by Higgs (38) and Kokini and Plutchok (22) to show that slip effects are significant. These results also suggest caution in using

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capillary viscometers for determining the flow properties of food suspensions.

## Conclusions

Determination of rheological properties of PFDs offers many challenges. The vane method provided reliable magnitudes of yield stresses for apple sauce; its applicability to other PFDs needs to be tested. Slip affected the flow properties of apple sauce. One can expect the effect of slip to be important in the case of other PFDs; therefore, quantitative studies are needed with other PFDs also.

The extent of heat treatment and particle size, as influenced by finisher screens, were shown to affect the rheological properties of tomato concentrates. The apparent viscosity ($\eta_{100}$) of 65 $^{o}$Brix concentrated orange juice was strongly dependent on temperature. The apparent viscosity ($\eta_{100}$) of tomato concentrates made from several cultivars increased in proportion to total solids concentration raised to a power of 2.0 to 2.5. The pulp content plays a major role in the rheological behavior of PFDs and the relative viscosity of apple sauce, tomato concentrates, and concentrated orange juice increased in proportion to pulp content raised to a power of 1.26 to 1.49. More experimental studies are needed with respect to fundamental rheological properties and on the role of composition of the PFDs on the magnitudes of the properties.

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Chapter 14

# Quality Maintenance in Fresh Fruits and Vegetables by Controlled Atmospheres

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Postharvest quality maintenance in fresh fruits and vegetables is greatly influenced by the temperature, relative humidity, and atmospheric composition (oxygen, carbon dioxide, and ethylene concentrations) of their environment. The optimum controlled atmosphere (CA) conditions for maintaining quality between harvest and consumption vary among commodities and cultivars. CA conditions retard loss of chlorophyll (green color), biosynthesis of carotenoids (yellow and orange colors) and anthocyanins (red and blue colors), and biosynthesis and oxidation of phenolic compounds (brown color). Textural quality is affected since the activity of polygalacturonase and solubilization of pectins that cause fruit softening and lignification leading to toughening of vegetables are delayed under CA conditioning. CA also influences flavor quality by reducing loss of acidity, starch to sugar conversion, interconversions of sugars, and biosynthesis of flavor volatiles. Retention of ascorbic acid and other vitamins result in better nutritional quality to fruits and vegetables kept under CA conditions.

The term quality used in reference to fresh fruits and vegetables may refer to market quality, shipping quality, edible quality, desert quality, nutritional quality, internal quality, or appearance quality (1). Different aspects of quality are of primary importance to producers, shippers, sellers and consumers of fresh fruits and vegetables. The rate of postharvest deterioration of flavor and nutritional quality is generally faster than that of appearance and texture quality of fresh produce.

Maintaining the quality of any fresh horticultural commodity begins with producing and selecting a good quality product. Subsequent handling to minimize physical injury, insure proper sanitation, and maintain proper temperature and humidity will contribute to the postharvest maintenance of quality. Once these

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requirements have been fulfilled, additional postharvest life may be achieved through modification of the atmosphere surrounding the product. Reducing the oxygen ($O_2$) concentration and/or increasing the carbon dioxide ($CO_2$) concentration can reduce the commodity's respiration and sensitivity to ethylene ($C_2H_4$), thereby maintaining its freshness and quality longer than might otherwise be possible. However, if any environmental parameter varies outside the tolerance limit of the product, quality will rapidly decline.

Several aspects of the quality of fresh fruits and vegetables have been recently reviewed (1-8).

Controlled atmosphere (CA) or modified atmosphere (MA) storage refers to reduction of $O_2$ and/or elevation of $CO_2$ to levels different from those in air. The addition of carbon monoxide (CO) or removal of $C_2H_4$ may also be involved. CA implies a greater degree of precision in maintaining specified levels of $O_2$ and $CO_2$ than MA.

The beneficial effects of CA in prolonging the postharvest life of apples have been recognized for more than 60 years (9). During that time, a large body of research literature has accumulated, most aimed at identifying optimum CA conditions for various commodities and cultivars. This paper presents an overview of the relatively small portion of this literature that addresses the mechanisms by which CA affects the metabolism of plant tissues and the specific effects of CA on fruit and vegetable quality.

## Effects of CA on Postharvest Physiology

Reducing the $O_2$ concentration around fresh fruits and vegetables reduces their respiration rate to an extent that varies with temperature, commodity, cultivar, and physiological age at harvest. Below a minimum of about 1 to 2% $O_2$, a shift from aerobic to anaerobic respiration occurs with a concomitant increase in $CO_2$ production (10). The reduction in aerobic metabolism associated with reduced $O_2$ levels is apparently not mediated by cytochrome oxidase, which has a $K_m$ value of $10^{-8}$ to $10^{-7}$ M $O_2$ (11,12). It is more likely that reduction of aerobic respiration results from diminished activity of other oxidases such as ascorbic acid oxidase, polyphenol oxidase (PPO), and glycolic acid oxidase, whose affinities for $O_2$ are 5 to 6 times lower than that of cytochrome oxidase (12).

Elevating the $CO_2$ concentration can also have a suppressive effect on respiratory metabolism, depending upon temperature, commodity and cultivar. Levels of $CO_2$ above the limits of tolerance of the particular commodity can also result in accumulation of acetaldehyde and ethanol within the tissues indicating a shift to anaerobic respiration (13). Elevated $CO_2$ atmospheres have been shown to result in accumulation of succinic acid due to inhibition of succinic dehydrogenase activity, and reduced formation of citrate/isocitrate and $\alpha$-ketoglutarate in apples (14-16), pears (17), and lettuce (18). $CO_2$ levels above 6% stimulated oxidation of malate but suppressed oxidation of citrate, $\alpha$-ketoglutarate, succinate, fumarate, and pyruvate in mitochondria isolated from apples (19). Ultrastructural changes in 'Bartlett' pear similar to those associated with senescence, such as fragmentation, reduction in size, and changes in shape of mitochondria, were caused by elevated $CO_2$ (20). Kerbel et al. (21) found that 10% $CO_2$ was associated with declines in protein

content and fructose 1,6-bis-phosphate and in the activities of ATP:phosphofructokinase and PPi:phosphofructokinase while levels of fructose 6-phosphate and fructose 2,6-bis-phosphate increased. They concluded that the inhibitory effect of elevated $CO_2$ on both phosphofructokinases in the glycolytic pathway could account, at least in part, for the observed reduction in respiration.

Respiration is not the only biological activity of plant tissues that requires $O_2$. Furthermore, since senescence can continue without an increase in respiration, low $O_2$ must exert its effect on processes other than respiration (22). Oxygen concentrations below about 8% can decrease $C_2H_4$ production and sensitivity to $C_2H_4$ of fresh fruits and vegetables (13). The decreased production of $C_2H_4$ may be due to inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to $C_2H_4$ which requires $O_2$ (23). $CO_2$ inhibits autocatalytic production of $C_2H_4$ in climacteric fruits, such as avocado, apple, and tomato. Injurious levels of $CO_2$ can, however, result in increased production of $C_2H_4$ (13). Many fresh fruits and vegetables respond to $C_2H_4$ with accelerated softening, increased abscission and induced physiological disorders, all of which have a direct effect on quality (24).

It is clear that CA profoundly influences the metabolism of plant tissues in several different ways. These effects are reflected, either favorably or unfavorably, in the quality of fruits or vegetables. The extent to which CA is beneficial depends upon the commodity, cultivar, maturity stage, initial quality, concentrations of $O_2$, $CO_2$, and $C_2H_4$, temperature, and duration of exposure to those conditions.

## Effects of CA on Postharvest Quality.

### Appearance Quality

Chlorophyll. Elevated $CO_2$ and/or low $O_2$ levels reduce chlorophyll loss in many fruits and vegetables (7,25). How this occurs is less certain. The disappearance of green color in processed and stored green vegetables is due to the replacement of magnesium in chlorophyll by hydrogen to form colorless pheophytin (26-28). High cellular pH caused by elevated $CO_2$ may reduce the breakdown of chlorophyll to pheophytin. Lowered sensitivity of plant tissues to $C_2H_4$ in the presence of elevated $CO_2$ and/or reduced $O_2$ is presumably partly responsible for the reduced chlorophyll breakdown. Wang and Ji (29) found that the retardation of senescence of Chinese cabbage by storage in a 1% $O_2$ atmosphere was associated with preventing increases in abscisic acid and ACC levels.

Carotenoids. Carotenoids are fat-soluble pigments comprised of isoprene units and, in plants, are generally associated with membranes (30). Carotenoids are generally classified as either carotenes, which are structurally related to hydrocarbons, or xanthophylls which are hydroxy, epoxy and oxy derivatives and are frequently esterified. The carotenoids most important in imparting color to fruits and vegetables are derivatives of $\alpha$- and ß-carotenes and lycopene. Carotenoids vary in their stability but, due to their unsaturated nature, they are generally susceptible to oxidation. Carotenes are important to

nutrition, flavor and appearance as precursors of vitamin A, precursors of some flavor volatiles and as pigments (6).

Low $O_2$ generally delays or inhibits the synthesis of lycopene, ß-carotene, and xanthophylls in tomato fruit (31,32). In sweet pepper, high $CO_2$ delayed development of red color equally whether combined with 21% or 3% $O_2$ (33). $C_2H_4$ is known to accelerate the biosynthesis of carotenoids (34).

Lipoxygenase appears to catalyze the direct oxidation of certain unsaturated fatty acids with the concurrent bleaching of carotenoids (30). Carotenoids are also sensitive to nonenzymatic oxidation with concurrent loss of color. Carotenoids can lose their color through bleaching after loss of moisture in the presence of $O_2$ (35,36). The low $O_2$, high moisture conditions common in CA may ameliorate some of these changes.

Anthocyanins. Anthocyanins are flavonoid, phenolic-based, water-soluble compounds located in the cell vacuole of the epidermal cells of many fruits and vegetables (37). They confer the characteristic red and blue colors of many flowers and fruits and as such are important components of visual quality. The anthocyanins are pH indicators and are red at low pH, colorless at intermediate pH and blue at higher pH. The blue colors at high pH are the result of co-pigmentation and metal chelation rather than a direct effect of pH. This can result in discoloration of fruits packed in metal containers or handled with metal implements (38).

Anthocyanins accumulate relatively late in the maturation process of fruits. CA conditions, which delay maturation, can thus be expected to delay the development of anthocyanin pigments. This may be mediated through reduced sensitivity to $C_2H_4$, which has been shown to accelerate biosynthesis of anthocyanins (39,41). CA conditions can also alter cellular pH in plant tissues (42) and consequently induce changes in anthocyanins.

$O_2$ and high temperature were found to be the most important factors in the degradation of anthocyanins in blueberry, cherry, currant, grape, raspberry and strawberry juices (43). Polyphenol oxidases can degrade anthocyanins in the presence of other phenolic substrates such as catechol or chlorogenic acid (44,45). Because polyphenol oxidase is estimated to have a $K_m$ many times higher than that of cytochrome oxidase, $O_2$ may become insufficient for its activity at ambient concentrations as high as 2% $O_2$. As this level of $O_2$ is common in CA, preservation of anthocyanins ought to be possible.

Oxidation of Phenolic Compounds. Phenolic compounds are widespread throughout the plant kingdom and are prevalent in fruits where they are important contributors to color and flavor (46). Phenolic compounds, particularly flavonoids and derivatives of chlorogenic acid, play a crucial role in the development of a number of postharvest disorders through their oxidation to brown compounds that discolor many fruits and vegetables and substantially reduce their quality. A number of enzymes catalyze the biosynthesis or oxidation of phenolic compounds, among them phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), cinnamic acid-4-hydroxylase (CA4H), polyphenol oxidase (PPO), and catechol oxidase (CAO). The chemistry

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of phenol oxidases in fruits and vegetables has been recently reviewed (47,48). The activities of these enzymes, and thus their mediation of desirable or undesirable changes are affected by CA. Storage of mushrooms in 0% $O_2$ inhibited PPO activity and browning (49). At higher $O_2$ concentrations, browning occurred. $CO_2$ can influence phenolic metabolism at a number of steps (42). $C_2H_4$-induced browning in pea seedlings was inhibited by $CO_2$ concentrations above 5% (50). $CO_2$ (20%) inhibited browning of mechanically damaged snap beans and reduced activity of PPO (51). Conversely, 15% $CO_2$ caused brown stain to develop in lettuce, but symptoms developed only after the removal of the $CO_2$ (52). Elevated $CO_2$ (15%) was associated with an increase in PAL activity in lettuce but the authors believed that this was a stress reaction and not the primary cause of browning (52). They speculated that the prevention of symptom development by the presence of $CO_2$ was related to reduced PPO activity, since $CO_2$ has been shown to be a competitive inhibitor of PPO (49). $CO_2$ suppresses the production of phenolic compounds, but $CO_2$ injury (brown stain) scores did not correlate well with total phenolic content in lettuce (52).

## Texture Quality

Fruit Softening. The softening of plant tissues is usually accompanied by the breakdown and solubilization of pectic materials and by catabolism of cell wall polysaccharides (5). Polygalacturonases (PG) specifically hydrolyze the ß-D-(1,4) linkages between galacturonic residues. Such residues constitute a major structural portion of the middle lamella and their hydrolysis is thought to be largely responsible for fruit softening during maturation and storage (53). The effect of CA storage on firmness has been evaluated for many fruits and vegetables and appears to vary with the commodity (7). Generally, any treatment that retards ripening, such as CA does, retards softening of fruits (54). A 24-hour exposure of snap beans to 40% $CO_2$ reduced titratable acidity and increased pH, apparently due largely to a reduction in malic acid (55). Degradation of pectic substances in the middle lamella increased with increasing pH, which may account for increased softening of snap beans associated with high $CO_2$ (though not with 2.5% $O_2$) exposure (55). The water-soluble polyuronides (WSP) in pears stored in air or in 1% $O_2$ increased substantially during 6 months of storage at 1°C. After removal to air at 20°C, pears previously stored at 1°C showed a decrease in WSP while those stored in 1% $O_2$ showed an increase in WSP (56). Development of a juicy, buttery texture in stored pears is thought to result from an increase in soluble pectic substances in the pulp (57). Anoxic conditions arrested softening, the increase in soluble pectin and the decline in total pectin in Conference pears, perhaps due to an inhibition of the synthesis of wall-degrading enzymes (58). Pectin solubility in high-bush blueberries increased during storage in air, but was unchanged during storage in high $CO_2$ (59). Bananas kept in 2.5% $O_2$ exhibited a larger increase in pectinmethylesterase activity compared to air (22). In the case of avocados, PG activity decreased 40-50% in 2.5% $O_2$, compared to air (60). The appearance of PG was prevented by storing tomatoes in 5% $O_2$ + 5% $CO_2$, but on removal to air, PG was synthesized (61). CA (2%

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$O_2$ + 5% $CO_2$) storage of kiwifruit at 0°C retarded softening compared to storage in air. In addition, the CA-stored fruit maintained an increased level of water insoluble uronic acids and the associated neutral sugars galactose, arabinose and rhamnose (62). There was no clear relationship between ß-galactosidase activity and softening rates of air or CA-stored McIntosh apples (63).

Toughness of Vegetative Tissue. Toughening of vegetative tissue during storage appears to be associated with phenolic metabolism and lignification (30). During the key steps of phenolic metabolism, phenylalanine is deaminated by PAL to cinnamic acid. Cinnamic acid can then be hydroxylated into various phenolic compounds. In the presence of $O_2$, PPO can oxidize these phenolic compounds into quinones which can polymerize into a brown pigment (52). Alternatively, cinnamic acid can be hydroxylated by cinnamic acid-4-hydroxylase to coumaric acid and on to lignin via the shikimic acid pathway (30). Exposure to elevated $CO_2$ levels can stimulate an increase in PAL, PPO and peroxidase (POD) activities resulting in increased lignification (cell wall thickening) and the accumulation and oxidation of soluble phenolic compounds leading to tissue browning (64). Low $O_2$ combined with high $CO_2$ partially reduced $CO_2$-induced PAL activity and soluble phenolic activity and reduced or retarded brown stain development in iceberg lettuce (65). Lignification is associated with increased PAL and PPO activity in asparagus and can be retarded in 7% $CO_2$ (66). Holding asparagus spears at 2°C in 15% $O_2$ + 15% $CO_2$ retarded lignification and toughening (67,68). High $CO_2$ (10%) reduced toughening of broccoli (69).

## Flavor Quality

Taste. Taste is the human perception of chemicals in the mouth due to their interaction with receptors on the tongue. Taste consists of four dimensions: sweet, salty, sour and bitter. Taste is affected by odor and texture, which makes it a complicated, subjective quality attribute, difficult to measure objectively (70). In fruits and vegetables, taste is mostly determined by the types and amounts of carbohydrates, organic acids, amino acids, lipids and phenolics (5,71). CA combinations, to the degree that they modify changes in these constituents, can affect the taste of stored fruits and vegetables. Usually, extremely low $O_2$ or high $CO_2$ will result in off-flavors and reduced quality due to anaerobic respiration. The specific effect of CA on flavor depends on the crop involved (7).

Both reduced $O_2$ and elevated $CO_2$ can slow starch-to-sugar conversion, thus inhibiting the development of sweet flavors. In bananas, phosphatase activity was reduced and sucrose, fructose and glucose accumulated more slowly when kept in 2.5% $O_2$ relative to those held in air (72). Storage of potatoes in 2.5% $O_2$ inhibited increases in fructose and glucose and reduced the content of sucrose (73). Low $O_2$ apparently inhibits the conversion of starch to sugars in potatoes (74). Keeping 'Granex' onions at 1°C in 3% $O_2$ + 5% $CO_2$ maintained a higher sugar content and a lower pungency than those kept in air (75) In sweet corn, loss of sucrose was retarded by 2% $O_2$, but elevated $CO_2$ (15% or 25%) nullified this benefit (76). Moderate $CO_2$ levels (up to 5% for brussels sprouts and 7.5% for horseradish) inhibit the

metabolism of sugars. Elevated $CO_2$, above optimal levels, has been associated with increased sucrose or glucose decomposition in horseradish (3), brussels sprouts (77), and carrots (78). High $CO_2$ concentrations stimulate respiration in many root crops, perhaps due to injury (79), and this elevated respiration may deplete carbohydrates.

Organic acids and their salts, esters, and glycosides are important both as sources of respiratory energy in the plant cell, and as flavor and aroma compounds, particularly in fruits. Malic acid is a major flavor component in apple, cherry, plum, apricot, broccoli and celery and citric acid is predominant in strawberry and raspberry. Both malic and citric acid are found in tomato and pear and malic and tartaric acid are found in grape. In most fruits organic acids are lost during ripening after harvest (7). Both reduced $O_2$ and elevated $CO_2$ can slow the loss of many organic acids, perhaps through interference with the conversion of pyruvate to citrate or through suppression of succinic acid dehydrogenase and the tricarboxylic acid cycle and associated accumulation of succinic acid (7,13,17,80). Different organic acids can be affected differentially by CA (7). Storage of mature-green tomato fruit in 5% $O_2$ + 5% $CO_2$ did not influence malic and citric acids (61). The metabolism of radiolabeled citrate in mangoes was suppressed by high $CO_2$ more than the metabolism of succinate, aspartate, acetate or malate, although all organic acids accumulated compared to controls (81). CA retarded loss of organic acids in stored McIntosh apples. As titratable acidity level is considered a prime quality determinant in apple juice, the authors considered that CA significantly preserved apple quality (82). CA storage of lettuce, spinach and broccoli resulted in lower levels of organic acids, compared to air storage (7).

Although most fruits and vegetables have relatively small amounts of proteins and amino acids, these constituents may play an important role as synergists and primary flavor components, as well as influence oxidation of ascorbic acid and of other flavor compounds (7). Peroxidase has been associated with "off-flavors" in raw vegetables (83). Several amino acids are considered to have unpleasant tastes (84) but glutamic acid is widely used as a flavor potentiator (85). Others are rated as sweet, salty or bitter (86). However, differences in amino acid concentrations during tomato ripening do not appear to be directly related to flavor differences (87). Synthesis of proteins is less in fruits stored in CA than in air (7). In pears (88), cauliflower (89), and lettuce (90) stored in CA this is reflected in a slowed reduction in free amino acids. However, in cherry (91), chestnut (92), and shiitake mushrooms (93), free amino acids were not retained during CA storage. Goodenough et al. (61) found no change during CA storage in the pattern of several respiratory enzymes, including alcohol dehydrogenase, malic enzyme, isocitrate dehydrogenase, phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, and NADH dehydrogenase. He suggested that CA did not interfere with changes requiring enzyme regulation but that it did interfere with de novo enzyme synthesis.

CA can prevent $C_2H_4$ induced formation of bitter isocoumarins in carrots (94) and can aid in removal of tanins and resulting astringency from persimmons (95,96).

Aroma. Aroma is considered to be much more complex than taste and has many dimensions (97). While the number of compounds conferring aroma in fruits and vegetables is large, they can generally be classified as hydroxy compounds, aldehydes, ketones, acids, esters, sulphur-containing compounds, $O_2$ heterocyclics and pyrazines (97). Several hydrolases and proteases involved in the synthesis of fatty acid and amino acid precursors to volatiles are synthesized or activated during the respiratory climacteric (98-100).

Production of volatiles is lower after CA storage of apples than after air storage (71,101-02). Knee and Hatfield (103) found that low levels of esters in apples stored in low $O_2$ atmospheres were a result of low rates of alcohol synthesis, which they thought was the precursor to esters and aldehydes responsible for apple flavors. This effect was reversible upon removal to air. Very low $O_2$ (about 1%) may suppress development of volatiles after removal to air (104). Fruits picked at the preclimacteric stage and stored in 1 to 2% $O_2$ may loose their ability to produce sufficient volatiles to achieve proper aroma (103). CA storage of apples suppressed production of butanoates, 2-methylbutanoates, pentanoates, and hexanoates, but had little effect on aldehydes and acetates (71). Golden Delicious apples stored 3 to 9 months in 11 combinations of low $O_2$ and high $CO_2$ had measurably lower production of volatiles than apples stored in air (105). Decreasing $O_2$ to 3% had little effect on volatile production, but a further decrease to 1% caused a significant reduction in volatile production. At $O_2$ concentrations above 2%, elevated $CO_2$ became important in reducing volatile production. The relationship between the effects of reduced $O_2$ and elevated $CO_2$ on volatile production was neither additive, nor linear, nor synergistic. There was a residual suppression of volatile production after removal to air, but a 3-week treatment in air at 1°C before removal to shelf temperature partially restored volatile production (105).

Tomato volatiles are important to both aroma and flavor and their concentration increases with ripening (106). Because many of the volatile aroma compounds of tomatoes apparently result from enzymatic breakdown of carotene pigments (107), CA, which delays pigment synthesis, can be expected to delay volatile production.

An inappropriate atmosphere can result in off odors and flavors. Too low $O_2$ or excessive $CO_2$ can cause fermentative metabolism leading to accumulation of ethanol and acetaldehyde. Such off odors can be particularly offensive in broccoli (108).

## Nutritional Quality

Vitamins. Vitamins, which are important nutritional components of fruits and vegetables, can also act as antioxidants, pigment relatives and cofactors for numerous enzymatic reactions. They are subject to decomposition after harvest so proper postharvest treatment is crucial to their preservation. The role of vitamins in the postharvest physiology of vegetables has been recently reviewed (6).

Ascorbic acid (vitamin C) is utilized as a cofactor to stabilize the chloroplast stroma, in quenching free radicals and reacting with hydroxy radicals and in the biosynthesis of tartaric acid and oxalic acid (6), important organic acids in grapes, and many vegetables. The effect of CA on ascorbic acid content differs with commodity,

atmosphere and temperature (6). Degradation of ascorbic acid is associated with wilting in green leafy vegetables (109). For many commodities, such as lettuce, parsley, corn salad, Chinese cabbage, spinach, green bean, kale, broccoli, brussels sprouts, apples, red currants, cress, and sweet pepper, 1 to 4% $O_2$ generally slows ascorbic acid degradation, presumably through prevention of oxidation (6,110-12). Elevated $CO_2$ can accelerate ascorbic acid degradation in green bananas, potatoes, spinach, asparagus, peas, apples, red currant, corn salad, parsley, cress, sweet pepper, and leeks (6,7,111-15). However, the effect of $CO_2$ on ascorbic acid depends upon temperature, $CO_2$ level, and storage time (7). Packaging of spinach and green beans in polyethylene bags prevented ascorbic acid degradation but this was attributed primarily to the maintenance of high relative humidity (116).

There is little vitamin A in fruits and vegetables but carotene, which is a precursor to vitamin A, can be affected by storage conditions (109). Carotenes are subject to oxidation and to degradation associated with wilting of leafy vegetables (109). CA conditions, which maintain low $O_2$ and high relative humidity, can be expected to preserve carotenes. $CO_2$ (5%) atmospheres accelerated losses of carotenes in carrots while 7.5% $CO_2$ resulted in higher carotene levels than at the beginning of storage (7). In leek, 10% $CO_2$ combined with 1% $O_2$ resulted in higher carotene levels than at the beginning of storage (117).

Minerals. Fruits and vegetables can be rich sources of minerals important in human nutrition. Minerals are not lost during storage, but can be translocated and concentrated. Thus, low $O_2$ storage of cauliflower resulted in decreased potassium in the curd compared to the leaf but increased calcium in the curd (7). Low $O_2$ also favors translocation of molybdenum from the leaves to the curd (7). Significant redistribution of calcium occurred in Cox's Orange Pippin apples during storage but the patterns of redistribution were similar cin CA and air-stored samples (118).

## Safety Considerations

Plant Pathogens and Mycotoxins. The effects of CA and MA on decay in fruits and vegetables after harvest are difficult to assess due to conflicting reports (119). It is clear that CA/MA can change the general microbial profile of foods (120). CA and MA can delay senescence and maintain the good physiological condition of fresh produce and in this way help maintain inherent resistance to decay organisms. Conversely, inappropriate atmospheres can serve to erode the plant's resistance to microorganisms and in this way increase decay. There is evidence that CA/MA can have a direct suppressive effect on some plant pathogenic microorganisms. Disease suppression can be attributed to both the effect of MA on host resistance and on the altered growth of the pathogen (119). Consequently, the improvement in quality obtained through the use of CA/MA may be in part due to the effect on the physiological condition of the commodity and in part due to the inhibition of spoilage organisms (121). The effects of CA/MA on postharvest pathogens of fruit and vegetables have been reviewed (119,122).

Lowering $O_2$ concentration to 2-3% evidently suppresses pathogen activity very little. Most pathogens are suppressed only at $O_2$ concentrations below 1%, conditions that are damaging to most fruits and vegetables (119). Levels of $CO_2$ commonly used in MA (1-5%) are only moderately suppressive to plant pathogens. Levels of $CO_2$ above 10% have been used successfully to suppress pathogens on those commodities that will tolerate it. Examples are suppression of *Botrytis* on strawberries and suppression of *Botrytis* and *Monolinia* on sweet cherries (119).

In some cases CA/MA can favor development of plant pathogens. This may often be due to the maintenance of high relative humidity associated with established atmospheres, but it has been reported that high $CO_2$ favors growth of gram-positive bacteria (123). Percent decay of tomatoes inoculated with *Geotrichum candidum* was greater in 3% $O_2$ with or without added $CO_2$ than in air (124). MA can retard periderm formation and wound healing in potatoes, thereby giving pathogens additional time to establish infections and colonize the wound (119).

Mycotoxins are toxic secondary metabolites of fungi, some of which are carcinogenic to animals and humans. Those of major concern are patulin, produced by several species of *Aspergillus* and *Penicillium*, and aflatoxin, produced by *Aspergillus flavus* and other species of *Aspergillus*. Aflatoxin is found primarily on nuts and patulin can be prevalent on apples (119), pears, and stone fruits (125). Levels of $O_2$ of 2% or higher were only modestly suppressive of *A. flavus* growth and aflatoxin production. Little suppression was noted in 10% $CO_2$ but 20% or higher was very effective in suppressing fungal growth and aflatoxin accumulation (119).

*Human Pathogens.* Bacteria pathogenic to humans have been isolated from a number of fresh vegetables and in some cases have been responsible for disease (126-31). The environment in a MA package can create a high humidity, low $O_2$ environment that may be favorable to pathogenic microorganisms that would not otherwise thrive on vegetables. *Clostridium botulinum* was able to grow and produce toxin in the low $O_2$ environment of packaged mushrooms (132). Growth of *Listeria monocytogenes* is known to be enhanced by elevated $CO_2$ concentrations (133) and has been found on fresh vegetables (129). Another psychrotrophic pathogen, *Aeromonas hydrophila*, has been isolated from vegetables (127) and could be of concern on products held in chilled, MA conditions (120).

## Future Research Needs

MA and CA storage, transport, and marketing of fresh fruits and vegetables continue to present a technical challenge and opportunity to researchers and those involved in the fresh produce industry. The gains to be made are substantial, as are the risks. Technical competence in the handling of these commodities will be more important than ever before. In order to ensure that the commodities are handled properly to maintain the highest quality possible while ensuring their safety, the following issues will have to be stressed in future research programs:

1. The effect of CA/MA on quality parameters; rather than evaluating postharvest life only on the basis of appearance, workers should

pay more attention to the effect of CA/MA on texture, flavor, and nutritional quality.

2. The need to determine the likelihood that human pathogens, particularly toxin-producing anaerobes, can grow and produce toxins in MA packages before other spoilage organisms make the produce, either by appearance or by odor, offensive to the consumer.
3. The effects of minimally processing fruits and vegetables on their physiology and deterioration rate and the potential use of CA/MA conditions for their preservation.
4. Efforts to develop a mathematical model that can be used successfully to select the best package for creating and maintaining the optimum MA for each commodity.
5. Research to elucidate the mode of action of reduced $O_2$ and elevated $CO_2$ levels on changes in chemical composition of fresh fruits and vegetables.

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# Chapter 15

# Modeling Gaseous Environment and Physiochemical Changes of Fresh Fruits and Vegetables in Modified Atmospheric Storage

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There has been an increasing interest in using polymeric films for packaging of fresh horticultural commodities to provide several benefits such as protection against physical injuries, improved sanitation, inclusion of light, and more importantly modificaton of atmospheric composition. This article presents a review of empirical and non-empirical modeling approaches employed in estimating the changing gaseous composition of the micro-atmosphere sorrounding respiring produce when packaged in polymeric film. In addition the work done in our laboratory is also discussed where ripening tomatoes at 'turning' stage were used as model commodity. The approach taken involved obtaining experimental data for respiration rate, and surface color change at twenty different but constant gas compositions. The experimental data obtained was used in developing predictive models. The models were then validated against experimental conditions other than those used in developing the models.

Modified atmosphere or controlled atmosphere storage are the terms used in describing the storage environments where removal or addition of gases from the surroundings of the commodity in storage results in an atmospheric composition different from that of air (1–4). The term modified atmosphere is used when the composition of the storage atmosphere is not closely monitored and controlled, whereas controlled atmosphere generally indicates more precise gas compositional control. Typically, the modified atmosphere storage involves establishing or approaching to establish a desired gaseous environment around the product after a reasonably short transient period due to the interplay of the commodities' physiology and the physical environment. One particular technique in creating modified atmospheres for storing horticultural commodities deals with packaging the product in a polymeric film such that the

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actively respiring and metabolizing product reduces $O_2$ and increases $CO_2$ level by restricting the exchange of air between the micro-atmosphere inside the package and the environment outside. With recent technical advances in manufacturing polymeric films it should be possible to produce a film of desired physical and gas exchange characteristics for packaging fresh produce. However, the limiting factor is identifying the appropriate packaging film, which is dependent upon the fundamental knowledge of the gas exchange characteristics of both film and commodity.

Numerous researchers (5-7) in the past have attempted to match the packaging needs to the commodity to be stored. The approaches taken have been largely empirical in nature which involved testing of a large number of films produced by various film manufacturing companies. Several other researchers have subsequently recognized the importance of using mathematical and computer modeling techniques in developing predictive equations for identifying polymeric films in terms of permeability characteristics such that desired modified atmospheres are obtained. This article reviews the modeling approaches reported by various investigators which have implications in predicting the appropriate characteristics of packaging materials to maintain or create known and optimal level of gaseous atmosphere surrounding the commodity and within the commodity.

## Modeling of Gas Exchange in Fruit and Vegetable Tissues

The work done in this area is of significance both in controlled and modified storage applications. It deals with the rates at which metabolic gases diffuse into and out of various commodities. Concentration of metabolic gases inside the tissue affects its metabolic activity (respiration rate). The metabolic activity along with the diffusion characteristics of the tissue has bearing on the gaseous concentration throughout the tissue.

Diffusion Equations. Application of Fick's law of gas diffusion in fruits was first investigated by Burg and Burg (8). The Fick's first law of diffusion (9) is defined as:

$$J = AD \frac{\partial c}{\partial x} \tag{1}$$

which states that the flux J (μmoles/s • kg of fruit), of a gas, diffusing normally through a barrier of area A ($cm^2$) is dependent on the diffusion coefficient D ($cm^2/s$), and the concentration gradient $\partial c/\partial x$. In order to estimate the diffusion coefficient it is necessary to determine the concentration gradient of the gas in the tissue. Further complicating the matter is that one has to solve the equation of Fick's second law of diffusion which is needed not only in knowing the change of the gas concentration at a given location ($\partial c/\partial x$) in the plant tissue but also to know how the concentration is changing with time ($\partial c/\partial t$). Such an equation for a metabolically active gas in three dimensions (cartesion coordinates) is given by Crank (9):

In Quality Factors of Fruits and Vegetables; Jen, J.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}\right) \pm E \quad (2)$$

where E is the constant rate of gas consumption or evolution. The factor E is zero for metabolically inactive or inert gas. For one dimensional diffusion in x-direction the Equation 2 simplifies to the following form:

$$\partial c/\partial t = D\,\partial^2 c/\partial x^2 \pm E \quad (3)$$

The above equations are based on the assumption that the diffusion coefficient D is independent of the gas concentration. However, that may not be necessarily true. Also, the diffusion coefficient is usually dependent on temperature following an Arrhenius type relationship. For a sphere, the Equation 2 can be represented in terms of concentration gradient in radial direction ($\partial c/\partial r$) as follows:

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r}\frac{\partial c}{\partial r}\right) \pm E \quad (4)$$

General solutions of non-steady state problems (Equations 2 to 4) and of steady state problems when concentration is independent of time, i.e., $\partial c/\partial t = 0$, are available in the literature (9). For more complex problems, numerical solutions based on finite difference and finite element methods using high speed computers can be obtained (10-13).

Solomos (14), in a review of principles of gas exchange in bulky plant tissues suggested that apart from the mathematical complexities, determining the resistance to diffusion of the peel fruits, roots, and tubers from the measurement of the efflux of the inert gas introduces number of uncertainties due to differential diffusion resistant between the skin, fruit surface and the flesh (15). Thus, it cannot be always presumed that the concentration of the metabolically inert gas is uniformly distributed throughout the fruit.

Solomos (14) further illustrated that the concentration gradient can be substantial in non steady-state cases. For an example it was assumed that a gas diffuses through an infinite cylinder of unit cross-section area, with a diffusion coefficient similar to that of the average flesh of 'Gala' apples (0.0014 $cm^2/s$). Then the distribution of M molecules deposited at time t = 0 at one end (origin, x = 0) is given by:

$$c(x,t) = 1/(4\pi Dt)^{1/2} \bullet \exp(-x^2/4Dt) \quad (5)$$

Equation 5 is solution of Equation 3 when E = 0 (9). From Equation 5 it can be estimated that after t = 5 min % of M at x = 0, 2.5 and 3.5 cm is 42, 1.2 and 0.047, respectively. Similarly, % of M after t = 20 min at x = 0, 2.5 and 3.5 cm is 24, 8.83 and 4.00, respectively (14).

Solution of a governing diffusion equation depends upon the assumptions made in solving it. As illustrated in an example above

numerical values of gas concentration at given time and location can be then estimated from the solution. However, the solution(s) is only meaningful if it can be verified with experimental data.

Measurement of Internal Concentration of Gases. Several techniques have been reported in the literature (15, 16) for determining intercellular gas concentration in horticultural commodities. These techniques include evacuation, manometric procedures, gas (oxygen) microelectrodes, and removal of plugs of tissues followed by sealing in air tight vials. None of these methods are really non-destructive to the extent they do not alter the conditions the investigator may be interested in measuring. Uncertainties associated with measuring true internal concentration of gases makes verification of mathematical models a difficult task.

Diffusion Barriers. Another degree of complexity in modeling gas diffusion in fruits may come from the non-uniformity of resistance to diffusion form one part of the fruit to the other. For example in case of apples the diffusion coefficient at the center may be different from that of the fleshy part which may be then different from that of the skin. Nature of the diffusion barrier is another variable which can play a significant role in exchange of gases in fruits and other bulky organs as the diffusion of gases can occur through gaseous pores, lenticels, or stomata, or through aqueous or waxy layers of the epidermis. Water diffuses through different channels than gases (8, 17, 18). Gases diffuse mainly through channels filled with air whereas water vapor transport takes place through aqueous medium.

## Modeling of Gaseous Composition of Horticultural Commodities Stored in Polymeric Films

Produce packaged in a polymeric film is a dynamic system involving respiration and permeation.

Permeation, $P_G$ (ml/hr), of a gas G in the film package can be expressed as follows:

$$P_G = k_G A \Delta G \qquad (6)$$

In Equation 6, $k_G$ is film gas permeance constant (ml/hr•m$^2$•atm), A is film area (m$^2$) and $\Delta G$ is concentration (atm) difference of the gas G across the film thickness (fraction).

Respiration, $R_G$ (ml of gas G/hr • kg of fruit), of a respiring product depends on number of factors such as gaseous composition surrounding the products, characteristics of the product (gas diffusion characteristics discussed earlier), storage period and temperature, etc. Thus, $R_G$ may be represented as a function f of those factors as given by:

$$R_G = f(c(O_2), c(CO_2), c(E), t, T, M, CV, X) \qquad (7)$$

where subscript G refers to gas associated with respiration (oxygen, carbon dioxide or ethylene etc.). In the above equation $c(O_2)$ is oxygen concentration, $c(CO_2)$ is carbon dioxide concentration,

c(E) is ethylene concentration, T is temperature, t is storage time, M is product maturity, CV is cultivar type, and X is any other factor(s) not explicitly expressed.

By mass balance the rate of change of concentration of a particular gas of respiration in the package is given as:

$$\Delta c(G)/\Delta t = P_G/V - R_G W/V \tag{8}$$

where, V is total free volume in the package (ml), and W is weight of the product in the package (kg). Equation 8 can be written in the following differential forms for rate of change of oxygen and carbon dioxide concentrations in the package:

$$\frac{dc(O_2)}{dt} = k_o A(0.21-c(O_2))/V - R_o W/V \tag{9}$$

$$\frac{dc(CO_2)}{dt} = k_c A c(CO_2)/V + R_c W/V \tag{10}$$

The solutions of the above two equations will yield $O_2$ and $CO_2$ concentrations within a produce package at any time between the start of the experiment and the time steady state conditions are achieved. The knowledge of respiration functions $R_o$ and $R_c$ (Equation 7) is essential to solving Equations 9 and 10 and degree of complexity of obtaining those solutions depends upon the mathematical form of the respiration functions. Equation 8 is expressed into Equations 9 and 10 for only oxygen and carbon dioxide, a similar equation can be written for any other biologically active gas (for example ethylene); but again solution of that equation can be obtained only if the corresponding rate of production or utilization function is known.

For steady-state conditions, that is when permeation equals respiration, Equations 8-10 take a simpler form:

$$P_G = R_G W \tag{11}$$

$$P_o = k_o A(0.21 - c(O_2)) = R_o W \tag{12}$$

$$P_c = k_c A c(CO_2) = R_c W \tag{13}$$

However, solutions of the above equations can be obtained only if the respiration functions are known.

Work published by various investigators in handling equations resulting from Equations 7 and 8 is reviewed below. They have taken different approaches and made different assumptions in modeling modified atmosphere packaging.

Some of the earlier work was done at the Massachusetts Institute of Technology. Jurin and Karel (19) investigated optimum packaging conditions for apples, and Karel and Go (20) for bananas. They assumed $R_o$ to be a function of oxygen concentration only and obtained oxygen consumption rate curves from experimental data. They also found that respiration quotient, $R_c/R_o$, was equal to one for oxygen level greater than 3%. They

dealt with steady state conditions only and solved the respiration-permeation equation graphically. Graphical solution for 350 gm of bananas packaged in polyethylene film (package area = 0.095 $m^2$) is presented in Figure 1, which shows steady-state oxygen concentration of 6.9%. Steady-state concentration of carbon dioxide was easily calculated from Equation 13, where $R_c = R_o$ and $R_o$ was obtained from Figure 1. Changes in the package characteristics will reflect on the location of the diffusion (permeation) curve in Figure 1, thus a desired gas composition in the package, at steady-state conditions, can be obtained by appropriate selection of package characteristics.

Karel and Go (20) also investigated the effect of temperature on the rate of respiration and assumed Equation 7 to be $R_o = f(c(O_2), T)$. They found that at a given oxygen concentration level, temperature dependence of respiration rate conformed to the Arrhenius type equation.

Veeraju and Karel (21) followed up on an earlier work of (19) and tried to use two films of different permeabilities for controlling oxygen and carbon dioxide levels in a package and met with reasonable success. They worked with apples and focussed on steady-state concentration values. They simultaneously solved the following two equations, while using the respiration rates, as needed, from the experimental curves for $R_o$ and $R_c$:

$$R_oW = (k_{o1}A_1 + k_{o2}A_2)(0.21 - c(O_2)) \quad (14)$$

$$R_cW = (k_{c1}A_1 + k_{c2}A_2)c(CO_2) \quad (15)$$

Equations 14 and 15 are manifestations of Equations 12 and 13, where additional subscripts 1 and 2 refer to two different packaging films.

Henig and Gilbert (22) used finite difference method in numerically solving the two first-order differential equations (Equations 9 and 10). They assumed $R_o$ and $R_c$ to be functions, f and g, of both oxygen and carbon dioxide concentrations, $R_o = f(c(O_2), c(CO_2))$ and $R_c = g(c(O_2), c(CO_2))$, and obtained experimental respiration curves for tomatoes (Figure 2) to attain the solutions. Accuracy of computer predictions were tested against experimental data (Figure 3). A good agreement was found.

Hayakawa and his colleagues (23) reported complex analytical solutions using Laplace transformations for transient and steady state conditions simulating the gas exchange of a fresh product package. Although they assumed $R_o$ and $R_c$ to be functions of oxygen and carbon dioxide as done by (22), they expressed respiration rates as combination of linear equations of the following form:

$$R_{oi} = p_i + q_ic(O_2) + r_ic(CO_2) \quad (16)$$

$$R_{ci} = l_i + m_ic(O_2) + n_ic(CO_2) \quad (17)$$

where, $c(O_2)_{i+1} \leq c(O_2) \leq c(O_2)_i$; $c(CO_2)_i \leq c(CO_2) \leq c(CO_2)_{i+1}$; $(i = 0, 1, 2, \ldots, n)$; and $l_i$, $m_i$, $n_i$, $p_i$, $q_i$, $r_i$ are constants of the ith segment. Respiration rate data obtained by

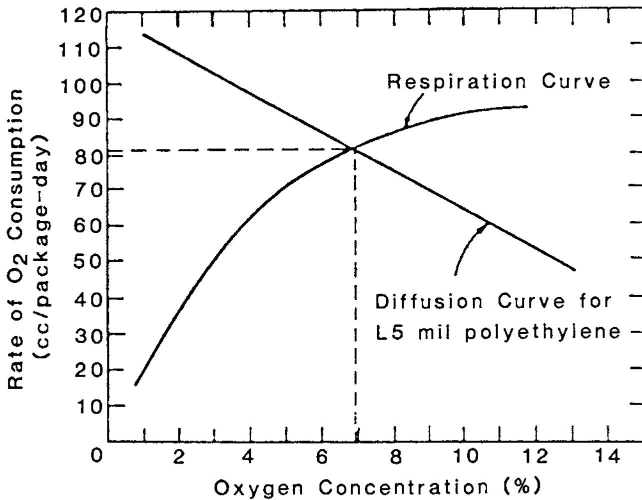


Figure 1. Graphical estimate of steady state concentrations of $O_2$ and $CO_2$ in polyethylene bags containing bananas. (Reproduced with permission from Ref. 20. Copyright 1964 Modern Packaging.)

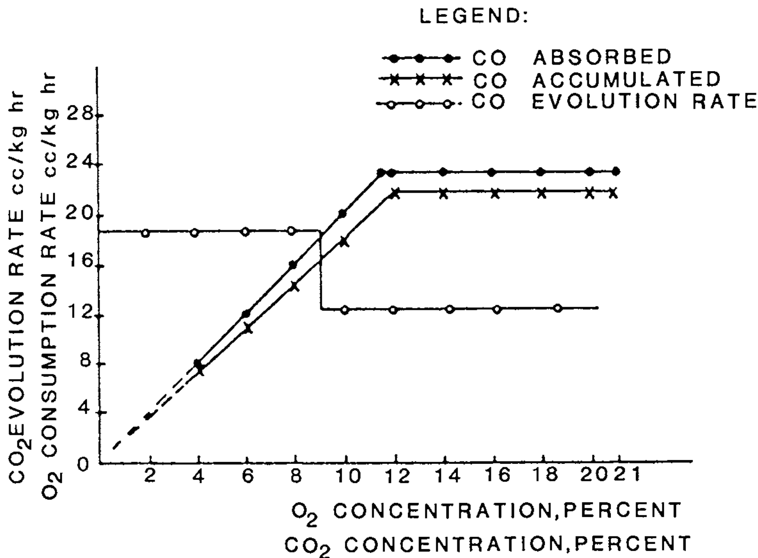


Figure 2. Effect of $O_2$ and $CO_2$ concentrations on $O_2$ consumption rate and $CO_2$ evolution rate of tomatoes. (Reproduced with permission from Ref. 22. Copyright 1975 Institute of Food Technologists.)

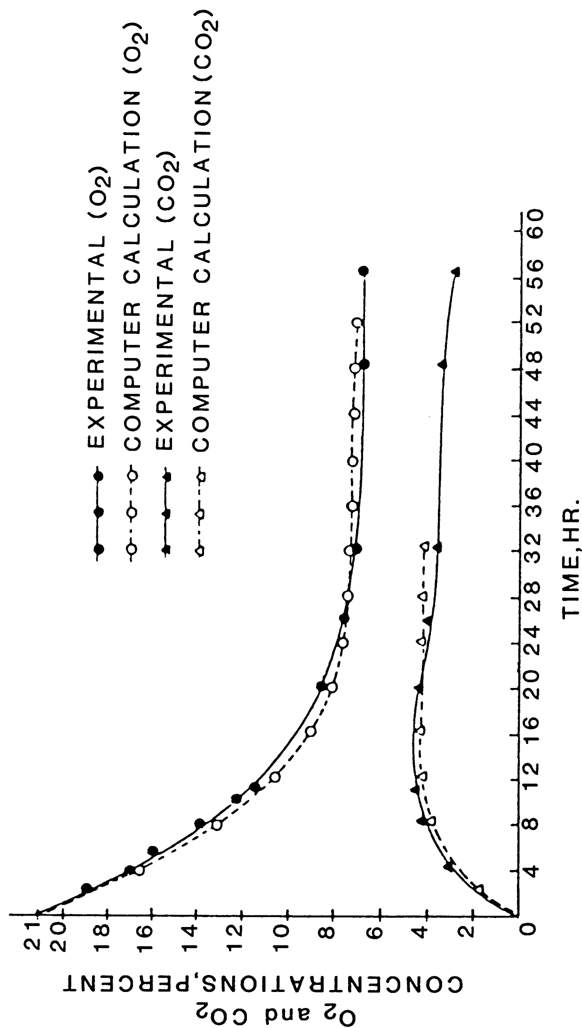


Figure 3. A comparison between computer calculation and experimental results of the atmosphere change in RMF-61 package. (Reproduced with permission from Ref. 22. Copyright 1975 Institute of Food Technologists.)

(22) was used by (23) in constructing appropriate linear equations. A fair agreement has been reported between the estimated gas composition values of (23) and the experimental values of (22). They (23) also proposed solutions for two film packages, details of which are not presented here.

Deily and Rizvi (24) derived analytical solutions, similar to (23), solutions of Equations 9 and 10 to optimize packaging parameters or to estimate transient and equilibrium gas concentrations in polymeric packages containing peaches. The respiration rate functions based on experimental data were of the following form, which were used in obtaining the analytical solutions:

$$R_o = \text{constant for } c(O_2) > 5\%$$

$$= -a \exp(-bt) \text{ for } c(O_2) < 5\% \qquad (18)$$

$$R_c = \text{constant for } 1 < c(CO_2) < 27\% \qquad (19)$$

Cameron and his colleagues (Cameron, H. C.; Boylan-Pett, W.; Lee, J., Michigan State University, unpublished data) obtained respiration data for tomatoes at three different stages of maturity, breaker, pink and red. For each stage they first obtained oxygen depletion curve with storage time and fitted a function of the following form:

$$c(O_2) = a_1 \{1-\exp[-(a_2 + a_3 t)^{a_4}]\} \qquad (20)$$

They calculated $R_o$ from Equation 20 using the following relationship:

$$R_o = -\frac{dc(O_2)}{dt}\frac{V}{W} \qquad (21)$$

where, V is free space volume of experimental container used in obtaining oxygen depletion data (ml), and W is weight of fruit (kg). They were not concerned with the transient stage of gas exchange and developed only the prediction models for oxygen concentrations within sealed packages of tomato fruit. To achieve that goal they obtained the solution of Equations 12, 20 and 21 for various combinations of W, $c(O_2)$ and film permeability parameters. Details of methodology in designing a packaging system is given in (25).

Yang and Chinnan (26) developed an integrated model to predict gas composition and color development of tomatoes stored in polymeric film packages. They conducted their investigation in several phases (26-28). First they obtained models to describe the effects of gas composition, oxygen and carbon dioxide, and storage period on respiration rate (oxygen consumption rate and carbon dioxide evolution rate). To obtain the mathematical expression of the following equation (Equation 7):

$$R_G = f(c(O_2), c(CO_2), t) \qquad (22)$$

they experimentally observed the respiration rates at 20 different but constant gas compositions involving four levels of oxygen (5, 10, 15 and 20%) and five levels of carbon dioxide (0, 5, 10, 15 and 20%) and fitted polynomial equations (28). Validation of models was done by testing the adequacy of predictions against the experimental data of two arbitrarily selected constant gas compositions (28). The next step taken was to predict steady-state oxygen and carbon dioxide concentration of the environment of ripening tomatoes packaged in film. The method used is summarized below.

It was assumed that for a short time period, $\Delta t$, the net amount of $O_2$ and $CO_2$ change in the package, based on Equations 12 and 13, can be expressed as follows:

$$a = R_oW\Delta t - k_oA(0.21 - c(O_2))\Delta t \tag{23}$$

$$b = R_cW\Delta t - k_cAc(CO_2)\Delta t \tag{24}$$

It is obvious that at steady-state conditions a and b will be theoretically zero, but it is difficult to obtain an explicit solution for $c(O_2)$ and $c(CO_2)$ from Equations 23 and 24 when $R_o$ and $R_c$ are polynomial functions of $c(O_2)$, $c(CO_2)$ and t. As a result, Yang and Chinnan (26) employed an iterative method to determine $c(O_2)$ and $c(CO_2)$ values at any given time, t, such that $a^2 + b^2$ had the smallest possible value. Algorithm and further details are given in (26). Experimental and predicted values of the atmosphere changes in four scenarios are illustrated in Figure 4, which showed a good agreement between estimated and observed values.

In modeling physio-chemical changes in tomatoes stored in modified atmosphere storage, Yang and Chinnan (27) went one step further than predicting respiration rates. They used tomato surface color development as an indicator of physiological changes affected by environmental gas composition. Tomatoes were held at 20 constant gaseous compositions, as mentioned above for the respiration rate data, and the surface color development was periodically monitored with a Hunter color instrument. Color data were presented in terms of a/b ratio, where 'a' and 'b' are Hunter color parameters. A mathematical equation of the following form was proposed based on a study reported by (29).

$$(a/b)_t = \frac{(a/b)_g + (a/b)_r[\exp(C_o + M(G))t]}{1 + \exp[C_o + M(G)t]} \tag{25}$$

where, subscripts t, g and r, refer to values at any color, green and red stages of tomatoes, respectively; M is color development index depending on gas composition G. M was considered a quadratic function of $O_2$ and $CO_2$ concentrations. $C_o$ and coefficients of function M in Equation 25 were determined from experimental data. The mathematical model given by the above equation was validated for tomatoes stored in three constant gas compositions (Figure 5).

Yang and Chinnan (27) adapted the TTT hypothesis of Thorne and Alvarez (29) for predicting color development when the gaseous

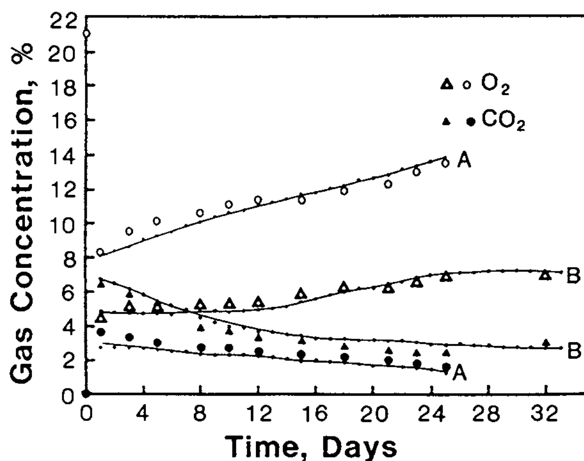


Figure 4. A comparison between experimental (O△●▲) and computer estimated ( – ) results of the atmosphere changes in two packages (A and B) made from Cryovac E-type film, both packages had same surface area (1342 $cm^2$), package A and B contained 2 and 4 tomatoes, respectively. (Reproduced with permission from Ref. 26. Copyright 1988 Institute of Food Technologists.)

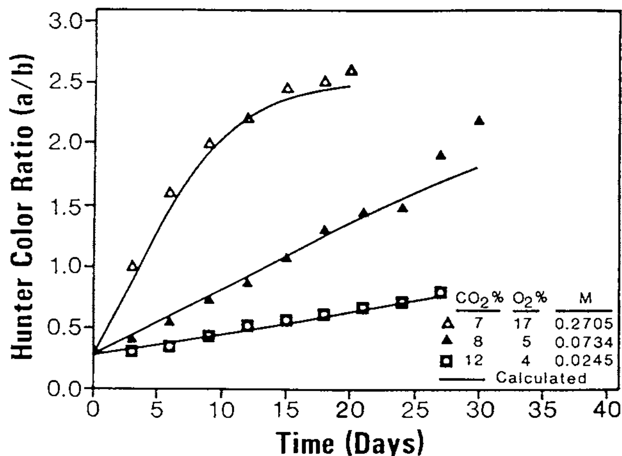


Figure 5. Experimental and calculated a/b values for tomatoes stored in three arbitrarily selected constant gas compositions at 21°C. (Reproduced with permission from Ref. 27. Copyright 1987 American Society of Agricultural Engineers.)

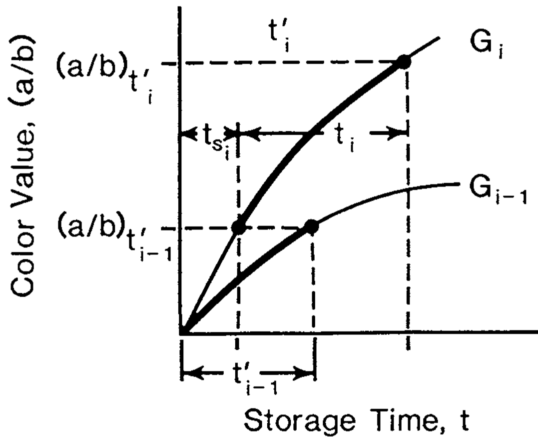


Figure 6. Concept of applying commutative and additive properties to surface color development of tomatoes stored under two different storage environments. (Reproduced with permission from Ref. 27. Copyright 1987 American Society of Agricultural Engineers.)

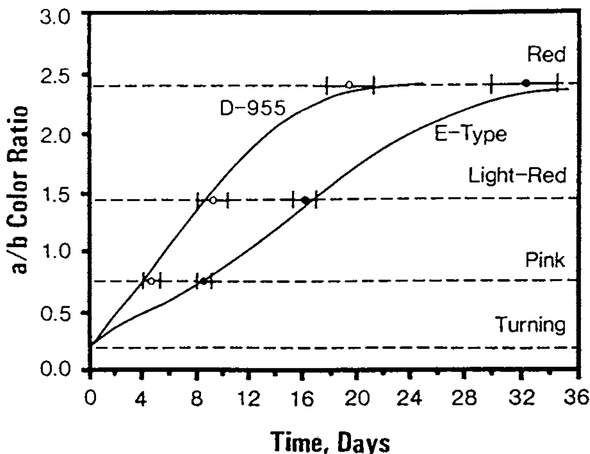


Figure 7. Mean number of days (O●) and the standard error ( |-| ) to reach specified USDA color stage and a/b color ratio of four tomatoes packaged in two types of Cryovac film. The D-955 film package (surface area = 1007 $cm^2$) and E-type package (surface area = 1342 $cm^2$) contained 623 and 645 g tomatoes, respectively. (Reproduced with permission from Ref. 26. Copyright 1988 Institute of Food Technologists.)

composition changed from one state to other. The concept of applying additive and commutative properties of the TTT hypothesis is illustrated in Figure 6. It is assumed that characteristic curves for color change, a/b, are known for two different environmental conditions, $G_{i-1}$ and $G_i$. The product is placed in environment $G_{i-1}$ for time $t'_{i-1}$ followed by placing the product product in environment $G_i$ for time $t_i$. Total real storage time $t_T$ is $t'_{i-1} + t_i$. Figure 6 shows that the color development of the fruit is same if it is stored in $G_{i-1}$ for $t'_{i-1}$ or in $G_i$ for $t_{si}$. Thus, a/b value after $t_T = t'_{i-1} + t_i$ is estimated from the curve for $G_i$ for time $t'_i = t_{si} + t_i$. Yang and Chinnan (27) developed an iterative procedure to use on a computer for calculating a/b ratios in varying gaseous environment. The follow up study (26) involved combining the models from two studies (27, 28) to develop and test an integrated model to predict the gas composition and the color development of tomatoes ripening in polymeric film package. The results from one experimental set demonstrating the adequacy of the integrated model are given in Figure 7.

## Concluding Remarks

The fundamental equations describing the gas exchange within the horticultural commodities and between the micro-atmosphere inside the package and the environment outside the package are not very complex. However, the challenging aspect of the modeling process is 1) obtaining solutions of those equations while employing realistic and meaningful assumptions, and 2) validation of the models with experimentally obtained data. Researchers have been successful with obtaining models and validating them but the models obtained are of limited scope. One of the limiting factors in developing models for modified atmosphere storage and packaging systems is the availability of respiration data in the form to be able to be used in predictive methods.

Future research needs to be focused on obtaining more comprehensive models describing the dynamic respiration and permeation system. The factors indicated in Equation 7 need to be considered to obtain greater understanding of the respiration component of the system. Data are needed on permeability characteristics of packaging films as a function of wide range of temperatures and relative humidities so that the product and film characteristics could be matched for different storage, handling and distribution needs.

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Chapter 16

# Physicochemical Changes and Treatments for Lightly Processed Fruits and Vegetables

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Consumer preferences for fruits and vegetables with fresh-like quality and convenience have lead to a new category of foods, called minimally processed or lightly processed fruits and vegetables. Acceptability of lightly processed fruits and vegetables depends upon their flavor, texture and appearance. Lightly processed fruits and vegetables are usually more perishable than the fresh produce from which they are made. To reduce their perishability, lightly processed fruits and vegetables are subjected to a combination of mild processing treatments, which are designed so that their preservative effects are complimentary, and the fresh-like quality of the products remains intact.

Consumers demand high quality in their food products. For fruits and vegetables, high quality usually implies fresh-like quality characteristics of flavor, texture, and appearance. Simultaneous with the desire for fresh-like quality, changes in consumers life-styles have lead to an increased demand for convenience in food products. Convenience is an attribute that is usually associated with some type of processing, such as peeling, coring, trimming and slicing. The combined demands for fresh-like quality and convenience have given rise to a new category of fruit and vegetable products that are called "lightly processed" or "minimally processed" (1).

Because of the great variations in fruit and vegetable commodities and the ways in which they are prepared for consumption, it is almost impossible to make an all-inclusive, precise definition of lightly-processed, or

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minimally processed, fruits and vegetables. For purposes of this communication, lightly-processed fruits and vegetables are those with more fresh-like quality than is associated with fully-processed products, but with the convenience of fully processed products. Fresh apple slices, shredded lettuce, and trimmed broccoli florets are examples of lightly processed fruits and vegetables. Lightly-processed foods are perishable. No single treatment is available that will maintain the fresh-like quality of lightly-processed fruits and vegetables and simultaneously render the products imperishable. The usual approach to decreasing the perishability of lightly processed fruits and vegetables is to apply a combination of "light" preservation treatments, on the assumption that their combined effects will, at least, be additive. No single light treatment should alter the product to the extent that its quality is no longer considered fresh-like.

The shelf life that is required for lightly-processed products is dependent upon the marketing and distribution system. When shipping distances and storage times are short and the product is consumed within a few days refrigerated storage and handling may provide sufficient shelf-life for the products, without any additional preservation treatment. For most situations some product treatment must be combined with good refrigerated storage and handling in order to maintain quality during distribution and marketing. When lightly-processed products are produced near the location where they are grown shipping times and distances are greater than when the products are made in the region of consumption. Thus large centralized processing facilities will usually process for a longer shelf life than small, dispersed processing plants.

Availability of the fresh product will also affect the kind of light processing that must be used. Products such as apples and potatoes are always available in fresh form. However, products such as fresh apricots and peaches do not store well and are available for only a short season. The required shelf-life of lightly processed products made from products such as apples and potatoes may be about 2 to 4 weeks, but in order to extend the marketing period for crops that are only seasonally available several months shelf life may be needed. For the latter situation more, or more intense, preserving treatments, will be required. Use of more preserving treatments, or more intense preserving treatments, usually causes the products to lose some of their fresh-like quality. However, even the more intensely treated lightly processed products should remain more fresh-like than their fully processed counterparts.

The degree of stabilization required in a lightly processed food also depends upon the market to which it is being sent. Generally, there is more product control in institutional and remanufacturing markets than in restaurants. Retail grocery and produce markets provide the least control over product handling abuse.

The kind of process-distribution train to be used will result from consideration of the above factors: 1) locations of production and consumption, 2) availability of fresh unprocessed product, and 3) the kind of marketing channel for the lightly-processed products. There are two basic process-distribution trains that may be used: the direct train and the interrupted train. In the direct process-distribution train the product is given the appropriate light process, packaged, and shipped directly to market. The interrupted process-distribution train involves an interruption in the system. The interruption may be to repackage the product, to blend several products, to remanufacture the products, or to otherwise reconstitute or modify the products. Shredded lettuce that is cut from whole heads of lettuce, packaged in market sized bags, and shipped directly to the point of consumption is an example of the direct train. Lettuce that is pre-cored and trimmed and then shipped in bulk to some location for cutting and packaging, is an example of the interrupted train. In general, the direct train is used for products that are only slightly modified by light processing, where a minimal shelf-life is required, and where unprocessed fresh material is continually available.

## Quality Factors

Regardless of the kind of process-distribution system that is used, the same quality factors must be maintained in the lightly-processed products, namely flavor, texture, and appearance. Microbiological quality and nutritional quality are also important, but they will not be dealt with in this communication.

While appearance, texture, and flavor are each important for most products, their relative importance will vary between products. For leafy salads, for example, texture and appearance may be more important determinants of quality than flavor. For pre-sliced fresh fruit, which is to be used in remanufactured foods, flavor and texture may be more important than appearance. The shelf-life of a product is measured as the time from preparation until one quality factor degrades to a level where it is unacceptable. Therefore it is necessary to treat or process the product so that all quality factors have about the same stability even though one factor is considered

more important to overall quality than another factor. It is not uncommon to find that a treatment which enhances the stability of one quality factor causes another factor to become unacceptable. A chemical additive, such as calcium chloride, may improve the textural quality of a fruit product, but it can also produce unacceptable flavors. Blanching, to inactivate enzymes that cause darkening, destroys the fresh-like textural quality that is desired in lightly processed fruits and vegetables.

Flavor. Flavor is one of the most difficult quality factors to maintain in fruit and vegetable products. Flavor change may result from the loss of compounds that provide good flavor or from the accumulation of compounds that produce off-flavors. Aroma components of flavor are most important for some products, especially fruits. Aroma quality may be lost by either the disappearance of good aromas or by the development of bad aromas. Light-processing systems should supply products which maintain the original flavor quality of the material from which they are made, and the systems must avoid conditions that lead to off-flavor development. Development of off-flavors may be due to either physiological or microbial degradation. Loss of original flavor in fresh fruits is not well-understood, but it has been shown that production of volatiles by apples is reduced or arrested by conditions of controlled atmosphere storage (2). Also, apples stored in air with under 2% $O_2$, at 3°C, took up ethanol but formed little ethyl acetate (3). Storage conditions that maintain high quality texture and appearance may lead to production of off-flavors. Anaerobic storage may improve both texture and maintain appearance of a product, compared to aerobic storage, but if $O_2$ levels are reduced too much the products may suffer from anaerobiosis, leading to formation of off-flavors (4). Microbial metabolites may also lead to off-flavors in products that are undergoing microbial spoilage, but when microbial spoilage occurs, texture and appearance are likely to be degraded simultaneously with the development of off-flavor. Use of chemical additives to prevent microbial or physiological spoilage may also impair the flavor of lightly processed fruits and vegetables. Flavor development in fruit has been hypothesized to occur simultaneous with the decomposition of cellular constituents as fruit matures. Treatments used to inhibit cellular decomposition, as occurs in the ripening process, may simultaneously inhibit the generation of compounds that produce good flavor. This illustrates the problem of the conflicting effects of light processing treatments. Treatments designed to maintain textural quality may prevent the generation of good flavors (5).

Texture. Texture is a quality factor that differentiates fresh from processed foods. Fresh fruits and vegetables have textures that are described as "crisp" or "firm" which are considered desirable. Processed foods have textures that are "soft" or "chewy", which are considered undesirable. The cells of high quality fresh fruits and vegetables have a high "turgor".

Plant cells contain semipermeable membranes. In order for many plant products to be crisp and turgid the cells must maintain a high turgor pressure. This requires that membranes remain semipermeable and yet contain a high turgor pressure. Processing stress result in loss of turgor. Enzyme catalyzed reactions cause depolymerization of cell membranes and cell walls. Pectinesterase, polygalacturonase, pectin-methylesterase, and ∂-galacturonase are examples of some of the enzymes that contribute to loss of texture. Processing and treatment systems which inactivate, or at least minimize the activity of, these enzymes will tend to maintain fresh-like textural qualities (6,7).

Texture of specific fruits and vegetables vary according to their composition, cell structure, and process treatments. The processes that affect the textural quality of some raw and cooked vegetables have been summarized (8).

Texture has been used as an index of fruit and vegetable quality grades in both the fresh and processed segments of the markets. Devices for measuring the textural properties include the Magness-Taylor firmness meter, that is used primarily for fresh fruit, and the Kramer Shear Press and the Instron Universal Testing Machine, which are used for all forms of products (9).

Appearance. Processed foods are highly evaluated on their appearance, based upon criteria such as color, piece integrity, absence of peel fragments, and uniformity. For lightly processed products appearance is highly important, and the criteria for measuring it depends upon the product form.

Discoloration of lightly processed fruits and vegetables is often the factor that determines their shelf-life. Enzyme catalyzed reactions which convert phenolic compounds to brown melanins are the main causes of discoloration of lightly processed fruits and vegetables. When products are peeled, cut, or otherwise injured, cellular compartmentation is lost, enzymes and substrates come in contact with each other, and discoloration occurs (10). The products of these reactions may also produce off-flavors, but the appearance is usually unacceptable at

lower levels of browning than are required for off-flavors to be detected.

In addition to browning, the breakdown of chlorophyll results in loss of appealing green color. As the chlorophyll is broken down, the carotenoids are exposed, giving rise to a yellowing of the products. In addition to senescence, chlorophyll may be transformed to pheophytin by the action of heat, as in blanching. The conversion of chlorophyll causes the product to lose its bright green color and become a yellowish-green or an olive-green color (11). Depending upon the process or treatment that is applied, lightly processed green vegetables may lose color quality by either the action of senescence or heat.

## Preparation

Preparation operations for lightly processed fruits and vegetables, such as peeling, slicing, coring, and pitting, must be done with care to avoid bruising and other cellular damage. When cells are bruised they lose their physical integrity, enzymes and substrates come together, and darkening and other breakdown reactions occur (10). Bruising is a more serious problem for lightly processed products than for fully processed fruits and vegetables, because for lightly processed fruits and vegetables there is seldom a processing step that can be used to inactivate the enzymes without diminishing the fresh-like quality of the products.

The effect of different cutting parameters on the storage life of shredded lettuce is shown in Table I where a sharp knife using a slicing action was best.

Table I. Quality Scores for Cut Lettuce made by Slicing or Cutting with Sharp or Dull Knives. 10 = Excellent, 1 = Unacceptable

| Storage Time | Slice | | Chop | |
|---|---|---|---|---|
| (days) | Sharp | Dull | Sharp | Dull |
| 0 | 9.0 | 9.0 | 9.0 | 9.0 |
| 7 | 9.0 | 8.8 | 8.5 | 8.7 |
| 11 | 8.3 | 6.0 | 5.8 | 5.0 |
| 14 | 7.7 | 5.0 | 3.5 | 2.8 |
| 18 | 6.5 | 3.2 | 1.5 | 2.8 |
| 21 | 6.5 | 1.8 | | |
| 25 | 5.2 | | | |
| 28 | 4.3 | | | |

Source: Data are from ref. 13.

Preservation Treatments

Minimally processed fruits and vegetables may be subjected to a variety of treatments and conditions to improve shelf-life. Potential preservation treatments and conditions are:

- Low temperature storage;
- Use of chemical additives;
- Mild heat treatments;
- pH modification;
- Reduction of water activity, aw;
- Ionizing radiation;
- Controlled/Modified storage atmospheres.

As previously noted, a common strategy is to apply a combination of treatments rather than rely on a single treatment to prevent all types of degradation. This is called the "hurdle" approach, where each treatment presents a hurdle to the degradation process.

Low Temperature Refrigerated Storage. Low temperature refrigerated storage has been used to maintain quality in many fresh fruits and vegetables, provided the commodities are not susceptible to chilling injury. Because many of the degradation reactions are enzyme catalyzed, very low refrigerated storage temperatures, but above the freezing point of the products may provide a high degree of preservation for some forms of lightly processed fruits and vegetables. Some enzymes exhibit the so called "temperature anomaly" which is the effect that enzyme activity diminishes at low temperatures at a rate that is greater than expected from Arrhenius kinetics (12). The temperature anomaly is most pronounced at low temperatures, approaching the freezing point.
Low temperature storage was found to be the most effective treatment for the preservation of cut lettuce (13) and citrus salads (14). Table II shows the effect of storage temperature on the quality of cut lettuce.
The quality scores remain higher for the lower temperature storage. Similarly the shelf life of citrus salads was enhanced by lower storage temperatures (14). The shelf life of citrus salads was less than 1 week at 50°F (10°C), 5-6 weeks at 40°F (4.5°C), and 12-16 weeks at -1°C. Fresh broccoli has a storage life of up to 3 weeks when held at 0°C in air. The freezing point of broccoli is -1.7°C, and when held exactly at the freezing point, without freezing, the shelf life is doubled over that of 0°C (15). The broccoli example illustrates the potential of extending the shelf life of lightly processed fruits and vegetables

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by holding them at temperatures several degrees below normal refrigerated storage. One approach to exploiting this potential is to condition the products to allow lower temperature storage without freezing.

Table II. Quality Scores for Cut Lettuce Stored at Different Temperatures. 10 = Excellent; 1 = Unacceptable

| Storage Time (days) | Temperature °C | | |
|---|---|---|---|
| | 2 | 5 | 10 |
| 0 | 9.0 | 9.0 | 9.0 |
| 7 | 9.0 | 8.9 | 7.7 |
| 14 | 8.2 | 6.8 | 3.2 |
| 20 | 7.3 | 3.8 | 1.2 |
| 28 | 4.2 | 2.0 | --- |

Source: Data are from ref. 13.

Chemical Additives. Chemical additives may be used to extend the shelf life of some minimally processed foods, but they must not affect flavor or be perceived to impair product safety. When chemical additives are used they should be used as an aid to preservation, not as the primary mode of preservation.

Ascorbic acid and calcium chloride were shown to have a synergistic effect in maintaining color and texture in refrigerated fresh apple slices (16). Sodium benzoate, potassium sorbate, diethylpyrocarbonate, and sodium bisulfite were added to refrigerated fresh peach slices, and sodium benzoate was the best preservative at 32°F, (0°C) (17). The other preservatives were either ineffective or had detrimental effects on flavor, texture, or appearance. Citrus salads were treated with sodium benzoate or potassium sorbate (17). No extension of shelf life was observed when these chemicals were added at storage temperatures of 50°F and 40°F, but when the additives were present at 30°F storage substantial improvements in shelf life were observed, demonstrating the synergistic effect of chemical additives and low temperature storage.

Chemical preservatives added to water for washing shredded lettuce had no effect on extending shelf life of the products. Removing cellular contents from the cut surfaces of the shredded lettuce and removing all free liquid from the product extended the shelf life of cut lettuce, but this can be accomplished without using chemical additives (13).Sulfites were traditionally used to maintain color quality for both fresh-cut and processed foods (18). The recent ban on the use of sulfites for fresh salad items has lead to a search for alternative

chemicals that will give color protection similar to that of sulfites. Finding chemical replacements for sulfites is not an easy task because the chemicals must not only be effective they must also be proven safe. Some organic acids, phosphates, or combinations thereof, are used to retard browning and there are other potential synergistic compounds such as calcium and zinc ions (19). Combinations of $CaCl_2$ and $ZnCl_2$ were shown to have a synergistic effect in maintaining color quality. The combined calcium-zinc treatments are most beneficial when the cut product is to be exposed to air for an extended time, perhaps several days.

Mild Heat Treatments. Mild heat treatments may be used to extend shelf life for some lightly processed fruits and vegetables. Peaches heated in syrup containing sodium benzoates or potassium sorbate at 130°F (54.4°C) to 145°F (62.7°C) became soft and ragged after 18 to 20 weeks storage at 32°F (0°C), but the heated samples had slightly better flavor than the controls (17). The flavor improvement was not considered sufficient to offset the loss of texture due to heating. Peaches, containing sodium sorbate, were heated in a jar for 5 to 15 min at 120°F (49°C) to 130°F (54.4°C). The heat treated peaches held for up to 92 days at storage temperature of 50°F (10°C) and 73°F (22.7°C), and the untreated samples spoiled within 3 days at 73°F storage and 12 days at 50°F storage (20). These examples illustrate the beneficial effect of using a combination of treatments such as heat plus chemical additives and heat plus chemical additives plus refrigerated storage. The examples also show the upper limit of temperature that can be used. For peaches 55°C appears to be the upper limit for heat treatment. At higher temperatures the fruit loses its fresh-like character. The primary beneficial effect of heating appears to be a reduction in the initial microbial load, resulting in reduced microbial spoilage, but some degradation enzymes may also be inactivated.

Modification of pH. Modification of pH may be beneficial in extending the shelf life of products which have a natural pH in the range of 5 to 7, where spoilage is rapid. Reducing the pH of vegetables to about 3.5 to 4.0 by the addition of acids will reduce the rate of microbial spoilage or allow for a reduced heat treatment. Acidification of fruits and vegetables often leads to some flavor modification, which limits the use of the treated product to items where slight off flavors can be tolerated.

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Reduction of Water Activity. Reduction of water activity, aw, has been used to preserve many foods (21-24). Various classes of microorganisms will not survive below specific aw levels. Reducing aw in combination with another treatment may extend the shelf life for some fruit and vegetable products. For aw control to be the sole mode of preservation, either the products would become traditional dried fruits and vegetables, or some additive, such as a salt or a sugar, would be required in such large amounts that the products would no longer have fresh like quality. Removing the moisture from products or adding a material to them that reduces their water activity will also reduce their freezing temperatures. This provides one technique for exploiting the shelf life enhancement of very low temperature refrigeration, which was previously described.

Ionizing Radiation. Ionizing radiation is often cited as a potential treatment for extending the shelf life of fresh foods. For fruits and vegetables ionizing radiation sufficient to kill microbial organisms usually diminishes shelf life, because it induces tissue softening, and hence loss of textural quality, at dose levels required to prevent microbial spoilage (25).

Controlled/Modified Storage Atmospheres. Controlled atmospheres, (CA), and modified atmospheres, (MA), are widely used to extend the storage life of fresh fruits and vegetables (26). Controlled atmospheres are maintained at predetermined concentrations of gases by removing components that build up, such as $CO_2$, and adding components that are depleted, such as $O_2$. Modified atmospheres are those that form within a closed container due to interaction between the atmosphere and the product. Respiration by the products consumes $O_2$ from the storage atmopsphere and replaces it with $CO_2$. The equilibrium gas concentrations in a modified atmosphere depend upon the nature of the product respiration, the initial composition of gases in the container, and the relative permeabilities of the container for the different gases.

In CA/MA storage the $O_2$ and $CO_2$ levels are adjusted to inhibit certain degradative reactions, related to respiration, senescence, and tissue softening. The optimal levels of $O_2$ and $CO_2$ must be established for each commodity, and this has been done for many fresh fruits and vegetables (27).

In the application of CA/MA all quality factors must be considered. Apples have been shown to lose flavor when $O_2$ is held too low for extended periods of time (3) When $O_2$ becomes too low or $CO_2$ becomes too high in a storage

atmosphere the product may undergo anaerobic respiration, leading to undesirable flavors and aromas (4).

If CA/MA can be used to extend the shelf life of lightly processed products for only a few days it may have economic significance. Currently there is much interest in using CA/MA within packages of fresh fruits and vegetables during distribution, and this preservation concept is being applied to lightly processed products also. This approach to maintaining quality through the marketing chain will receive continued interest in the future, (1, 28-31).

Maintaining precise control of atmospheres within small packages may be difficult to achieve, but controlled atmosphere shipping vans have been available for a number of years. When lightly processed fruits and vegetables are to be shipped in bulk, for subsequent remanufacturing or further processing, CA/MA may be used for the entire shipping container.

The use of CA/MA will become more widespread in the lightly processed foods industry as optimal CA/MA conditions are established for each product and as methods for attaining and maintaining these conditions are developed. The CA/MA approach to preservation is receiving much attention because of its potential efficacy and because it is considered innocuous to the product. It leaves no potentially harmful residues on the products. For some applications, however, CA/MA must be applied with extreme caution. Low acid foods held under atmospheres approaching anaerobic conditions may support growth of pathogens while inhibiting growth of spoilage organisms. For any application of CA/MA to lightly processed foods a thorough microbiological analysis of the system should be made.

Summary.

Lightly processed fruits and vegetables are new forms of fruit and vegetable products intended to meet the consumers desires for convenience and fresh-like quality. Lightly processed products are highly perishable, more so than the fresh materials from which they were made. The quality factors that are important for lightly processed products are: flavor and aroma, texture, and appearance, and the shelf life is determined as the time required for any one of these factors to become unacceptable. A variety of potential treatments exist to maintain one or more of these quality factors, but a treatment to preserve one quality factor will often be detrimental to another factor. The key to designing practical light processing systems is to select a set of treatments that will

uniformly preserve all quality factors while maintaining overall fresh-like quality in the products.

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# Chapter 17

# Chemistry and Safety of Acidified Vegetables

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Acidified vegetables means low acid vegetables to which acid(s) or acid food(s) are added; these vegetables include, but are not limited to, beans, cucumbers, cabbage, artichokes, cauliflower, peppers and tropical fruits, singly or in any combination. Acidified vegetables have a water activity greater than 0.85 and a finished equilibrium pH equal to or smaller than 4.6. Acidified vegetables may be called, or may purport to be "pickles" or "pickled". Increasing the acidity of canned vegetables reduces required processing times and temperatures, thus contributing to enhancement of flavor, color, texture and nutritive quality. Addition of organic acids improves the microbiological, color, structural and flavor stability of select fresh, minimally processed or canned vegetables.

One important step in the development of the canning process was the recognition that adding acid to the brine of canned vegetables to a pH of less than 4.6:

1) reduced the thermal death time of vegetative cells;
2) inhibited the germination and growth of microbial spores;
3) inhibited enzyme activity and stabilized color and flavor quality; and
4) prevented the germination and outgrowth of _Clostridium botulinum_ spores.

Food and Drug Administration (FDA) Good Manufacturing Practices make significant distinctions between acid, low acid, and acidified foods based on a finished equilibrium pH of 4.6.

## Acidified Foods

Acidified foods are defined as "low acid" foods to which acid(s) or acid food(s) are added; these foods include, but are not limited to

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beans, cucumbers, cabbage, artichokes, cauliflower, onions, peppers, tropical fruits and fish, singly or in any combination. Acidified foods have a water activity greater than 0.85 and a finished equilibrium pH of 4.6 or less (1).

Spoilage Microorganisms. The spoilage organisms of concern in acidified foods are aciduric bacteria with no special heat-resistant qualities (2). *Bacillus coagulans*, an aciduric, anaerobic spore-former, is responsible for flat-sour spoilage of tomato products (3). *Clostridium pasteurianum* is also a troublesome aciduric, anaerobic sporeforming microorganism in acidified foods. *Clostridium pasteurianum* produces butyric acid and gas that will swell and spoil canned acid foods. Vegetative cells of *Clostridium pasteurianum* are heat labile in acidified vegetables.

*Clostridium botulinum* growth normally does not occur in foods with a pH less than 4.8, yet the FDA and the food processing industry historically recognized the discriminatory pH inhibiting *Clostridium botulinum* growth as 4.6. The discriminatory pH of 4.6 established by regulation is an equilibrated pH in the finished vegetable product as determined from a ground or pureed vegetable. Townsend et al. (4) reported a minimum of ten days are required for *C. botulinum* growth at a pH of 5.0 in vegetable products.

However, pH is not the overriding factor for growth of all microorganisms, especially in relation to vinegar and acetic acid (5). Lactobacilli, Leuconostoc, yeasts and molds may proliferate in acidified vegetables depending on pH, may spoil the vegetable food, but will not endanger the health of the consumer.

Vegetables have commonly been fermented to produce pickles or pickled foods, or acidified directly by the addition of lemon juice or vinegar. Recent economic instability in the Pacific Northwest, occurring simultaneously with excellent yields of many low acid vegetables, has resulted in entreprenurial development of many small regional vegetable producer/processors. Many of the entrepreneurs are marketing conveniently processed acidified vegetables - asparagus, beans, onions, garlic, peppers and/or tomatoes.

Purposes. The primary purpose of acidifying low acid vegetables to a finished equilibrium pH of 4.6 or less is to prevent the growth of *Clostridium botulinum* and to reduce the thermal resistance of microorganisms which in turn reduces processing time and temperature. Acidifying vegetables permits thermal processing in a boiling water bath at atmospheric pressure rather than processing under steam pressure. The advantages of shortened processing times and reduced processing temperatures are exhibited as acceptable uniform texture, color, flavor and nutritive value of vegetables. Many of the advantages could not be achieved with thermal processing that accompanies application of steam pressure. Potential advantages other than quality and safety include reduced energy costs and increased production rates.

Acidulants are added to food directly or indirectly for more than 25 separate purposes (6). Gardner (6) suggests the major functions of acidification are to enhance flavor and reduce thermal processing time. Tartness, if not excessive, adds subtle character to the overall flavor of vegetables. Acids also intensify some

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flavoring agents, enhance aromas, act as flavor fixatives, and provide seasoning or blending properties that result in uniformity of taste. Flavor enhancement characteristics of acids are often related to both the hydrogen ion and undissociated acid concentration in solution (7,8).

Thermal resistance of microorganisms and other living organisms is reduced as acidity increases and pH decreases (4). Shillinglaw and Levine (9) demonstrated that acetic acid is more growth inhibitory and bacteriacidal than citric acid at comparable pH. Edible organic acids are more toxic to bacteria and yeasts than highly dissociated inorganic acids such as hydrochloric acid at the same pH. Acetic acid toxicity for various microorganisms is not confined to hydrogen ion concentration alone, but seems to be a function of the undissociated acetic acid molecule (5).

Contamination of fruits and vegetables by vegetative microorganisms is often accompanied by spores. Microbial spores are very resistant to thermal inactivation. Acids contribute a preservative capacity in addition to reducing thermal resistance, inhibiting the germination and growth of spores. The addition of acids to vegetables is only an aid to thermal processing, and is not a solution to heavy microbial contamination or inadequate sanitation (6).

Acidulants also act as synergists to antioxidant inhibition of rancidity, slowing catalysis of oxidation by chelating metals and providing a reducing environment of hydrogen ions which serve to partially regenerate antioxidants. Inhibition of oxidation results in stabilizing of food colors, reducing turbidity and stabilizing flavors (6). Acidulants inhibit enzymes by reducing pH below the optimum for activity of specific enzymes. Enzymatic discoloration can be controlled by the addition of acids to vegetables (10).

Acidulants are also added to foods for several other specific applications. Acids protonate galacturonic acid to enhance gelling of pectin, fruit and sugar mixtures. Leavening acids promote the production of carbon dioxide from sodium bicarbonate. Acids promote crosslinking of cereal proteins to enhance the texture of baked goods. Ascorbic acid and phosphoric acid often provide nutrients for microbial growth during fermentations. Acids can act as emulsifying agents in combination with alcohols and/or monoglycerides. Acids modify the consistency or texture of foods by chelating minerals and enhancing crosslinking of proteins. Addition of acids to sucrose solutions results in production of invert sugar and increases the sweetness of the solution.

Acidulant use. Acetic, citric and phosphoric acids are the most commonly used acidulants, although lactic, fumaric, adipic, succinic, malic, benzoic, tartaric, ferulic and gluconic acids are sometimes used. Lemon juice, 5% citric acid, and vinegar, 5% acetic acid, are the most commonly used natural acidulants. The acidity of fermented vinegars is often questionable, and should be determined prior to use as an acidifying agent. The acidity of juice from fresh lemons may vary from 4.0 to 8.0% expressed as citric acid, and should also be used carefully.

Comparison of the physical and general properties of commercially available food acids is often necessary. Acid

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solubility in water (6), dissociation (Table I) and flavor are the most relevant properties to successful food acidification. Solubility of food constituents in acid solution may also be important. For example, the solubility of rutin is apparently less in solutions of acidified asparagus than in the brine of canned asparagus, so undesirable rutin precipitation in acidified asparagus is prevalent and difficult to control (11).

Table I. Dissociation constants of organic acids

| | |
|---|---|
| Citric Acid | $K_1$ - $7.1 \times 10^{-4}$ |
| | $K_2$ - $1.7 \times 10^{-5}$ |
| | $K_3$ - $6.4 \times 10^{-6}$ |
| Acetic Acid | $1.8 \times 10^{-5}$ |
| Ascorbic Acid | $K_1$ - $7.9 \times 10^{-5}$ |
| | $K_2$ - $1.6 \times 10^{-12}$ |
| Benzoic | $6.5 \times 10^{-5}$ |
| Lactic | $8.4 \times 10^{-4}$ |
| Malic | $K_1$ - $3.9 \times 10^{-4}$ |
| | $K_2$ - $7.8 \times 10^{-6}$ |
| Succinic | $K_1$ - $6.9 \times 10^{-5}$ |
| | $K_2$ - $2.5 \times 10^{-6}$ |
| Tartaric | $K_1$ - $1.0 \times 10^{-3}$ |
| | $K_2$ - $4.6 \times 10^{-5}$ |

Vegetables. Vegetables are generally low acid foods with a pH greater than 4.6. Vegetables are acidified to reduce the times and temperatures required to reach commercial sterility, to inhibit enzymatic and nonenzymatic discoloration, and to enhance flavor. Vegetables that are commonly acidified include asparagus, green beans, cabbage, artichokes, pimentos, cucumbers, peppers, onions, mushrooms and peppers.

Vegetables are acidified by soaking in an acid solution prior to processing, bulk storage in acid solutions, blanching in acid solution or direct addition of an acid brine. Acidifying soak water with citric or malic acid enhances the quality of canned dry beans (12) and dry lima beans (13). The occurrence of splits, drained weights, shear values, color and taste of dry beans were improved. A 0.25% citric acid soaking solution improved the color of canned lima beans. Acidified soak water improves color by retarding the retention of Fe and Cu salts with sulfides or polyphenols that contribute to discoloration (13).

Bulk storage of snap beans and peas at a pH of 1.2-1.6 in hydrochloric acid solution for 4 mo. resulted in quality vegetables (14,15). Acidified bulk storage was employed to lengthen the processing season and enhance quality. Following acidified bulk storage, blanched peas and snap beans had better quality than unblanched vegetables. Some cultivars of green beans changed color from green to red during acidification. The color change was attributed to changes in leucoanthyocyanins (anthocyanins). Blanched green beans stored in a hydrocholoric acid solution at a pH

of 1.5 changed color from dark green to a light more acceptable green color (15). The color changes were attributed to changes in pheophytins and pheophorbides as described in native chlorophyll. Cauliflower sometimes becomes pink and garlic sometimes exhibits a blue-green color when acidified.

Tomatoes were covered with a hydrochloric acid solution, pH of 1.2-1.6, to inactivate microorganisms and plant enzymes (16). Pre-peeled tomatoes were stored in acid solutions successfully. Fructose and carotene degradation in the presence of oxygen were practical disadvantages. The processing and acidified bulk storage of semifinished fruits and vegetables reduced costs and improved quality (17).

Diffusion. Acid diffusion in pH modified foods was investigated with fluorescent dyes or color indicators (18). Colorimetric pH profiles compared well with electromagnetic measurements. Effective mass diffusivities for acetic, citric, gluconic and phosphoric acids in potatoes were on the order of $10^{-10}$ $m^2$/sec. Diffusion occurs mainly in an unsteady state in heterogeneous, multilayer, cellular systems (19). Diffusion can be seen mathematically predicted by one-dimensional solutions of the second Fick equation:

$$\frac{dc}{dt} = \mathrm{div}(D \cdot \mathrm{grad}\ C)$$

$$\frac{C(y \cdot t)}{C^*} = \mathrm{erfc}\ \frac{y}{2\sqrt{D_a \cdot t}}$$

where:

Da = Apparent Diffusivity
C = Concentration of tracer at height y and at time t

## Acidified Vegetables

Acidified or fermented green beans were observed to have acceptable firmness, color and flavor (20). The acidified and fermented beans were much firmer with less sloughing than conventional canned beans. Acidified or fermented green beans are suitable for use in casseroles and salads.

Palmito, the young tender leaves of select palm trees, often called heart-of-palm, are acidified with citric acid to inhibit discoloration of the naturally white leaves and inhibit C. botulinum growth (21). Diffusion of citric acid into palmito required more than 33 days; vacuum acidification and biological transport of acid into palmito were unsuccessful.

Processing, type of acid, ripeness, post-harvest holding period, acidification technique and peeling method influence the quantity of acid required to acidify pimiento peppers to a given pH (22). Several organic acids have been evaluated as acidulants for pimientos. Fumaric acid was most effective for reducing the pH of pimientos (23) and tomatoes (24). Succinic acid imparted undesirable flavors when added in large quantitites. Powers et al. (25) reported acidification did not affect the color of pimientos,

but did modify the flavor. Acidification increased the drained weight and firmness of canned pimientos (25,26). Addition of an acidulant and calcium chloride resulted in a highly significant synergistic increase in drained weight and firmness of canned pimientos. Addition of 0.05% monosodium glutamate (MSG) to acid-treated pimientos increased organoleptic acceptability slightly (25). Pimientos of different maturity exibit different pH after processing when acidified by immersion, whereas pimientos acidified in the jar exhibited no pH differences due to maturity (27). An equation predicting pimiento pH from acid bath concentration, temperature and duration of dip was used to illustrate that acid bath concentration was by far the most important determinant. Some garden grown peppers are more highly buffered than others, requiring additional acid to reach an acceptable pH for acidified home-canned peppers (28). The addition of no less than 1 tablespoon of vinegar (5% acetic acid) per pint (15 mL/473 mL)of canned peppers was recommended.

Mushrooms, pearl onions and cherry peppers were packed with sufficient citric or acetic acid to reach an equilibrium pH of 4.8 (29). Equilibration with acetic acid required 1 day for mushrooms, 15 days for onions and 30 days for cherry peppers. Larger equilibration times were required for citric acid. The time required for the pH inside the vegetables to decrease to 4.8 or less was a function of acid type, initial acid concentration and the vegetable being acidified.

Crispness of cucumber pickles is an important quality characteristic (30), whether the pickles are derived from a genuine brine-stock fermentation or from the fresh-pack process. Enzymatic softening of cucumber pickles is inhibited by acidification, but nonenzymatic softening is enhanced by addition of acids. Oxalic acid reached an equilibrium very quickly in cucumbers, but was very damaging to texture. Acetic and lactic acid equilibrated more slowly than oxalic acid, but still reduced pickle firmness. Citric acid penetrated at the slowest rate and also reduced firmness. Enhanced softening at reduced pH was ascribed to hydrolytic cleavage of glycosidic bonds in the neutral carbohydrates of the cell wall (31). Enhanced softening at neutral pH was ascribed to polymer cleavage, in this case through beta elimination reactions involving pectins (32). Mold-induced softening of cucumber pickles was inhibited in fermentations reaching an equilibrium pH of 4.0 or in fresh-pack pickles with addition of 0.16% acetic acid (33).

Direct acidification of cabbage with acetic, citric, glucono-delta-lactone and lactic acids produced saurkraut-like products with diminished flavor and aroma (34).

Acidification of canned tomatoes with citric, fumaric and malic acids reduced pH effectively (24). Fumaric acid was most effective. Adipic acid was least effective. Succinic acid was an effective acidulant, but induced an undesirable taste. Acidulation tended to reduce pH more when salt was added to tomatoes than when salt was omitted. Acids also tasted less sour in the presence of salts.

Acidification of mung bean sprouts with acetic, citric, gluconic, lactic or malic acid enhanced texture (35). Treatment of mung bean sprouts with acetic acid was detrimental to flavor. The best acidified mung bean sprouts were obtained by acidification with gluconic acid (35).

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Addition of 1% or 3% ascorbic or citric acid to processed sweet potatoes did not improve the color (36). With a pH change from 8.0 to 3.0 elicited by a citrate buffer, sweet potatoes' color and attractiveness improved (37).

## Summary

Acidified vegetables provide the consumer with a safe, wholesome, quality food; and the entrepreneur producer/processor with a convenient way to market a regional low-acid vegetable.

Standardized lemon juice, citric acid solutions or vinegar appear to be the most convenient and effective acidulants. Acidified vegetables equilibrated to a pH of 4.5, or preferably to a pH of 4.0 or less and adequately processed in a water bath will exhibit a firmer texture, increased drained weight, and more stable color and flavor than adequately processed pressure canned low acid vegetables.

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# Chapter 18

# Effect of Freezing Conditions and Storage Temperature on the Stability of Frozen Green Beans

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We have followed biochemical indices of quality in fast or slow frozen blanched green beans whilst in frozen storage at a range of temperatures, and simultaneously determined ice crystal sizes, in order to correlate structural and biochemical indices. Ascorbic acid was determined by HPLC and chlorophyll by spectrophotometric methods to characterise biochemical degradation. Isothermal freeze fixation and SEM were used to visualize the ice crystals in the frozen tissues. Fast freezing gave a higher quality product, but the difference in quality between fast and slow frozen green beans decreased with extended storage times. Storage temperature markedly affected the rate of quality loss. Ice crystals were larger for slowly frozen materials and for materials stored at higher temperatures, even if these were initially rapidly frozen. Cryomicroscope observation confirmed that slow freezing extracted more water from cells, leading to large extracellular ice crystals. Fast freezing led to less cell dehydration, and smaller intracellular ice crystals.

It is well-known that the freezing process is very complex and many interrelated changes take place. During subsequent frozen storage even more changes occur. Our understanding of many of these changes is still incomplete. In food systems, the overall result of several of these changes is collectively referred to as "freezing damage". Freezing damage is a result of ice crystals forming and rupturing membranes, of concentrated electrolyte solutions denaturing proteins, of pH changes denaturing proteins, of the constituents of enzymatic reactions being dislocated, of protein denaturing at ice/solution interfaces, of intracellular ice damaging organelles and of non-enzymatic reactions happening, etc.

Much of the background knowledge of freezing in use today dates back to the 1960's. Objective tests to measure adverse

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changes in several frozen vegetables were studied by a Dietrich and others in USDA, Albany (2,5-9) . Their data revealed that the deterioration followed a well defined course that is described by loss of reduced ascorbic acid, conversion of chlorophyll to pheophytin, changes in color and flavor. In addition, storage temperature is very important, the rates of chemical reaction which cause changes in quality are related exponentially to temperature. Deterioration is negligible at -29°C; and at -18°C the products which are initially of high quality maintain good quality for about a year. As the temperature is increased above -18°C, storage life becomes more and more limited. In these studies commercial products were used, and consequently the effects of freezing conditions on storage life were not investigated. Since then several conflicting statements have been published regarding the effect of freezing conditions on the quality of frozen fruits and vegetables, Jul,(10). When products are frozen rapidly, numerous small ice crystals are formed, propagating from many active growth centers under conditions where the movement of water molecules may be limited. In slow freezing, where there is adequate time for the translocation of water, a smaller number of large ice crystals are formed, propagating from fewer active growth centers. Where the barrier properties of cell walls and cell membranes are contributory factors, in fast freezing nucleation takes place within cells, in slow freezing nucleation is external to cells. Where no such barriers exist, the nucleation centers in fast freezing are apparent some distance into the freezing body, whereas in slow freezing the nuclei are close to the heat exchange surface. The effect of slow freezing on plant cell structure appears to be greater than the effect of fast freezing, Brown (4). However, it has been claimed that this structural difference between slow freezing and rapid freezing has very little or no effect on the final quality of most frozen foodstuffs. To evaluate the effect of different freezing conditions on the quality of frozen vegetables, it is useful and realistic to determine both the changes after initial freezing and during frozen storage. The advantages in quality of some products which might initially be achieved by rapid freezing could be subsequently lost during frozen storage, (10).

This study investigates whether different freezing conditions have any effects on the quality (as indicated by such biochemical changes as loss of ascorbic acid, alteration of chlorophyll) of frozen green beans after freezing and during storage at different temperatures and whether any changes in biochemical indices of quality are related to the structure of these frozen tissues, particularly the size of ice crystals formed. This requires that the chemical analysis and the microscopic technique for determination of ice crystal size in frozen samples be performed on samples taken at the same time. Some cryomicroscopic observations of plant cells are included to help illustrate the different processes of ice formation in the plant cells during freezing under different cooling regimes.

MATERIALS and METHODS.

Sample Preparation. A batch of green beans obtained as fresh, Blue Lake Variety, were processed in our pilot plant. Blanching utilized a steam blancher (98°C, 1.5 minute), with immediate cooling by immersion in ice water. It was followed by either fast freezing or slow freezing. Fast freezing used a Conrad Blast Freezer with an air blast temperature of -69°C, and a downward air velocity of 200ft/min. Freezing to a center temperature of -69°C took around 1.5hr. Blanched green beans were frozen unwrapped in a single layer on open mesh trays in order to allow proper air circulation. Immediately after freezing they were put into polyethylene (PE) bags, flushed with nitrogen gas and sealed and then were boxed for storage in cold rooms. Slow freezing was in still air in cold room (-20°C, 24 hrs). In this case the materials were put into PE bags, flushed with nitrogen gas and sealed prior to freezing. The beans in the bag were arranged in a flat, thin layer. Freezing to a center temperature of -20°C took around 14hr.

Frozen Storage. Frozen green beans were moved into storage at -20, -12 and -5°C. The changes which occur during freezing and frozen storage were assessed, with samples being taken immediately after freezing, and also being removed from storage for evalution at suitable time intervals.

Thawing. Since the method of thawing a frozen product can greatly impact the quality, one standardized thawing method was used. This was chosen for ease of repeatability, rather than as an optimum thawing procedure. An alternative would have been to cook beans directly from the frozen state to evaluate cooked quality. Frozen samples were thawed in plastic bags immersed in a cold water bath (4°C) for 1.5 hour.

Quality assessment : Biochemical Changes.

Ascorbic acid. Frozen samples were thawed and then homogenized in an extracting medium (0.01 N $H_2SO_4$ + 0.3% metaphosphoric acid). The resulting homogenate was then centrifuged (5000 rpm for 3 minutes). The supernatant fluid was filtered through 0.2 micron membrane prior to injection into a HPLC. An Aminex HPX-87 column with a Micro Guard ion exclusion cartridge was used. The mobile phase was 0.01 N $H_2SO_4$ and the detector was an ISCO V4 UV Absorbance detector set at 245 nm.

Chlorophyll. A spectrophotometric procedure described by Vernon (14) was followed except the thawed samples were homogenized in an cold environment. A Varian DMS 100 UV Visible Spectrophotometer with automatic recorder was used for absorbance readings at the different wavelengths of 536, 645, 662 and 666 nm.

Quality assessment : Structural Changes.

Ice Crystal Size Determination. Frozen green beans were taken from storage at times which corresponded to the biochemical assay points and put into the isothermal freeze fixative, Asquith and Reid (1).

This fixation was followed by washing, dehydration, sectioning, critical point drying and sputter coating. The size of ice crystals in the frozen samples can be qualitatively determined and compared by Scanning Electron Microscopy (SEM) of these prepared materials. Unambiguous measurement of ice crystal sizes has not, as yet, been achieved by this technique, as it can be difficult to differentiate between some of the smaller crystals, and the green bean cells. In appropriate circumstances it is possible to identify ice crystals unambiguously by freeze etch techniques, Bomben (3), but these do not allow for investigation of ice at higher temperatures. Also, Bomben determined dendritic spacings for directional freezing, rather than actual ice crystal numbers and sizes. Appropriate image analysis methods to identify all ice crystals in the green bean sections still have to be developed.

Cryomicroscopic studies on the relationship of freezing rate to ice formation in intact cells. As cultured green bean cells were not available, the processes of cell freezing were visualized on a cryomicroscope, Reid, (11,12) using suspension cultured pear cells. The pear cells, preequilibrated in the standard liquid growth medium, were placed on the cryomicroscope stage. One of several cooling rates was selected. Ice crossed the field of view of the microscope at the freezing point of the medium. Fast freezing of the cells (with intracellular ice formation) and slow freezing (with cell dehydration and consequently more extracellular ice formation) could be clearly observed and recorded by the video camera attached to the cryomicroscope and also by photomicrography. The time sequence of events was recorded, and the sequence of photomicrographs provides an illustration of the possible effect of different rates of freezing on the location of ice crystals, and the extent of cell dehydration in a tissue.

## RESULTS and DISCUSSION.

Biochemical studies. The preliminary experiments on one batch of frozen green beans employing HPLC for determination of ascorbic acid retention revealed that rate of ascorbic acid loss is temperature dependent and is most rapid at high temperature storage. This finding agrees well with the previous studies done by Dietrich and co-workers (5). As can be seen from Figures 1(a), 1(b) and 1(c), ascorbic acid retention went down to below 30% within less than a month in -5°C storage as compared to almost one year for a similar reduction in -20°C storage. Fast freezing in general appears to yield greater ascorbic acid retention and this advantage is retained to some extent during storage. The difference in effect of freezing rate is less pronounced in samples stored at higher temperature (-5°C), where the fast frozen samples average 10% of initial ascorbate content greater than slow frozen samples. Looked at over all storage regimes, the retention of ascorbic acid in fast frozen product is greater by 14 ($\pm$7)% of the initial ascorbate content than that of the slow frozen product.

Figures 2a, 2b and 2c illustrate the changes of chlorophyll a in frozen green beans stored at -5, -12 and -20°C respectively. Chlorophyll b did not show such a marked decrease as chlorophyll a. The results on chlorophyll a retention also show the parallel

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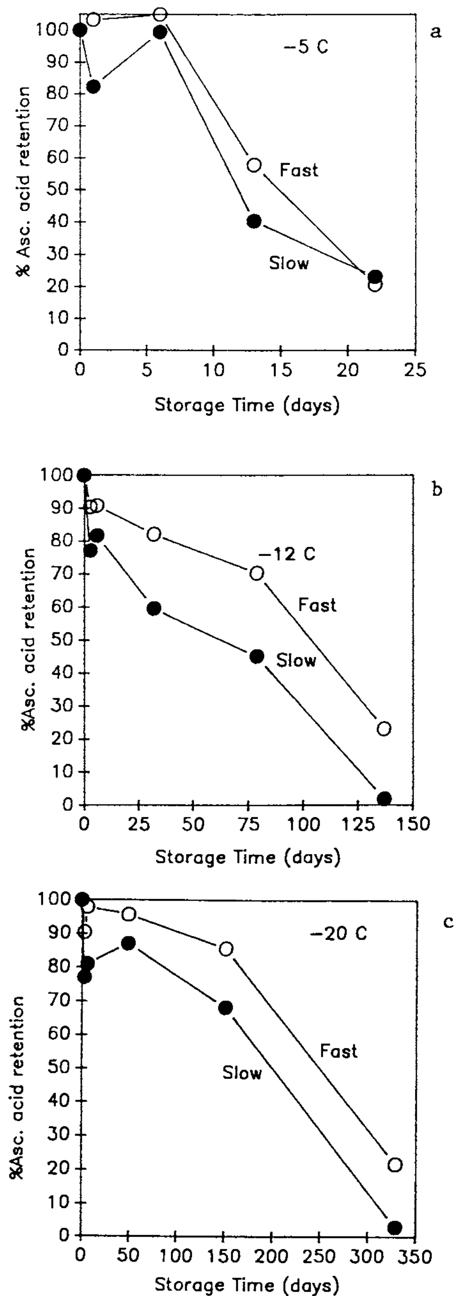


**Figure 1. Ascorbic acid retention in fast and slow frozen green beans stored at (a) -5, (b) -12, and (c) -20°C.**

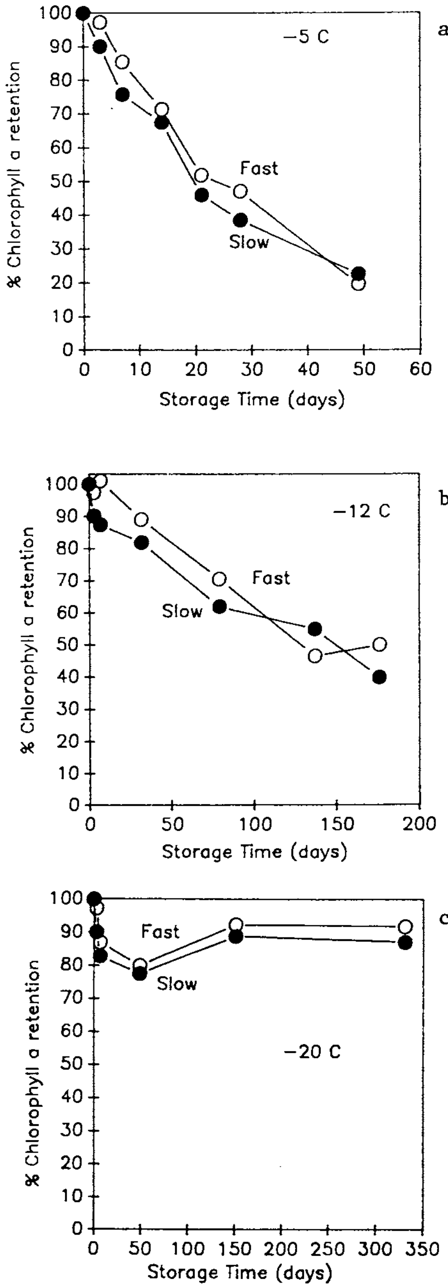


**Figure 2. Chlorophyll a retention in fast and slow frozen green beans stored at (a) -5, (b) -12, and (c) -20°C.**

effect of storage temperature. Evaluating over all storage times, the retention of chlorophyll in fast frozen product is greater by 5.5 (± 5)% of the initial content than that in slow frozen product under the same storage regime.

SEM studies. The photomicrographs of frozen green beans prepared by isothermal freeze fixation and SEM techniques are shown in Figures 3 (a-l). It is clear that, as expected, rapid freezing produces a large number of small ice crystals and that slow freezing produces smaller number of larger ice crystals in freezing comparable green beans (as compared in Figure 3a vs 3b, 3c vs 3d, 3e vs 3f). The difference in fast and slow freezing is more pronounced in unblanched samples (as compared in Figures 3g vs 3h). This is probably because fast freezing causes intracellular ice formation especially in intact cells (see cryomicroscopic studies section). As compared by the set of figure 3a vs 3c, fast frozen green beans stored at lower temperature (-20°C) contain smaller ice crystals than those stored at high temperature (-5°C). The slow frozen samples show slightly less difference in the effect of storage temperature, figure 3b vs 3d. As can be seen from the set of figures 3c, 3e vs 3d, 3f, the difference in size of ice crystals for fast and slow frozen samples stored at -5°C decreases for a storage time of 24 days as compared to 9 days. The overall size of ice crystals; of course, increases with time of storage. A similar conclusion can be reached for similar samples stored at -20°C, (see figures 3i, 3k vs 3j, 3l) though the time scale for change is much longer. These beans were from a different batch , and show differnt absolute sizes than the first batch, but the same progression in relative size changes. The gradual reduction in the structural difference between fast and slow frozen samples mirrors the reduction of the difference in the biochemical indices with time of storage. Fast freezing gives a slightly higher quality in both biochemical and structural aspects, but the difference in quality between fast and slow frozen materials decreases with extended storage times. The difference in the effect of freezing rate is less pronounced at higher storage temperature.

Cryomicroscopic studies. Since fast and slow frozen green beans showed different patterns of ice formation, it was helpful to use a cryomicroscope as a tool to observe the freezing of isolated pear cells in standard liquid growth medium. The photomicrographs taken from the cryomicroscope during the freezing events of pear cells under two different cooling regimes are shown in Figures 4(a-h). When the sample on the cryomicroscope is sufficiently cooled, ice forms first in the external medium, 4a, 4e. When intact cells are surrounded by frozen medium, they will tend to lose water through osmosis. If sufficient time is available, the internal cell contents will attain a concentration with the same water activity as the frozen external medium. If, however, the water loss is not sufficiently rapid, the internal contents will be more dilute than is required for equilibrium. They are, then, effectively undercooled. In rapid cooling, this undercooling may increase, and may exceed a critical undercooling, which allows the cell contents to nucleate and freeze internally. Internal freezing is easily observed on the cryomicroscope, as it is associated with flashing,

a sudden darkening of the cell contents. The freezing condition that results in internal freezin can be termed fast freezing. Figures 4(a-d) show the sequence of the process of fast freezing of pear cells cooled at 40°C min$^{-1}$. This is rapid enough to give internal freezing, as evidenced by the darkening in the cells. When the rate of change of temperature is slower, the osmotic transfer of water may keep the cell contents sufficiently close to equilibrium so that the critical undercooling is not exceeded. The water which transfers across the cell membrane is incorporated into extracellular ice crystals, and the cells shrink. This is slow freezing. Figures 4(e-h) show the sequence of slow freezing of pear cells cooled at 4°C min$^{-1}$. The shrinkage can be seen, there is extensive extracellular ice growth, and the cells remain unfrozen internally. By following the time course of the freezing events under different cooling regimes, the effective rate of osmotic transfer can be deduced, Reid and Charoenrein (13). This cryomicroscopic illustration of the effect of freezing rate is appropriate. The frozen structure (ice crystal size and location) in frozen green beans especially unblanched tissues (Figures 3g and 3h) i.e. fast frozen unblanched tissues, is small ice crystals formed internally and in slow frozen tissues, is larger ice crystals formed externally. This difference is less obvious in blanched tissues. The cell membrane in blanched tissue loses some of its ability to be a barrier between inside and outside environment.

## CONCLUSION.

There appears to be some correlation between the effect of freezing rate and frozen storage temperature on the structure of frozen green beans and on the change in biochemical indices of quality. Where there exist larger structural differences, there also are larger differences between the biochemical indices. This correlation could be a result of the parallel which exists between the rates of chemical reactions, and the rates of recrystallization processes as a function of temperature, both of which can exhibit Arrhenius behavior. It might also indicate that the frozen structure can influence the diffusional properties of molecules, in particular by modifying diffusional pathways, and hence affect the rate of kinetic processes. Clearly, the gradual elimination of the difference in frozen structure is accompanied by a similar decrease in the difference of biochemical quality. The cryomicroscope observations remind us that the freezing conditions can alter the physical location of water within the product.

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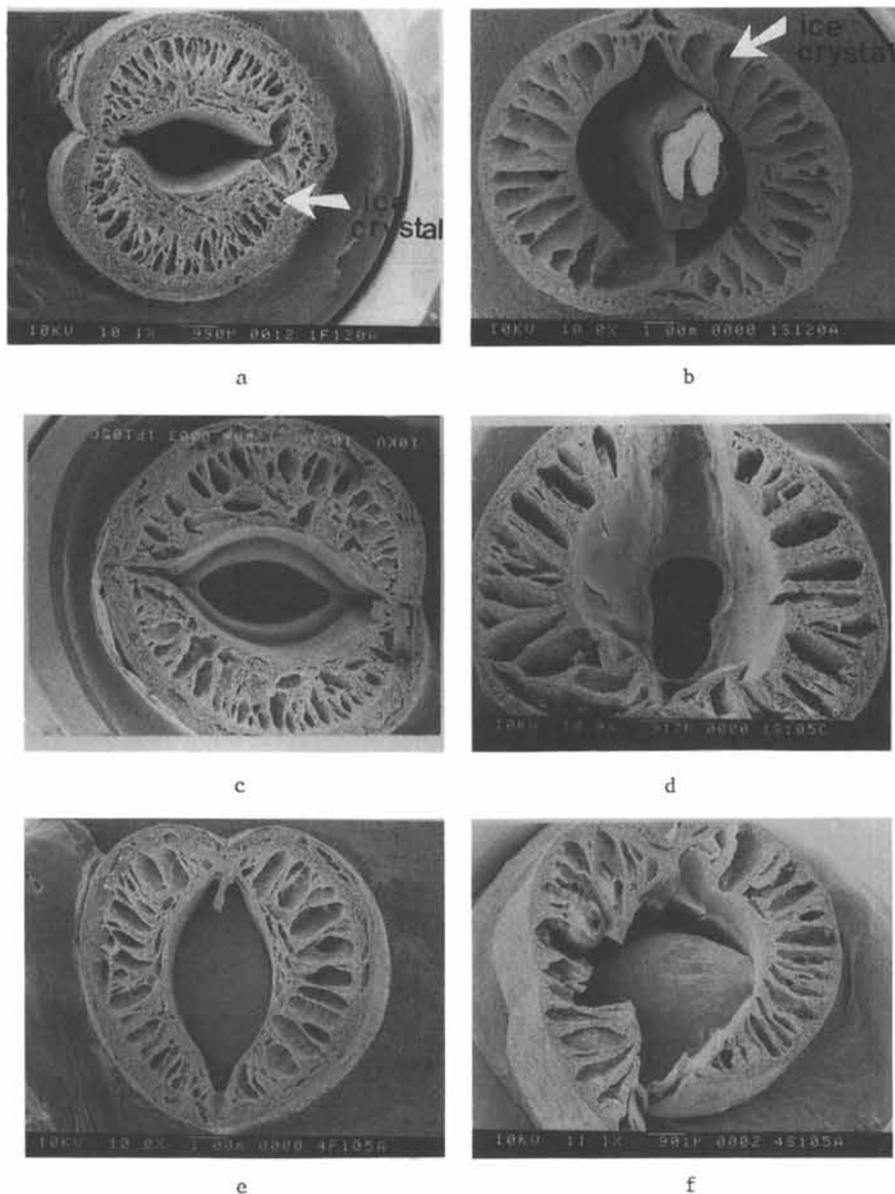


Figure 3. SEM photomicrographs of isothermally freeze fixed frozen green beans. Some ice crystals are identified by arrows. a, Fast frozen, stored at –20 °C for 9 days; b, slow frozen, stored at –20 °C for 9 days; c, fast frozen, stored at –5 °C for 9 days; d, slow frozen, stored at –5 °C for 9 days; e, fast frozen, stored at –5 °C for 24 days; and f, slow frozen, stored at –5 °C for 24 days. *Continued on next page.*

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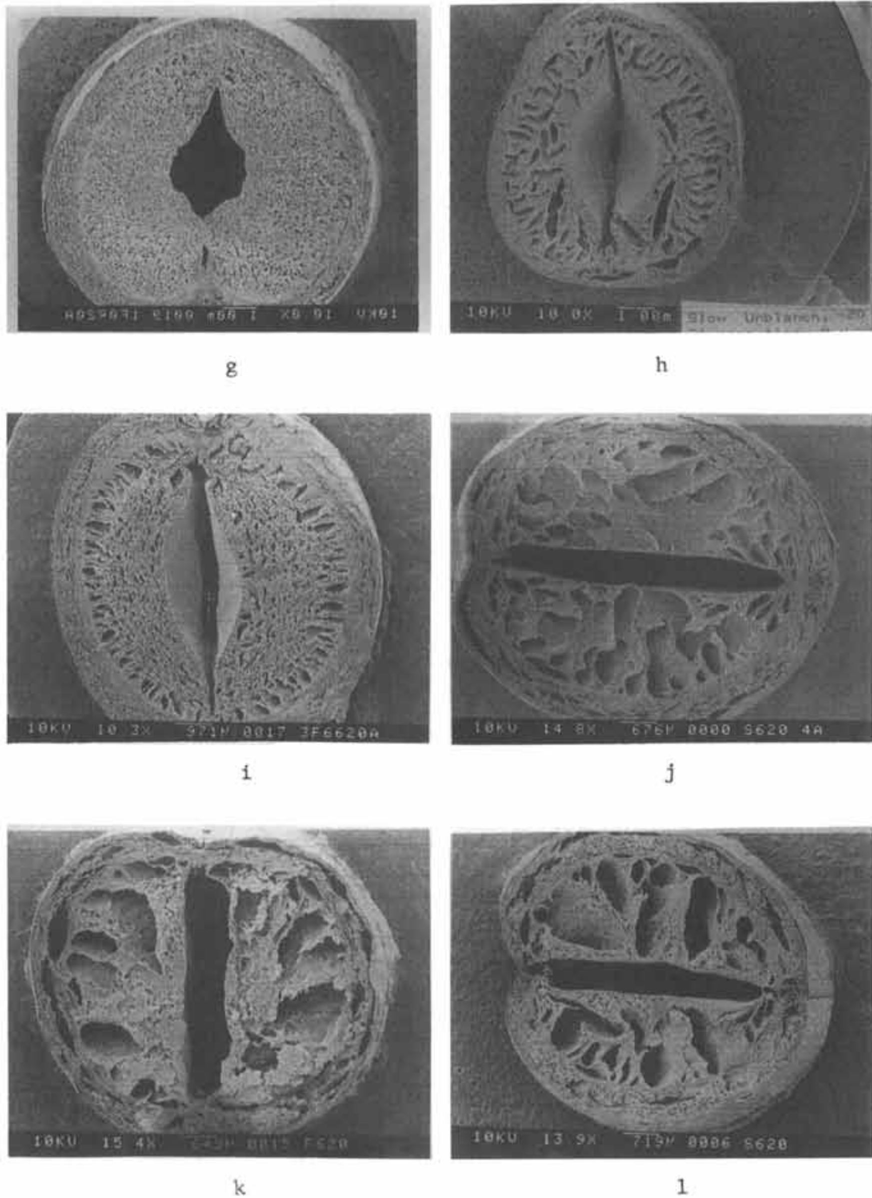


Figure 3. *Contined.* g, Unblanched, fast frozen, stored at –20 °C for 9 days; h, unblanched, slow frozen, stored at –20 °C for 9 days; i, fast frozen, stored at –20 °C for 51 days; j, slow frozen, stored at –20 °C for 51 days; k, fast frozen, stored at –20 °C for 10 months; and l, slow frozen, stored at –20 °C for 10 months.

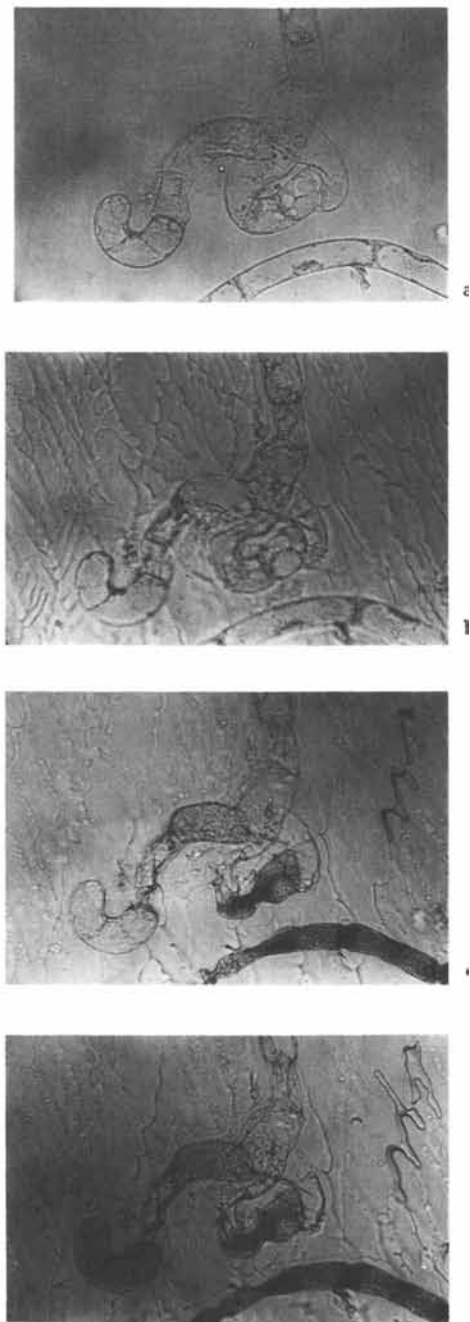

Figure 4. Photomicrographs of pear cells freezing under different cooling regimes on the cryomicroscope stage. a–d, fast freezing at a cooling rate of 40 °C/min. Photographs in time sequence, top to bottom. *Continued on next page.*

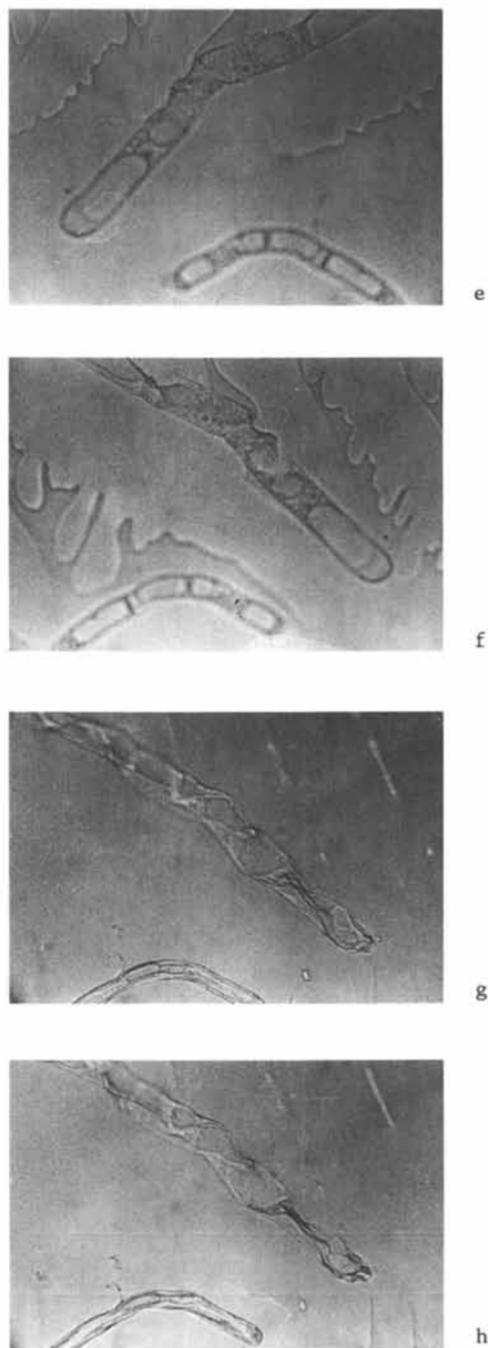


**Figure 4.** *Continued.* **e–h, Slow freezing at a cooling rate of 4 °C/min. Photographs in time sequence, top to bottom.**

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# Chapter 19

# Chemistry and Processing of High-Quality Dehydrated Vegetable Products

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The basis for structural collapse of vegetable tissues on dehydration and approaches to preserve texture and rehydration properties were reviewed. These principles formed the basis for producing high quality carrots and potatoes by a process of biopolymer infusion followed by high temperature short time fluidized bed dehydration. Infused biopolymers was shown to penetrate intracellular spaces and cell walls and may contribute to reduced cell collapse in the dehydration process. Deposition of infused biopolymer within the cells was elucidated using a convalently bound complex of biopolymer and colored dye which was visible upon histochemical examinations under a microscope. The dehydration process was optimized with response surface methodology. The resulting products have excellent quality, high rehydration ratio and a puffed structure.

## The Need for High Quality Dehydrated Vegetables

The current trend towards larger produce sections in supermarkets underscores increasing consumer demand for fresh fruits and vegetables and the willingness of the consumer to pay a premium price for quality products. The perishability of fresh vegetables, however, leaves a niche for processed products in competition for the consumer dollar. Quality conscious consumers demand quality products along with the convenience. Therefore, for processed foods to remain competitive, processes must optimized for quality. An understanding of the fundamental chemistry and physics involved in food processing operations is essential in optimization.

The use of microwave ovens has increased the convenience of dehydrated foods to the same level as that of frozen foods. Dehydrated foods can be produced, packaged and distributed at a fraction of the cost of frozen or canned foods. For these reasons, dehydrated soup mixes and pasta based mixes are one of the fastest

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growing grocery products. These items, which used to be available only in speciality stores catering to the relatively small group who go backpacking and camping, are now regular items on the supermarket shelves. The increasing popularity of dehydrated foods can be attributed to the improved quality of products now marketed in comparison with what was available in the past. Further growth of this sector of the grocery products line requires the availability of high quality dehydrated ingredients suitable for compounding into dinner entrees and soup mixes.

## Physio-Chemical Changes on Dehydration and Effects on Texture

Holdsworth (1) summarized the physico-chemical aspects of dehydrated food products which are important in determining the overall organoleptic quality. These include: structure and composition of the raw material, shrinkage during drying, loss of volatile components, browning reactions and moisture absorption and rehydration. Nutrient loss, degradation of color pigments and overall texture may also be added to this list. Except for structure and composition of the raw material, these factors are directly influenced by the conditions used during the dehydration process.

Structure, composition of raw material and shrinkage during dehydration influence rehydration and textural properties of the product on rehydration. According to Reeve (2), texture of fresh fruits and vegetables is determined by both histological structure, i.e., cell size, intercellular components, cell wall thickness and structure and cell wall composition. The cellulosic microfibrils, which comprises the basic architecture of the cell wall, may include some pentosans, vary greatly in orientation, degree of crystallinity and cumulative thickness. The spaces between the microfibrils are occupied by substances which encrusts the cellulose. These encrusting substances include primarily amorphous polysaccharides pectin and hemicellulose, and lignin, suberins and cutins of varying chemical make up depending upon the type of tissue. In addition, pectic substances and air may be present in middle lamella and intracellular spaces of cells. For example, the cortical zones of apples consist of 20 to 30% of intracellular spaces and is responsible for the spongy texture of apples. It is the reason apples are particularly suitable to be infiltrated by syrups or water during processing (3, 4).

The integrity of the cell wall and the relative mobility of cell wall content upon breakage of the cell wall determine the changes in textural characteristics of vegetables during processing. Texture, according to Brown (5), is the combined effect of the mechanical properties of the cells and contents and the manner of breaking up of the tissue during mastication. When raw, turgor, the internal pressure of the cell contents, allows the cell to resist mechanical deformation and reflect the perception of firmness. The sudden collapse of the cell wall at the bioyield point during the process of mastication results in the perception of crispness. When starch and protein are present inside the cells, they may bind water or hinder its release, therefore, maintaining a firm texture, although a crisp perception may not be

exhibited. The application of heat weakens the cell walls due to dissolution of pectin, resulting in cell break up and loss of turgor or a reduction of the bioyield point resulting in a loss of crispness and the onset of mushiness.

In the process of dehydration, intracellular water is removed, relieving turgor, resulting in collapse of the cell walls. The collapse can be total and irreversible in the case of high moisture vegetables such as celery, tomatoes and lettuce whose cell walls are very fragile, such that the rehydrated product only absorbs a fraction of the original moisture level. For these vegetables, the absorbed moisture of rehydration is only intercellular; therefore, the texture of the rehydrated product is mushy (6).

In a flesh vegetable such as carrots, the lignified cell walls of some of the cells and starch, oil and vacuoles in some cells limit shrinking, and although some cells shrink completely, a total collapse does not occur. The vacuoles and oil droplets inside cells and different degrees of shrinkage which occur among adjacent cells having different cell wall structures, produces intercellular spaces in the dried product (7). In the same study, the author also showed that in a starchy material such as potatoes, the starch content of the cells prevented total tissue collapse although a sizable reduction in cell size occurred as the tissues shrunk during dehydration.

## Formation of Intracellular Voids in Dried Vegetables

Based on these studies in microstructure and the mechanism of moisture removal on dehydration and moisture absorption during reconstitution, good quality dehydrated products which reconstitute well upon dehydration should have the following characteristics: cells must not be totally collapsed, cell walls must remain intact and intercellular spaces must be maintained in the dried product. The latter will allow capillary action to draw the water into the vicinity of the cells during the process of rehydration. Water can then diffuse across the intact cell wall and into the cells to reestablish turgor.

The development of intercellular spaces in the dried product is clearly manifested in the porous structure of freeze dried foods. In general, products which have undergone a minimum of shrinkage during dehydration rehydrate faster because of the presence of well-defined intercellular spaces. These products also have low bulk densities, both an advantage and a disadvantage. Consumers may perceive receiving more product with a higher bulk, but, on the other hand, more packaging is required to deliver a given weight of product.

Rate of moisture removal during dehydration has a strong influence in the shape and histological structure of a dehydrated product. Toledo (8) showed a diagram of the relationship between the moisture content of apple slices and the drying rate in conventional air drying (Fig. 1). At moisture content in excess of 1.0 on a moisture free basis (MFB, g water/g dry matter), water exists as free water and the vapor pressure of water in the product is the same as that of pure water ($a_w = 1$). In this moisture range, dehydration rate occurs in two stages as the moisture

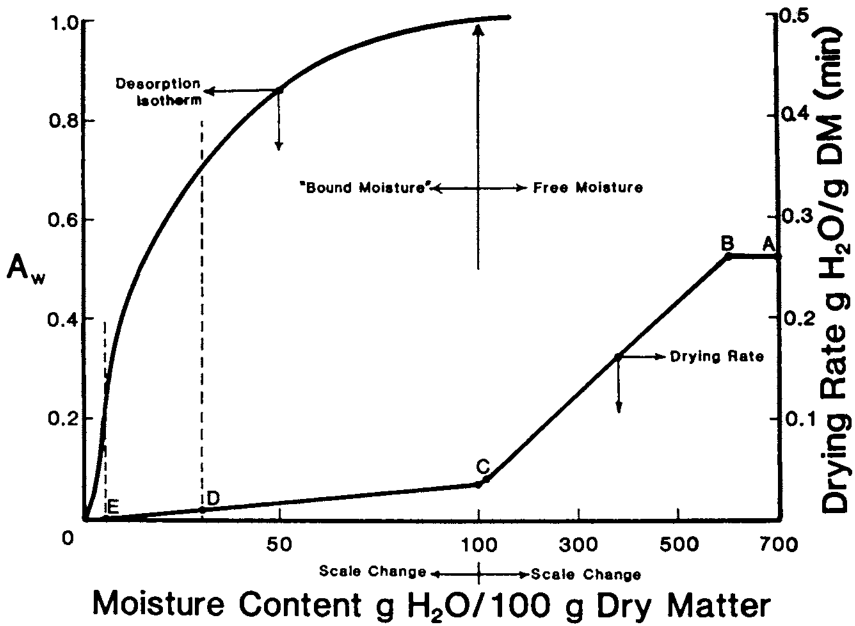


Figure 1. Desorption isotherm and rate of drying of raw apple slices. (Reproduced with permission from ref. 8. Copyright 1980 Van Nostrand Reinhold.)

content decreases. An initial constant rate stage occurs at moisture contents in excess of 6.0 MFB where surface moisture is removed and capillary flow of moisture from the interior of the solid replenishes surface moisture as soon as it is removed. A falling rate stage at moisture contents from 6.0 to 1.0 MFB occurs when the surface dries out and capillary moisture flow is no longer sufficient for the internal moisture to replenish the surface moisture. During this first falling rate period of drying, the surface would appear dry and because of a reduction in turgor with the removal of moisture from the interior cells, a slight deformation may be exhibited. In diced vegetables, the corners dry first and establish rigidity and as the interior sections lose moisture, causing the cells to shrink in size, the faces of the dice are drawn in exhibiting a concave appearance. When the moisture content drops below 1.0 MFB, most of the water is bound. Vapor pressure of water in the product decreases below that of pure water ($a_w < 1$) and a second falling rate period of drying is exhibited. If moisture removal from the surface is slowed such that moisture gradients are relatively low, the rate of shrinkage of cell size is uniform throughout the solid, and the whole solid will shrink into a very small size. The dried solid will have no intercellular spaces; it will be hard and the bulk density will be very high. On the other hand, when drying is rapid, particularly in the first falling rate period of drying, a rigid outer layer first forms. As the moisture content in the interior of the solid is reduced and cell size becomes smaller, the rigid outer layer resists deformation, limiting total shrinkage and intercellular voids are formed. Once the cell walls have dried to form a rigid structure, continued moisture removal can occur without further deformation.

The formation of intercellular voids in the dried product has been exploited by several investigators as a means of increasing rehydration rate of dehydrated foods. Techniques for imparting a porous structure into the dried product other than freeze drying include: high temperature short time pneumatic dehydration by fluidized bed (9, 10, 11) or centrifugal fluidized bed (12, 13, 14) and by conventional air dehydration followed by explosion puffing (15, 16, 17, 18). Sullivan et al. (19) showed that rate of dehydration in the second falling rate period of drying is much faster in apples which was explosion puffed compared to conventionally dried apples. Low bulk densities and rapid rehydration were characteristics common to dehydrated products which were subjected to explosion puffing or very rapid high temperature short time dehydration.

One problem with high porosity in dehydrated foods, in addition to increased packaging requirements, is the possibility of rapid oxidation because of increased surface area of exposure to oxygen if air is present within the pores. An approach which could be used to solve the problem was first suggested by Sinnam et al. (20) when they compressed explosion puffed carrots after dehydration and found that there were no differences in the rehydration rate or rehydration ratio compared to the original explosion puffed dehydrated carrots. This concept has been extensively exploited by the U.S. Army Natick Laboratories (21) in the development of

In Quality Factors of Fruits and Vegetables; Jen, J.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

compact rations by freeze dehydration and compression of vegetables. The process is based on the principle that at a certain moisture content range, the cell wall structure becomes flexible such that the cells can be collapsed without breaking. If the cell walls are collapsed when they are relatively dry, such that they do not stick together, rehydration will not be impeded and the solid swells back to the original shape upon rehydration.

Intercellular voids promote rehydration rate, but integrity of the cell walls promotes moisture retention and re-establishment of turgor upon rehydration. Approaches used to strengthen the cell wall to prevent damage during processing include: addition of calcium salts (22), control of pectic enzyme activity (23), addition of polyhydric alcohols (24), osmotic dehydration (4, 25) and *in situ* polyacrylamide polymerization (26). These approaches result in firmer texture on rehydration but could interfere with moisture absorption resulting in increased rehydration time and reduced rehydration ratio. Osmotic dehydration prior to high temperature short time fluidized bed dedydration has been shown by Kim and Toledo (11) to result in lower rehydration ratio and slower rehydration rate in dehydrated blueberries. Considerable amount of sugar exchange for water was observed in osmosis-vacuum dehydration of apple chips by Dixon and Jen (27). These previous works demonstrated the complexity of interactions among processing parameters in establishing final product characteristics. Improvements in the process by optimization is needed to create high quality dehydrated fruit and vegetable pieces.

## Process Development for High Quality Dehydrated Diced Vegetables

In our laboratory, a combined biopolymer treatment and high temperature short time (HTST) fluidized bed dehydration process was developed for diced carrots and potatoes. Carrots and potatoes were selected as experimental material due to commercial importance of these vegetables. Raw carrots (*Dansus carota*) of a hybrid cultivar and Russell Burbank potatoes (*Solanum tuberosum*) were purchased from local supermarkets. For carrots, the top and narrow bottom parts were removed after washing. The middle portion of the carrots were diced to three-eighth inch cubes in a vegetable dicer (Dito Dean Model TR-22). For potatoes, the skins were peeled before dicing. The carrot and potato dices were treated with a mixture of biopolymers. The treatments included vacuum infiltration, pressure cooking, blanching and dipping. The biopolymers included maltodextrins, pectins, polydextroses, gums and others, singly or in combination. After treatment, the vegetable cubes were first dried in a HTST fluidized bed dryer at a set temperature for a certain time period. The vegetable pieces were dried to a final moisture content of 3-5% in a tunnel drier set at 70°C and at 4 m/sec air velocity. Response surface methodology (RSM) analyses was used to optimize the following parameters: concentration of biopolymer, time of blanching, time and temperature of the HTST fluidized bed dryer. Product quality factors evaluated as responses were good color, high rehydration ratio and low bulk density. The time and temperature were controlled to within ± 5 sec and ± 1°C in all cases.

Literature reports showed that pretreated dehydrated carrots can have rehydration ratio (RR, total mass of rehydrated carrots per unit weight of dry matter) in the range of 5 to 7 (28, 29). The RR was calculated after boiling a known weight of dried carrots in 100 ml distilled water for 30 minutes. Currently commercially available samples tested in our laboratory possessed RR < 6.0. In the new process developed in our laboratory and pilot plant, the dehydrated diced carrots consistently attained RR of 9.5 or above. In addition, these high quality dehydrated carrots have low bulk density and minimum carotenoid loss (Mudahar, G. S. et al., J. Food Sci., In Press).

The theoretical basis of the new process are described in previous paragraphs. Incorporation of natural biopolymers into the cell structure may strengthen the cell walls and/or fill the intracellular spaces to prevent total cell collapse in the dehydration process. Furthermore, rapid drying in a HTST fluidized bed dryer develops a porous structure which may enhance water penetration on rehydration.

A three level four factor three response design of RSM was employed to optimize the carrot dehydration process. The four factors were drying temperature in HTST fluidized bed dryer, exposure time in HTST fluidized bed dryer, concentration of biopolymers and blanching time. The three responses were rehydration ratio, bulk density and carotenoid loss during the process. Second degree polynomial equations and Statistical Analysis System were used to fit the data for RSM.

Rehydration ratio was measured as previously described. Bulk density was determined as weight per volume basis (11). Carotene contents were determined by solvent extraction and spectrophotometric measurements (30). The amount of carotene loss was calculated according to the established formula (31).

In addition to high rehydration ratio of 9.5 or above, the rehydrated carrots have excellent color and texture approaching that of blanched fresh carrots. Analyses by RSM showed that the important factors in the process are the time and temperature of the high temperature drying step. For three-eighth inch carrot dices, the optimum conditions are 150°C and 12.5 minutes. The biopolymer treatment was important in minimizing the carotenoid degradation and in providing superior texture to the rehydrated products. A blanching time of 12 minutes and a biopolymer concentration of 1.4% were determined to be optimum for these three-eighth inch carrot dices. These processing parameters may change with different sizes, varieties of carrots or other uncontrollable conditions. However, modelling and optimization can easily be achieved by the RSM procedure. Optimum processing parameters identified by RSM were verified by pilot plant runs to produce dired carrots with characteristics predicted by the RSM model equation.

For dehydration of diced potatoes, a three level four factor four responses design was used. The four factors stayed the same as in the carrot study. The four responses were rehydration ratio, bulk density, non-enzymatic browning and water holding capacity. Optimal conditions were determined to be 145°C and 10 min in HTST fluidized bed dryer with blanching time of 4.5 min and biopolymer

concentration of 1.2%. A superimposed contour plot at T = 145°C and t = 10 min for the four responses is shown in Fig. 2. The optimum processing region is clearly shown by the shaded area in Fig. 2.

## Illucidation of Penetration of Infused Biopolymers in Vegetable Tissue

To observe the movements of the biopolymers into and retention in the vegetable cells, the biopolymers were modified into colored compounds suitable for use as markers in microscopic examinations. The biopolymers were reacted with Remazol brilliant blue R salt according to Rinderknecht et al. (32) with modifications. The reaction created covalent bonds between the blue dye and hydroxyl groups of the biopolymers. The dye-biopolymyer complexes have the same physical properties as natural biopolymers. These types of dyes have been used for cellulose materials (33) and galacto-D-Mannan (34).

Carrots treated with the dye-biopolymer complexes and processed under the optimum condition as determined by RSM were rehydrated and fixed for light microscopic examination. Thin slices of carrots (2-3 mm) were fixed on cork board and frozen in isopentane at -160°C for 15 sec. The frozen samples were warmed up to -20°C in a histostat cryostat microtome chamber and cut into sections of 28 micron thickness. The sections were fixed on slides with glycerol gel and dried in an oven at 35°C to remove any air bubble under the slides. The slides were examined and photographed under a light microscope at 500 and 785 times magnification.

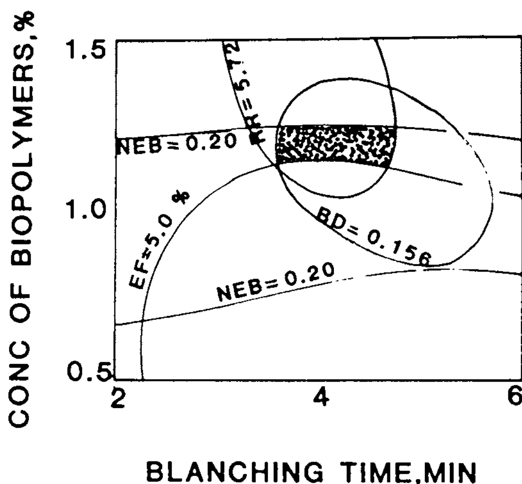


Figure 2. Superimposed plots of 4 contour plots at 145°C and 10 min for rehydration ratio (RR), bulk density (BD), non-enzymatic browning (NEB) and expressible fluid (EF) of dehydrated potato cubes.

The dye-biopolymer complexes were seen at intracellular spaces in intact carrot cells (Fig. 3a) and adhered to cell walls in broken cells (Fig. 3b). These pictures provided some evidence for our hypothesis that the biopolymers migrate on and around cell walls and their presence may have assisted in preventing or reducing cell collapse during dehydration. Much work needs to be done to elucidate the role of biopolymers on quality improvement of dehydrated vegetable pieces, and to define the proper size of molecules that would accomplish the desired texture.

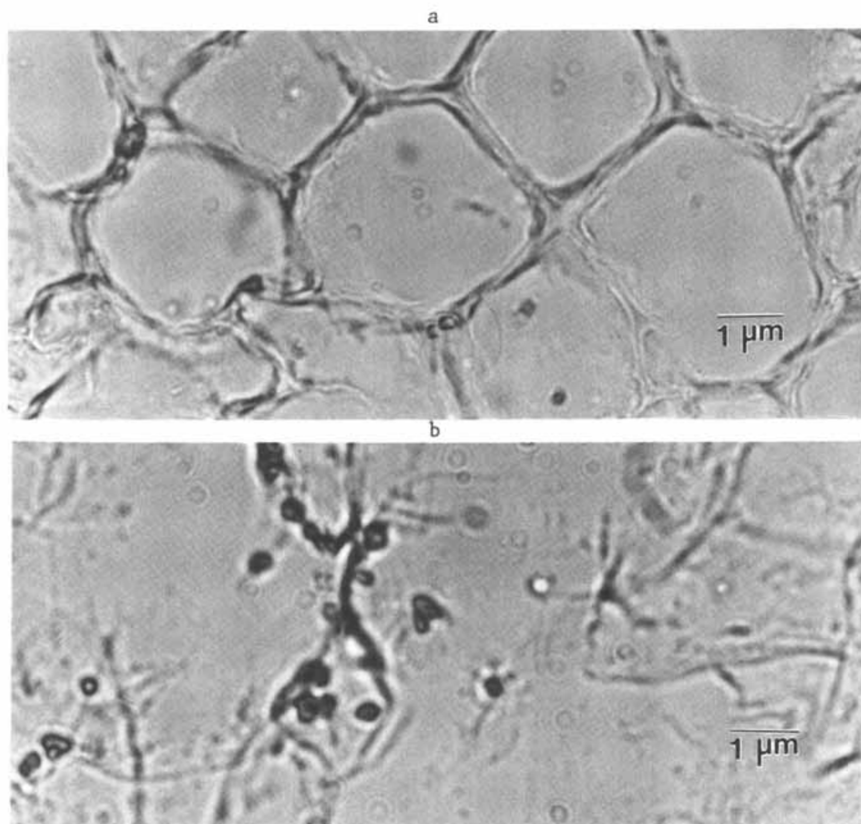


Figure 3. Microscopic pictures of (a) intact and (b) broken carrot cells after biopolymer-dye complex treatment, dehydration and rehydration. Bar in picture shows one micrometer in length.

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## Conclusion

Basic principles in physio-chemical changes occurring during dehydration provided an effective approach to improve quality of dehydrated products. Carrot and potato dices infused with biopolymers before dehydration and processed under optimal conditions had good texture, high rehydration properties, good color and puffed appearance. Infused biopolymers were deposited on cell walls and intracellular spaces and may contribute to prevent cell collapse.

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Chapter 20

# Effect of Enzyme Treatment on the Quality of Processed Fruit and Vegetables

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Pectinases, cellulases and hemicellulases are used for clarification of fruit juices, juice extraction, improvement of cloud stability of vegetable and fruit juices and nectars, liquefaction and maceration of fruits and vegetables, reduction of cooking time of pulses and improvement of rehydration characteristics of dried vegetables. The chemical basis of these treatments and of the consequential changes in sensory and nutritional quality are discussed.

In the processing of fruit and vegetables exogenous enzymes are used as processing aids to improve or maintain the quality characteristics of traditional products. The use of enzymes further provides possibilities for product and process development (1). Table I summarizes present uses and Table II shows the quality aspects involved.

Table I. Commercial pectolytic enzymes in fruit and vegetable technology

| |
|---|
| **Clarification of fruit juices**, e.g. apple juice; depectinised juices can also be concentrated without gelling and without developing turbidity. |
| **Enzyme treatment of pulp** of soft fruit, red grapes, citrus and apples, for better release of juice and colour pigments. |
| **Maceration of fruits and vegetables** (disintegration by cell separation) to obtain nectar bases and baby foods. |
| **Liquefaction of fruits and vegetables**, to obtain products with increased soluble solids content (pectolytic and cellulolytic enzymes combined). |
| **Special applications**. Treatments to improve rehydration and cooking characteristics of pulses and dried products. |

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Table II. Quality aspects of use of exogenous enzymes

|  |
|---|
| Clarity or cloud stability of juices. |
| Acid taste, aroma, mouth feel (changes). |
| Nutritional compounds. |
| Undesirable compounds. |
| Rehydration / cooking time of dried vegetables, pulses, potatoes. |

## Pectinases

Because pectic substances are important for the firmness (cell walls) and cohesiveness (middle lamella) of parenchymatic plant tissue (2,3) and for the properties of products derived from fruits and vegetables (4) pectolytic enzymes are mainly used. Commercial preparation are usually called pectinases and are derived mainly from Aspergillus niger. Table III (5) shows the specific activities of three different commercial preparations determined on polysaccharide substrates. The points of attack of the pectolytic activities are illustrated on a pectin model in Figure 1. Pectin lyase (PL) depolymerizes highly esterified pectin by splitting glycosidic linkages next to methyl esterified carboxyl groups through a β-elimination mechanism (Figure 2). Another depolymerization pathway is by the combined action of pectinesterase (PE) and polygalacturonase (PG). PE splits off methanol from highly esterified pectin transforming it into low ester pectin which is hydrolyzed by PG attacking glycosidic linkages next to a free carboxyl group. PG and PE occur also as technologically important endogenous enzymes of fruits and vegetables (6,7). Pectate lyase (PAL; Figure 2) also attacks glycosidic linkages next to a free carboxyl group so that PE also prepares a substrate for this enzyme. PAL was not found in the pectinases analyzed in Table III, because it is a bacterial rather than a fungal enzyme.

Table III. Specific enzyme activities of multi-enzyme complexes in technical fungal pectinase preparations expressed as International Units per g of Enzyme Preparation

| Enzyme activity | A | B | C |
|---|---|---|---|
| Polygalacturonase | 1982 | 3314 | 1878 |
| Pectin lyase | 43 | 53 | 74 |
| Pectin esterase | 548 | 448 | 227 |
| Combined pectolytic | 198 | 290 | 274 |
| $C_x$-cellulase | 998 | 180 | 1228 |
| $C_1$-cellulase | 99 | 1 | 22 |
| Arabanase: linear arabinan | 9 | 10 | 16 |
| Arabanase: branched arabinan | 14 | 14 | 16 |
| α-L-Arabinofuranosidase | 35 | 37 | 333 |
| Galactomannanase | 3 | 4 | 9 |
| Mannanase | 7 | 0 | 0 |
| Galactanase | 11 | 58 | 91 |
| Xylanase | 4 | 0 | 2 |

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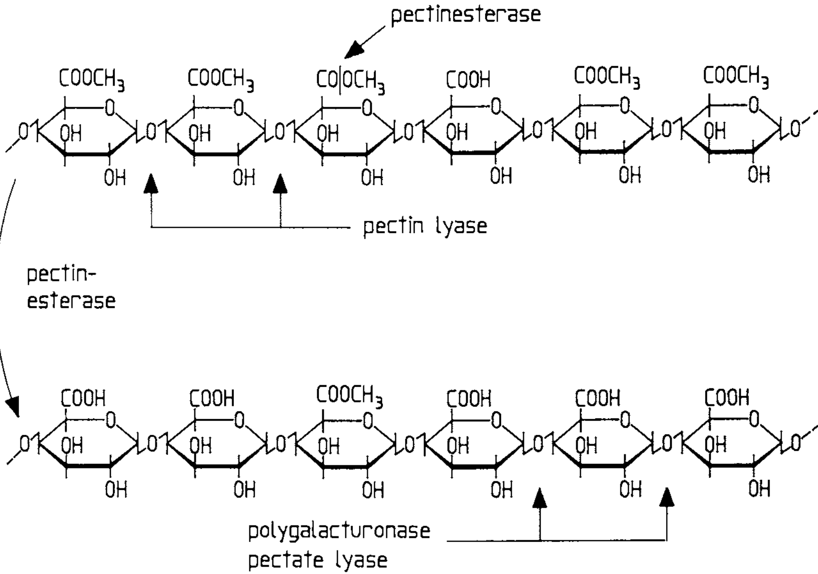


**Figure 1 : Fragment of a pectin molecule and points of attack by pectic enzymes.**

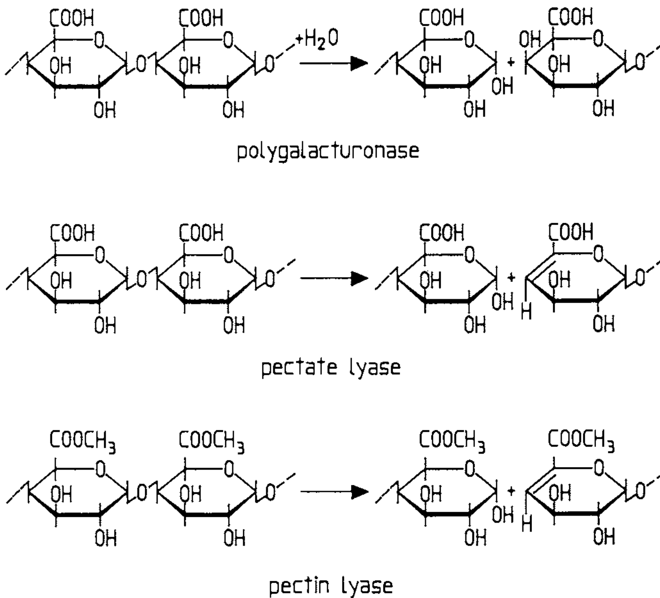


**Figure 2 : Splitting of glycosidic bonds in pectin by hydrolysis (polygalacturonase) and by β-elimination (pectate lyase and pectin lyase).**

The authors have previously (7-9) discussed activities, occurrence and microbial production of pectolytic enzymes. The hemicellulases listed in Table III are produced together with the pectolytic enzymes and are also important for pectin breakdown. The homogalacturonan presented in Figure 1 is only part of the pectin molecule. Pectin also carries "hairy regions" which are rich in neutral sugars: L-rhamnose in the main galacturonan chain and L-arabinose and D-galactose bound to the rhamnose and/or galacturonic acid molecules partly as complex side chains. D-xylose occurs as short side chains (2). Commercial pectinases therefore break down pectins to methanol, saturated monomers and oligomers of galacturonic acid (methyl ester), unsaturated oligomers of galacturonic acid (methyl ester), monomers and oligomers of arabinose, galactose and xylose and low molecular weight arabinogalactans. The $C_x$-cellulase in the preparations in Table III is also a side activity of the fungal culture. The $C_1$-activity of these preparations most probably comes from Trichoderma viride and has been added for special technological purposes (10,11).

## Clear fruit juices

Figure 3 is a general flow diagram of fruit juice manufacture. For clear juices this is a crushing, pressing and clarifying process. The use of fungal pectinases has been introduced more than 50 years ago to obtain sparkling clear juices from deciduous fruit and grapes (12,13). Enzyme treatment is necessary because the raw press juice obtained is viscous from dissolved pectin and has a persistant cloud of cell wall fragments and complexes of cell wall fragments with cytoplasmic protein. Addition of pectinase lowers the viscosity and aggregates cloud particles to larger units ("break") which sediment and are easily removed by centrifugation and/or (ultra)filtration. Indeed, in the early days pectinase preparations were known as filtration enzymes. 35 years after the introduction of this use of enzymes it has been explained by Japanese workers for apples, pears and grapes. The cloud particles are essentially composed of a protein nucleus carrying a net positive charge which is coated by negatively charged pectin molecules. A partial degradation of this pectin coat results in aggregates of oppositely charged particles (14). The reduction of the viscosity of the juice is due to depolymerization of the dissolved pectin (15,16).

## Pulp enzyming process

Pulping soft fruit like blackcurrants results in a semigelled mass which is very difficult to press. Treatment of such pulps with pectinases yields thin free run juice (17) and a pulp with good pressing characteristics. This pulp enzyming process is almost as old as enzymatic clarification (18). In the early seventies a start was made to apply it to apples with poor pressing characteristics due to cultivars (Golden Delicious) and the necessity for storage. An important aspect of this process (19; Figure 3) is aeration of the pulp. Phenolics are oxidized by endogenous polyphenoloxidase and subsequently polymerize to water insoluble compounds which are unable to inhibit the added pectinase. At the same time the danger

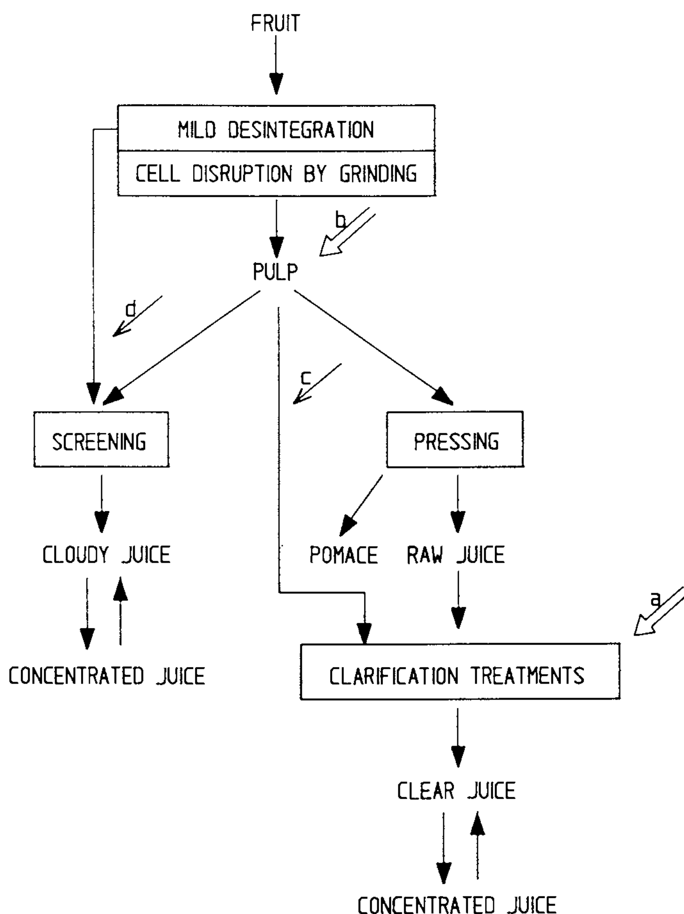


Figure 3 : Flow diagram of fruit juice manufacture. Arrows indicate eventual enzyme treatments:
a) pectinases for clarification of press juices;
b) pectinases for pulp enzyming;
c) pectinases and $C_1$ cellulases for liquefaction;
d) polygalacturonase or pectin lyase or pectate lyase for maceration.

of a polyphenol haze in the finished product is diminished (20). The good pressability of enzyme treated pulp (17) is shown in Table IV: High yields are always connected with enzyme treatment (21,22).

Table IV. Effect of enzyme treatment and storage on apple juice yields

| Variety | Storage | Enzyme treatment | Press yield (%) |
|---|---|---|---|
| **Treatment with 0.04% Pectinol flüssig® at 45°C, for 10 min (21)** | | | |
| Horneburger | + | - | 66 |
| Horneburger | + | + | 86 |
| Golden Delicious | + | - | 59 |
| Golden Delicious | + | + | 73 |
| Golden Delicious | - | - | 71 |
| Golden Delicious | - | + | 83 |
| Jonathan | - | - | 61 |
| Jonathan | - | + | 88 |
| **Treatment with 0.004% Ultrazym 100® at 40°C, for 20 min, after preoxidation (22)** | | | |
| Golden Delicious | + | + | 78 |
| Golden Delicious | + | - | 52 |
| Golden Delicious | + | + | 75 |
| Golden Delicious | + | - | 62 |
| Karmijn | + | + | 82 |
| Karmijn | + | + | 74 |

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One understands why pulp enzyming has become firmly entrenched within just 15 years in the European apple juice industry, even for good pressing apples - not to the delight of apple pectin manufacturers.

## Liquefaction

Enzymatic treatment of pulps can be carried thru to almost complete liquefaction (23), if $C_1$-cellulases are added to the pectinases which already contain $C_x$-cellulase. $C_x$-cellulase attacks amorphous regions in cellulose micels and $C_1$-cellulase acts as exo-enzyme splitting off cellobiose from the non-reducing ends (10,11,24). The cellobiose is split into glucose by cellobiase which is generally limiting in *Trichoderma viride* preparations, but present in high concentrations in *Aspergillus niger* preparations. Figure 4 shows the viscosity decrease of stirred apple pulp during treatment with pectinase, cellulase and a mixture of the two enzymes. A synergistic effect is seen when the enzymes act together (23). Low viscosity values reached correspond to complete liquefaction. Whether the juice is almost clear as in the case of cucumber or papaya, cloudy as in the case of apples and peaches or pulpy as in the case of carrots depends on the accessability of the cell wall compounds for the enzymes (lignin). Liquefaction products can then be clarified further by the usual techniques (25). Liquefaction is obtained by enzymatic breakdown of cell wall polysaccharides to low molecular

water soluble carbohydrates. Examination under the microscope (slides shown during oral presentation) clearly shows the disappearance of cell walls. The action of the enzymes can be followed chemically in enzyme treated cell wall material by comparing the diminishing amounts of galacturonic acid and neutral sugars in alcohol insoluble residues with the amounts in the control sample (26,27).

Table V. Neutral sugars and galacturonides released from apple AIS by pure enzymes. Percentage recovery, corrected for control

| Enzyme treatment | Neutral sugars | | | | | | | AGA | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Rha/fuc | Arab | Gal | Man | Xyl | Glc | Total | | |
| PE (citrus) | -- | -- | -- | -- | -- | -- | -- | -- | 0.1 |
| PG | 4 | 9 | 5 | -- | 4 | -- | 3 | 21 | 8 |
| PL | 19 | 39 | 27 | -- | 20 | 4 | 17 | 57 | 28 |
| PE+PG | 25 | 52 | 34 | -- | 13 | -- | 18 | 75 | 33 |
| $C_1$ | 9 | 3 | 12 | 26 | 20 | 22 | 16 | 5 | 13 |
| $C_1$+PE+PG | 58 | 89 | 69 | 84 | 71 | 79 | 78 | 82 | 80 |

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Table V giving results of experiments on apple cell wall material with purified enzymes, reveals that pectinase activity (PE+PG) alone releases 75% of the pectic material, including the pectin sugars arabinose, galactose, xylose and rhamnose. No glucose from cellulose is released. Cellulase alone had little influence on pectin and solubilized only 22% of cellulose. Combined cellulase - pectinase activities released 80% of the polysaccharides. This is the synergistic effect noticed in pulp viscosity experiments (Figure 4). A similar effect has been found for grapefruit segment membranes (28) when treated enzymatically. The breakdown products increase the soluble solids content of the juices (23, Table VI), so that yields of more than 100% are obtained on soluble solids basis. In addition lignefaction leaves no or very little insoluble residue holding juice as liquid phase. This is especially interesting for manufacturers of concentrated juices.

Tabel VI. Increase of soluble solids by enzymic liquefaction

| Product | Ground | Liquefied |
|---|---|---|
| Apple | 13.0 | 14.8 |
| Oranges | 13.0 | 14.7 |
| Pears | 11.4 | 12.7 |
| Apricots | 14.6 | 15.7 |
| Peaches | 14.6 | 15.8 |
| Carrots | 3.3 | 6.7 |

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Table VII contains a comparison between pressing and lignefying for apple juice. Liquefaction is also interesting for vegetables and fruits (29-31) from which pressing yields no juice or for which no presses have been developed (mango, guava, bananas, etc. (32). Therefore much interest is shown in this new, simple, low capital, easy maintenance technology in developing countries (Figure 3). Enzymic liquefaction is attractive to all countries in that costly waste disposal is virtually eliminated. In industrialized countries socio-economic factors are increasing the necessity to process stored apples during the whole year and this situation also generates interest in the process. Furthermore legal restraints with regard to definition of fruit juice and the use of *Trichoderma viride* cellulase eventually should be overcome.

Table VII. Comparison of yields from 1000 kg apples with good pressing characteristics

| | | |
|---|---|---|
| Pressing : | 786 | kg juice 12°Bx (750 litres) |
| | 131 | kg concentrate 72°Bx |
| | 1000 | kg 72°Bx concentrated juice from 7633 kg apples |
| Enzymic liquefaction : | 950 | kg juice 13.5°Bx (900 litres) |
| | 178 | kg concentrate 72°Bx |
| | 1000 | kg 72°Bx concentrated juice from 5618 kg apples |

Source: Reproduced with permission from ref. 29. Copyright 1988 International Federation of Fruit Juice Producers.

## Quality aspects of clear fruit juices

Considering quality aspects of press juices and of juices obtained by pulp enzyming and by enzymatic liquefaction, it can be stated that the primary quality requirement of the European consumer, namely for sparkling clarity can be achieved for all three types of juice provided pectins are sufficiently degraded. This is checked by a simple alcohol flocculation test: absence of flocculation upon the addition of 2 volumes of alcohol. The test is also assuring that no turbidity by pectin flocculation upon storage will occur in juice or in concentrated juice. Nevertheless haze has appeared in concentrated apple and pear liquefaction juices and sometimes in concentrated juices from pulp enzyming. Analysis of the haze material shows it to be 90% arabinose of which 88% is α-(1-5) linked (33,34; Table VIII). Structure work on non-haze apple juice arabans (35) and the study of mould arabanases (36) indicate the haze forming mechanism in Figure 5: The enzyme treatment of pulp releases a branched araban. The small uronide content indicates that the haze may come from the hairy regions of the pectin molecule. Its main chain is α-(1-5) and the side chains are α-(1-3). The authors and their co-workers have identified and purified various arabanases from commercial pectinase preparations. An exo α-L-arabinofuranosidase II preferentially splits the 1-3 linkages of the side chains (36), which results in debranching. Remaining (1-5) chains can then retrograde under conditions such as low water activity and low temperature and crystallize, generating haze.

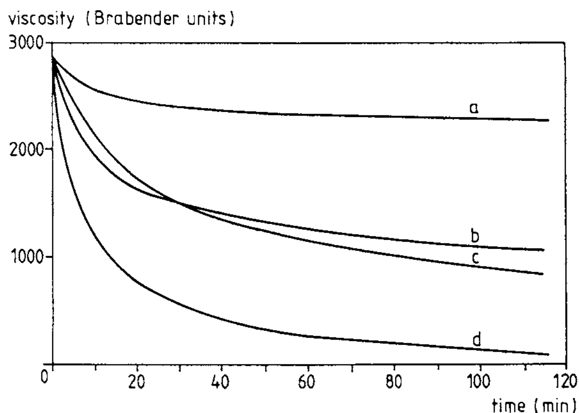


**Figure 4.** Viscosity decrease of stirred apple pulp at 25 °C. a, No enzyme added; b, cellulase added ($C_1$ + $C_x$); c, pectinase added; and d, cellulase + pectinase added. (Reproduced with permission from ref. 23. Copyright 1975 Fluessiges Obst.)

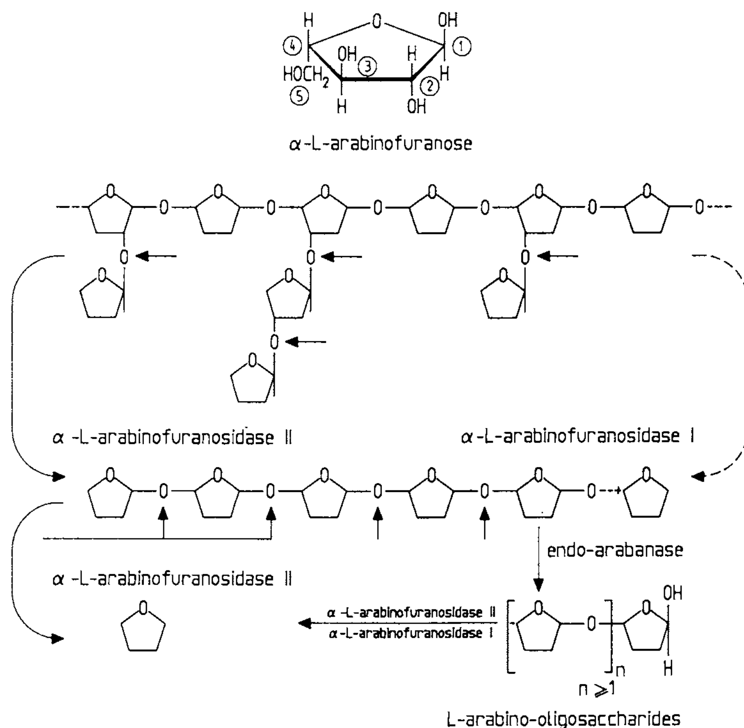


**Figure 5.** Points of attack of arabinan degrading enzymes. (Reproduced from ref. 35. Copyright 1986 American Chemical Society.)

Addition of sufficient endo-arabanase activity to depolymerize the 1-5 chain inhibits haze formation. Enzyme manufacturers are now providing for this in their preparation to minimize the problem.

Table VIII. Sugar composition of haze material and glycosidic linkage composition for arabinosyl residues

| | Amount of sugar g/100 g haze | Glycosidic linkages (a) arabinosyl units (%) |
|---|---|---|
| Rhamnose | 1.2 | 4.2% 1-linked, (terminal furanose units) |
| Arabinose | 90 | |
| Xylose | 0.3 | 0.2% 1-linked, (terminal pyranose units) |
| Mannose | 0.7 | |
| Galactose | 1.6 | 2.3% 1,3-linked, (unbranched furanose units) |
| Glucose | 0.1 | |
| Galacturonic acid | 3.9 | 88.4% 1,5-linked, (unbranched furanose units) |
| Total | 97.8 | 3% 1,3,5-linked (branched furanose units) |
| | | 1% 1,2,5-linked (branched furanose units) |

a) derived from methylation studies

Source: Reproduced with permission from ref. 33. Copyright 1982 Lavoisier.

Table IX. Amounts and chemical composition of polysaccharides (PS) isolated by dialysis from apple juices prepared by different enzymes (7,37)

| Analysis | Pressing | Pulp enzyme treatment | | | Liquefaction | |
|---|---|---|---|---|---|---|
| | | A* | B* | C* | C* | D* |
| Polysaccharides | | | | | | |
| Content (mg/kg pulp) | 155 | 1075 | 1860 | 1075 | 3000 | 4300 |
| Sugar composition Mol % | | | | | | |
| Rhamnose | 3 | 5 | 3 | 5 | 4 | 4 |
| Arabinose | 21 | 38 | 6 | 52 | 43 | 54 |
| Xylose | 2 | 5 | 2 | 5 | 6 | 8 |
| Galactose | 19 | 9 | 4 | 7 | 6 | 8 |
| Glucose | 2 | 1 | 1 | 1 | 1 | 0 |
| Anh.-galacturonic acid | 53 | 42 | 84 | 30 | 40 | 26 |

*) Different enzyme preparations used. A, B, and D were experimental preparations from Gist-Brocades, Delft, The Netherlands; A was a pectinase; B a pectinase rich in hemi-cellulase activity and D a pectinase-cellulase mix. C was ultra SP-L from Novo Ferment AG, Basel, Switzerland

Table IX shows the monomer analysis of freeze dried dialysis retentate of apple juices made in the laboratory with the three technologies. It provides information on the degree of

polymerization of the solubilized cell wall polysaccharides and the activities of the enzyme preparations used. Thus liquefied juices with a high araban content of a high total carbohydrate retentate may be susceptible of haze formation (37).

A simple test, such as the alcohol test for pectin or the iodine test for starch, is necessary and would also help to create confidence in the liquefaction process. Phenolic hazes by polyphenol-protein interaction are not expected in enzymatically extracted juice, provided enough aeration for endogenous polyphenoloxidase action has been applied (20). Enzymatically extracted apple juices even from aerated pulp have a yellowish colour because of yellow flavonol glucosides (quercitrine). These glucosides do not undergo enzymatic oxidation during processing. In the traditional press technology they remain in the press cake, but can diffuse into the juice in the enzyme processes. Colour difference between juices obtained by enzymatic and non enzymatic processes has never been characterized as a quality defect. Table X shows the total uronide content of apple juices obtained by different technologies (38). Uronides increase with intensified enzyme treatment and enhance acid taste which may be welcome. It also means an increase in titration acidity. Depending on the PL action of the enzyme preparations used, 20 to 40% of the uronides are present as unsaturated oligomers (Figure 2). These enzyme treated juices undergo more rapid browning than traditional juices. Figure 6 presents the results of model experiments from which it is seen that the unsaturated oligomer uronides are powerful browning precursors. The models for concentrated apple juice were made up as follows: Indicated quantities of oligo-uronides and fructose were brought to 70° Brix with sorbitol and to pH 3.5 with malic acid. In some experiments 0.5% amino acid mixture (Asn, Asp and Glu) were also added. Storage temperature was 50°C (39).

Table X. Uronide content of apple juices (12° Brix)

| Methods of preparation | Uronide content mg / ml |
|---|---|
| Pressing + clarification | 0.58 |
| Water extraction | 1.31 |
| Methanol extraction | 0.35 |
| Enzymic pulp treatment | 2.51 |
| Enzymic pulp liquefaction | 4.32 |

We have also researched the browning mechanism and have shown that the unsaturated oligomers are split into saturated oligomers with one unit less than the original compound. The unsaturated monomer reacts further to form a number of compounds among which we have identified 2-furoic acid and 5-formyl-2-furoic acid, both known browning precursors (39). The uronide content of Table X also means a methanol content (from PE action) of about 10% of these quantities, i.e. from 60 to 450 mg/kg. The higher figure would be about 100 mg/kg above the range found in all kinds of beverages (40). Of course, in modern fruit juice technology volatiles are

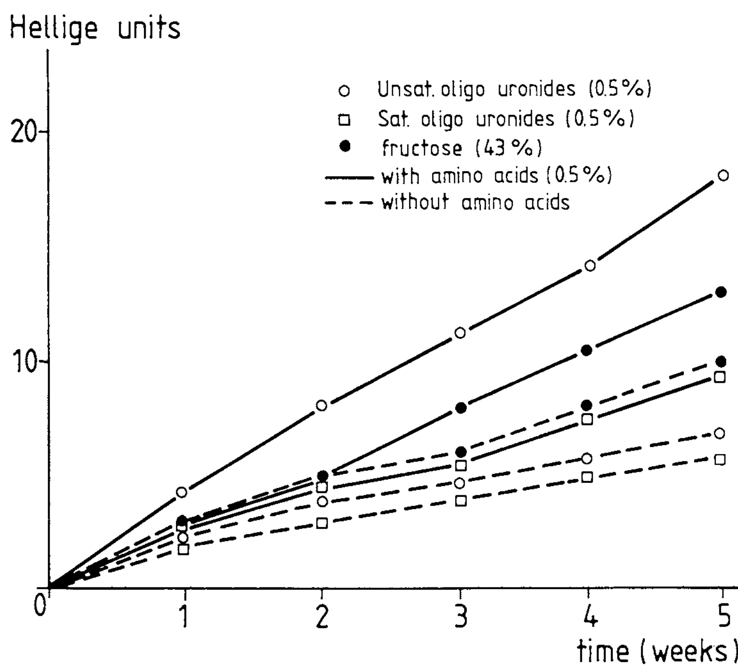


**Figure 6. Unsaturated galacturonides as nonenzymatic browning precursors. Hellige units are obtained by comparing color of sample with color of tinted glass discs according to the Lovibond principle. (Reproduced with permission from ref. 39. Copyright 1988 Springer.)**

stripped from juice and kept and traded separately and mixed for use. This means that in reconstituted juices methanol content can be controlled. Aroma: Fruit juices prepared by enzyme technology have often been investigated sensorially and chemically. Table XI shows impact compounds in volatiles of apple juices obtained by pressing and by liquefaction (42). One notices mainly saponification of esters and reduction of aldehydes to alcohols. The disproportionately large amounts of $C_6$ alcohols is explained by increased endogenous lipoxygenase activity which results in a higher initial concentration of $C_6$ aldehydes. In fact all the chemical and corresponding flavour changes are brought about by endogenous enzyme actions which proceed more rapidly because of better access of enzymes to substrate in the liquefied system. This has also been observed in tropical juices (43,44). Nevertheless appropriate technology can minimize these flavour changes (45-47). Furthermore the statement made earlier regarding the control of methanol by mixing volatiles also applies here.

Table XI. Aroma compounds of apple juices obtained from pulp by pressing and by enzymatic liquefaction. The pulp was not heat treated

| ppm | Pressing | Liquefaction |
|---|---|---|
| 2-Butylacetate | 0.15 | 0.05 |
| Butylacetate | 28 | 0.3 |
| 2-Pentylacetate | 0.6 | 0.25 |
| Butylbutyrate | 0.3 | 0.2 |
| Hexylacetate | 5.1 | 0.05 |
| Hexanal | 1.3 | 1.4 |
| 3-Hexenal | 0.3 | 0.15 |
| 2-Hexenal | 18 | 15 |
| Benzaldehyde | 0.05 | 0.2 |
| Butanol | 14 | 26 |
| 2-Methylbutanol | 0.1 | 0.65 |
| Pentanol | 0.05 | 0.2 |
| Hexanol | 3.7 | 14 |
| 2-Hexenol | 0.6 | 1.9 |

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## Cloudy fruit and vegetable juices and nectars

The preparation of cloudy juices from pulp is also indicated in the flow diagram in Figure 3. This type of juice is necessary when colour pigments (carotenoids) and flavour compounds (terpenoids) are bound to the pulp particles. Clear serum would not be recognizeable as juice of the particular fruit or vegetable. The cloud stability is therefore an important quality aspect. In the case of orange juice this has become one of the most intensively researched areas of food technology (48-50). Orange juice which has been pasteurized shortly after extraction has a stable cloud. Cloud loss has been shown to be a phenomenon of self clarification due to endogenous PE which deesterifies the pectin in the juice. At a certain degree of demethoxylation the pectin is sufficiently calcium ion sensitive to

precipitate with the calcium ions of the juice (51). The data of Figure 7 reveal that mold PG acting together with esterase prevents coagulation, obviously by degrading the deesterified regions in the pectin molecules as they are formed (52). Another possibility to protect cloud is the degradation of the initially highly esterified pectin by PL (53, Figure 8). These enzyme processes may be useful where full heat inactivation of PE is considered to harm flavour and frozen storage and shipping of concentrated orange juice is not possible. Apricot nectars exhibit another phenomenon of cloud loss. This nectar is made by mixing finely screened pasteurized apricot pulp with water, sugar and acid to contain from 30 to 50% apricot ingredient. On standing the slowly sedimenting pulp particles form a gel which contracts leaving clear syneresis fluid as supernatant (54,55). This separation can be avoided by grinding the pulp so finely that at least one third of the cells are broken open (56). This unfortunately is difficult to control during production. Fortunately cells can be broken open by the action of pectolytic enzymes (55; Table XII). A thorough study has shown that for this purpose an araban rich pectin fraction must be degraded (54,55). Pure PG or commercial preparations containing mainly PG are not suitable. The cell walls become thinner but do not break. Thus PG prevents gel formation but not sedimentation. If PE or an exo-arabanase is added, the enzyme preparation becomes a cloud stabilizing enzyme, capable of degrading the extracted araban rich pectin and of breaking open cells in the apricot pulp from which then a cloud stable product can be made. Figure 9 shows the elution patterns of gel filtration chromatography of the araban rich pectin after treatment with various enzyme systems. Treatments with cloud stabilizing enzymes (Table XII) indeed move the uronide peak into the included volume, indication depolymerization.

Table XII. Stabilizing action of various pectolytic enzymes on apricot nectar cloud

| | Gel prevented | Stable cloud | Cell walls |
|---|---|---|---|
| PG | + | - | thinner |
| PG + mould PE | + | + | broken |
| PG + exo-arabanase | + | + | broken |
| PL | + | + | broken |
| Pectinex | + | + | broken |

Pectinex is a commercial wide spectrum pectinase

Pulpy viscous products from fruits and vegetables are finding increasing interest for use as baby food, as fruit ingredients in milk puddings and in milk products, as basis for the preparation of vegetable juice and fruit and vegetable nectars. For manufacture from pulp (Figure 3; 57) heat treatment would have to be applied either by blanching before pulping or by heating the pulped product to soften up the tissues and to inhibit endogenous enzymes which would damage flavour, colour and ascorbic acid. This introduces the danger of heat damage; moreover finisher operation to remove coarse tissue results in mechanical losses of nutritive constituents and a

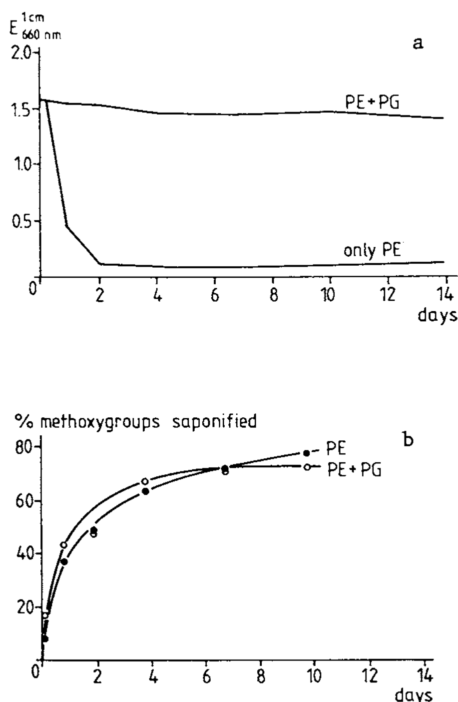


**Figure 7.** a, Cloud loss in enzyme-inactivated orange juice by addition of citrus PE. Stabilization of cloud by adding PG together with PE. b, The activity of PE in both cases is shown by methanol release. (Reproduced with permission from ref. 52. Copyright 1974 Forster.)

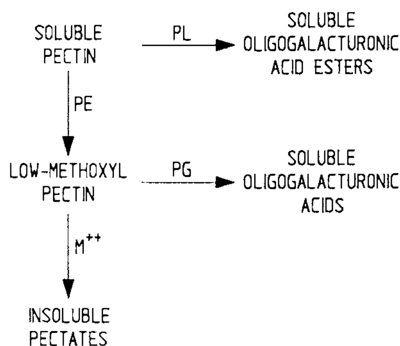


**Figure 8.** Possibilities for preventing PE-induced calcium pectate precipitation in orange juice by enzymatic pectin degradation. (Reproduced from ref. 53. Copyright 1980 American Chemical Society.)

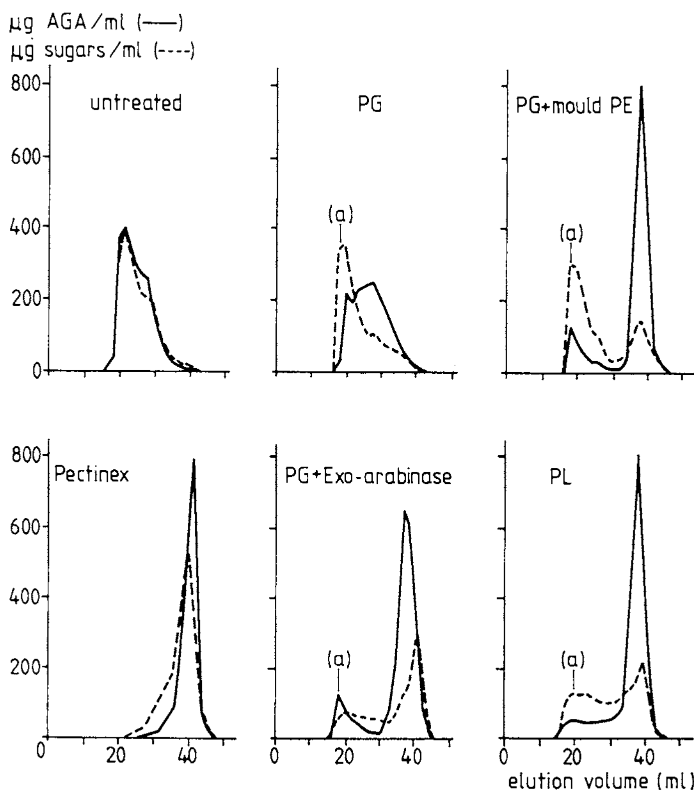


Figure 9. Gel filtration chromatography of the HCl-soluble apricot pectin fraction, degraded by various enzyme systems on a Sephacryl S—300 column (68 × 1.05 cm), eluent 0.05 M phosphate buffer pH 7. AGA is anhydrogalacturonic acid; Pectinex is a wide-spectrum commercial pectinase. (Reproduced with permission from ref. 54. Copyright 1985.)

smooth consistency is not always achieved. Enzymatic degradation of pectin after a mild mechanical treatment (Figure 3) often improves product properties if carried out to leave as many cells as possible intact. The aim of the enzyme treatment is to transform tissue into a dispersion of intact cells (58-60). The pectin degradation therefore should affect only the middle lamella pectin. Such a process is called **enzymic maceration**. The so called macerases are enzyme preparations with only PG (61-64) or PL activity (65,66). The effectiveness of these macerases on various substates has been compared (67). For maceration of vegetables bacterial PAL is interesting because of its high pH optimum (68,69). The restricted pectin degradation solubilizes rather than degrades the middle lamella pectin which may help the creamy mouth feel. Table XIII lists the advantages claimed for maceration treatment. The process is often used for carrots. Whether maceration or cell wall breakdown has occurred can be checked by microscopy (slides shown at oral presentation) and by the absence of enzymatic browning. Cloud stability is not always achieved (57). This is not surprising in view of the completely different mechanisms for cloud stability and clarification for apple juice, orange juice and apricot nectar discussed above. A very interesting use of maceration is pulp for the production of dried instant potato mash. It prevents starch leaking out of the cells, avoiding thus the quality defect of glueiness of the reconstituted product. Potatoes are blanched in order to gelatinize starch and to inactivate endogenous PE which together with the added PG would extend the possibilities for pectin degradation and turn the maceration process into pulp enzyming technology. Inactivation of endogenous PE is important for the maceration of most products (70,71).

Table XIII. Advantages claimed for macerated products

| |
|---|
| Avoid adverse effects of thermo-mechanical treatment |
| Minimize finishing losses |
| Avoid endogenous enzyme action |
| Better retention of nutrients |
| Minimize starch leakage in potato pulp |
| Creamy mouth feel |
| Cloud stability? |

## Non liquid processed products

Much work and probably industrial applications are carried out with regard to the improvement of the quality of products such as dried vegetable ingredients in instant soups which should rehydrate fully and quickly or for the decrease of cooking time of pulses such as dried seeds of peas or beans. However, no basic research in this promising field is known to the authors.

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Chapter 21

# Processing and Storage Influences on the Chemical Composition and Quality of Apple, Pear, and Grape Juice Concentrates

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Apples (Granny Smith), pears (d'Anjou, Bartlett, Comice) and grapes (Thompson Seedless) were processed into juice and juice concentrate and stored at 25°C. The influences of pear fruit maturity (hard vs. soft) and length of post-harvest storage of apple fruit (3 vs. 9 months) were examined, as were the following processing treatments -- addition of $SO_2$, and fining with bentonite-gelatin-silica sol. Browning and clarity of the finished products were the quality attributes of primary concern. Detailed compositional analyses (sugar, nonvolatile acid and phenolic profiles, total acidity, total protein, total phenolics, formol values, and gel electrophoresis of proteins) were performed on the 28 samples. $SO_2$ treatment protected phenolics, particularly procyanidins, as well as total soluble proteins. Formol values were better predictors of browning rates during storage (within fruit commodities) than were initial browning indices. Fining does not influence browning rates, but is effective in preventing haze formation.

Processing apples, pears and grapes into juice concentrate is a means of utilizing surplus fruit and defects such as under and oversized fruit. This allows for more complete utilization of these agricultural commodities, with economic and ecological benefits to growers, processors and consumers. In addition to being used as single strength juices, there is increasing demand for the use of apple, pear and white grape juice concentrates as ingredients for blended juices, syrups, juice drinks and soft drinks. There are different quality prerequisites for these various end products and there is considerable quality variation among the concentrates available in the commercial marketplace. There is no standard of identity for these concentrates in the USA.

In this investigation we processed pears, apples and Thompson seedless grapes into juice and juice concentrate and tracked the compositional changes through processing and storage. The quality

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attributes of primary interest were color and appearance, i.e. browning, sedimentation and haze. A major objective was to determine those compositional indices which could be predictive of product quality. We were also interested in finding compositional indices which reveal processing/storage history as well as those which are useful in determining authenticity. This chapter summarizes the results of this study, the original references (1-7) should be referred to for details of experimental methods and complete listings of compositional data.

## Processing and Storage Trials

Figures 1, 2, and 3 outline the unit operations for the different processing trials. $SO_2$ addition is commonly used in commercial processing of Thompson seedless grapes into concentrates. We were particularly interested in its influence on composition and quality and did parallel trials with and without addition of 70 ppm $SO_2$. All samples were given a prepress commercial pectinase treatment, a procedure which is being used in industry to increase juice yields. Trials with and without fining (addition of Bentonite, gelatin and Silica Sol) were conducted because of the interest in haze and sediment formation. Juices were pasteurized and bottled as single strength, and also concentrated to $71^{o}$ Brix. Juice and concentrates were stored at $25^{o}C$. All processing trials were replicated. Those points of processing and storage where samples were collected for compositional analyses were indicated by an asterisk in Figures 1, 2 and 3.

Granny Smith was the variety of apples selected for juice processing. The influence of long-term storage of fruit was investigated as apples are commonly held in cold storage for considerable periods of time before processing. Processing trials were carried out on fruit from the same lot which had been stored for three and nine months. All apple juice samples were given a prepress treatment with a commercial pectinase preparation. Trials with and without fining were conducted. The protocol for bottling of single-strength juices, concentration and storage regimes were the same as for the grape trials.

Three varieties of pears, Bartlett, Comice and d'Anjou, were included in this investigation. As commercial processors utilize both hard, unripe fruit and soft, ripened fruit in juice manufacture, the influence of ripening was investigated for all three varieites. Juice was pressed from green fruit as well as fruit from the same lot which had been ripened by removing from cold storage ($\sim 1^{o}C$) and holding at $15^{o}C$ for 5-7 days. The influence of $SO_2$ (50 ppm) addition was included for separate lots of unripe Comice and d'Anjou fruit. Fining was found to be essential for satisfactory filtration of all pear juice samples; therefore, there were no comparative unfined pear juice experimental trials.

## Sugar Composition

Sucrose, glucose, fructose and sorbitol were separated and quantitated by HPLC. The sample preparation procedure included removal of acids by percolating the juice through a minicolumn

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ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

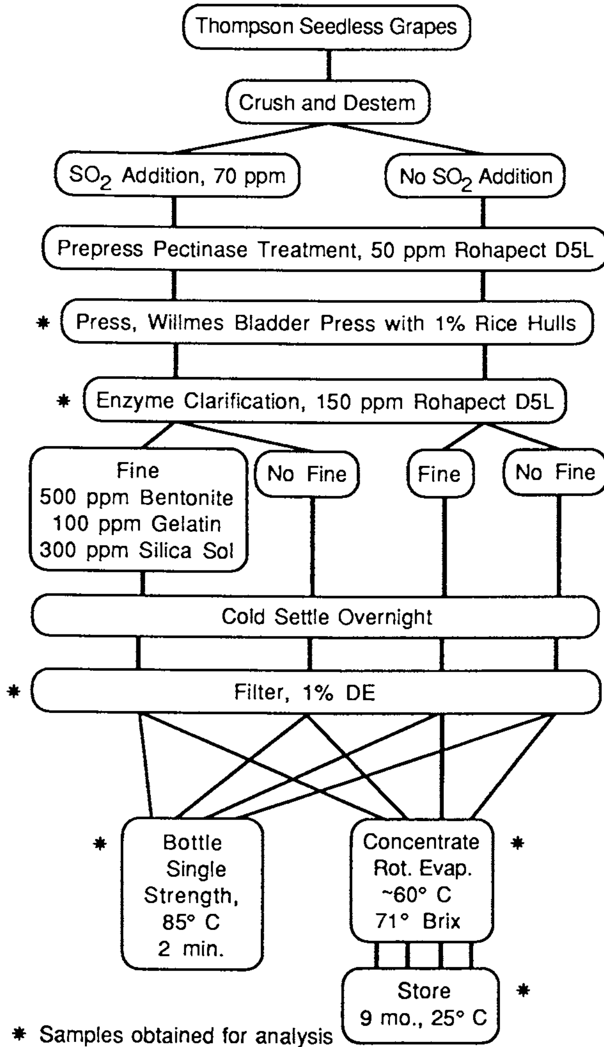



Figure 1. Unit operations for processing Thompson Seedless white grape juice samples. (Reproduced from ref. 4. Copyright 1989 American Chemical Society.)

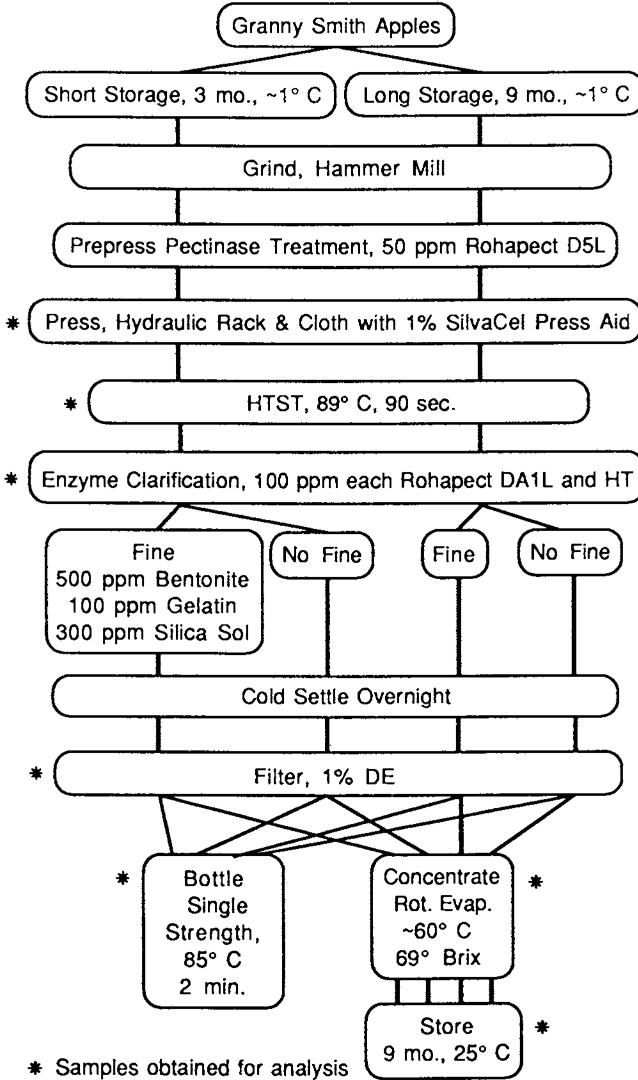



Figure 2. Unit operations for processing Granny Smith apple juice samples. (Reproduced from ref. 2. Copyright 1989 American Chemical Society.)

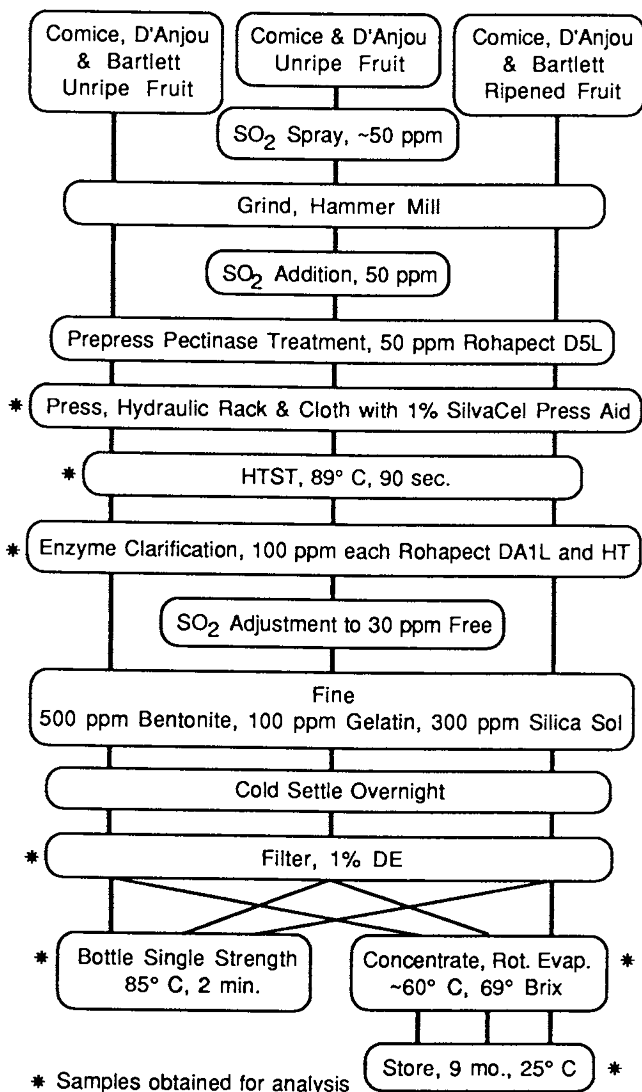



Figure 3. Unit operations for processing Bartlett, Comice, and D'Anjou pear juice samples. (Reproduced from ref. 3. Copyright 1989 American Chemical Society.)

containing anion exchange resin and removing phenolics by treatment with C-18 reverse phase cartridges (1). Mannitol was used as an internal standard. Compositional data for all pear samples was normalized to 11.0° Brix; apple and grape samples were normalized to 11.5° and 16.5°, respectively. Normalization to a standard °Brix value facilitates comparisons among experimental and commercial samples.

Table I summarizes the results for the pear juice samples. There is little difference in the °Brix value for juices from unripe and ripened pears. The values for the individual sugars are presented as percent of total sugars (summation of glucose, fructose, sucrose and sorbitol). Fining and $SO_2$ treatment had no influence on the sugar profile. This was true for the apple and grape samples as well (1). Ripening appears to have an influence on sucrose and sorbitol levels. Unripe Bartlett juice had higher levels of sucrose than juice from ripened fruit; the reverse was true for Comice and d'Anjou, however, with ripe fruit having lower levels of sucrose. Juice from unripe Comice and d'Anjou pears had higher levels of sorbitol than that from the ripened fruit. Chromatograms from all fruits showed trace (<1% of total peak area) amounts of one peak which was tentatively identified as arabinose on the basis of equivalent retention time. Its presence could be the result of hydrolysis of cell-wall polysaccharides by the prepress treatment with commercial pectinase preparations.

## Nonvolatile Acid Composition

The AOAC approved method for analysis of nonvolatile acids in cranberry and apple juice (8) was used for all juice samples. This HPLC procedure utilizes direct injection of juice samples on two reverse phase C-18 columns connected in series. Tartaric, quinic, malic, citric and fumaric acids were quantitated by the external standard method. Table II summarizes the results for the pear juice samples. Ripening had little influence on the total titratable acidity, however, quantitative differences in the compositional profile were evident. Most noticeably, the level of quinic acid was higher in the ripened fruit.

Varietal differences were evident, d'Anjou being higher in titratable acidity than Bartlett and Comice. Bartlett had higher proportions of quinic, citric and fumaric acids than d'Anjou and Comice. $SO_2$ addition, fining and other processing unit operations had no influence on the nonvolatile acid profile.

## Phenolic Composition

The phenolic compounds present in these three commodities fall into two general classifications, cinnamic acid derivatives and flavonoids. Included in the former are chlorogenic acid and its isomers, free cinnamic acids such as caffeic and p-coumaric acid and various esters of those two acids. Included among the following:

Flavan-3-ols (catechin, epicatechin)
Flavan-3,4-diols

Table I. Sugar Profile for Pear Juice Samples

| Sample | °Brix | Sucrose | Glucose | Fructose | Sorbitol |
|---|---|---|---|---|---|
| | | (% OF TOTAL SUGARS BY SUMMATION) | | | |
| Bartlett | | | | | |
| Unripe, fined | 11.7 | 6.1 | 8.5 | 56.3 | 29.1 |
| Ripe, fined | 12.0 | 3.0 | 11.5 | 56.5 | 29.0 |
| Comice | | | | | |
| Unripe, fined | 14.1 | 3.6 | 9.1 | 51.3 | 36.0 |
| Unripe, fined, +$SO_2$ | 13.8 | 3.6 | 8.9 | 51.9 | 35.6 |
| Ripe, fined | 14.2 | 7.0 | 5.4 | 58.2 | 29.3 |
| d'Anjou | | | | | |
| Unripe, fined | 12.1 | 2.1 | 13.0 | 49.3 | 35.6 |
| Unripe, fined, +$SO_2$ | 12.1 | 2.1 | 13.1 | 49.0 | 35.7 |
| Ripe, fined | 12.7 | 3.3 | 12.4 | 55.0 | 29.4 |

Table II. Nonvolatile Acid Profile for Pear Juice Samples

| Sample | % of Total Acids by Summation | | | | Titratable Acidity |
|---|---|---|---|---|---|
| | %Quinic | %Malic | %Citric | %Fumaric | g/100 mL[a] |
| **Bartlett** | | | | | |
| Unripe, fined | 22.1 | 53.1 | 21.4 | 0.22 | 0.16 |
| Ripe, fined | 36.6 | 40.3 | 14.6 | 0.39 | 0.15 |
| **Comice** | | | | | |
| Unripe, fined | 10.0 | 86.9 | 0.43 | 0.107 | 0.17 |
| Unripe, fined, $+SO_2$ | 11.8 | 85.1 | 0.34 | 0.11 | 0.18 |
| Ripe, fined | 26.7 | 70.6 | 0.58 | 0.08 | 0.16 |
| **d'Anjou** | | | | | |
| Unripe, fined | 4.44 | 94.3 | 0.51 | 0.07 | 0.27 |
| Unripe, fined, $+SO_2$ | 4.92 | 93.9 | 0.62 | 0.08 | 0.26 |
| Ripe, fined | 15.3 | 82.8 | 0.45 | 0.09 | 0.28 |

[a]Normalized to 11.0 $^{o}$Brix

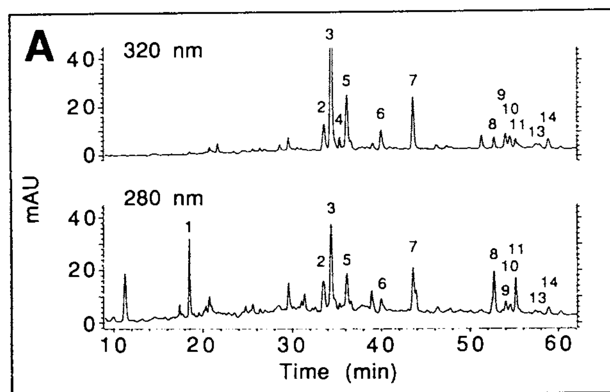
Flavonols (quercetin--common to apples, pears and grapes, isorhamnetin--found in pears)
Dihydrochalcones (phloridzin and phloretin--found in apples)
Procyanidins (condensed tannins, polymers of catechin and epicatechin)

Arbutin (Hydroquinone-ß-D-glucoside) fits neither category. It is not widely distributed in plants, but is found in pears.

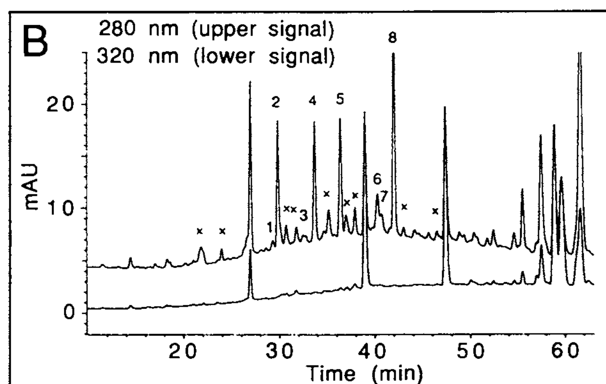
Analytical Methods. Diode array detection in combination with HPLC proved to be a powerful toolfor classifying peaks as to their compound class, making assignments as to peak identity, determining peak purity, and quantitation. The HPLC system utilized a reverse phase (Supelcosil LC-18) column and a gradient elution system of pH 2.5 phosphate buffer (0.07 M) and methanol. This solvent system offered the advantage of being transparent in the ultraviolet, a prerequisite for taking full advantage of the capabilities of the diode array detector. Figure 4 shows HPLC chromatograms of apple juice phenolics, the cinnamates, flavonols and dihyrochalcones being detected at 320 nm. Peak assignment for compounds previously identified in apple juice was done on the basis of their diode array spectra and retention time. While complete characterization for some of the peaks was not accomplished in that the glycosidic substituent was not identified, the peak could be categorized as to compound class. By assuming that detector response factors for compounds within a class would be similar, their quantities could be estimated by using a compound from within the class as a standard (2,7).

Coelution of procyanidins with cinnamics and flavonoids presented a major problem in quantitating the procyanidins. (Cinnamics and flavonoids could be measured in the presence of procyanidins by monitoring at 320 nm where procyanidins are nonabsorbing.) This was overcome with preliminary separation of compound classes with minicolumn gel filtration (4). Figure 4B shows an HPLC separation of apple juice procyanidins. Compounds absorbing at 320 nm which coeluted with catechin, epicatechin and the procyanidins have been eliminated by the cleanup procedure. The HPLC conditions (column, solvent gradient, etc.) are identical to those used for separation of cinnamics and flavonoids, the separation taking 60 min.

Apple Phenolics. Figure 5A shows the influence of processing unit operations and storage on the total cinnamics of apple juice. These compounds serve as substrates for polyphenoloxidase (PPO). Crushing and pressing provide opportunity for PPO action prior to its inactivation by high-temperature-short-time (HTST) treatment, 89°C for 90 sec. There is a higher level of cinnamics in the HTST sample than the sample from initial pressing. Enzymic degradation after sampling and/or thawing of the frozen samples most likely accounts for this difference; in addition, the heat process may have solubilized bound phenolics. Depectinization, and to a lesser extent fining, resulted in a reduction in the quantity of cinnamics. Filtering, bottling, and concentration had little or no effect. Storage of the concentrates for 9 months at 25°C resulted in loss of cinnamics.




Peaks: 1. HMF, 2. conjugated coumaric, 3. chlorogenic, 4. chlorogenic isomer, 5. caffeic, 6. p-coumarylquinic, 7. p-coumaric, 8. phloretin xyloglucoside, 9. quercetin galactoside, 10. quercetin glucoside, 11. phloridzin, 12. quercetin xyloside, 13. quercetin arabinoside, 14. quercetin rhamnoside




Peaks: 1. Procyanidin B3, 2. Procyanidin B1, 3. Procyanidin B4, 4. catechin, 5. Procyanidin B2, 6. trimer, 7. tetramer, 8. epicatechin, "x". unknown procyanidins

Figure 4. HPLC chromatograms of Granny Smith apple juice phenolics. A, Cinnamics, flavonols, and dihydrochalcones; B, procyanidins. (Reproduced from ref. 2. Copyright 1989 American Chemical Society.)

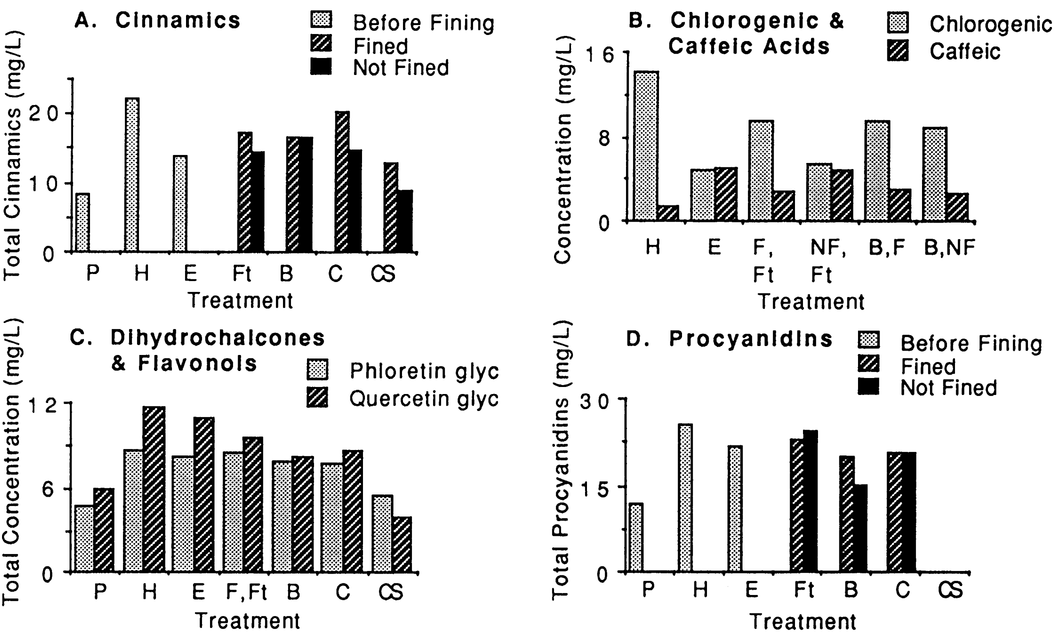



Figure 5. Effect of processing and storage on the phenolics of Granny Smith apple juice. Unit operations code: P, pressed juice; H, heat treatment; E, enzyme clarification; F, fined; NF, not fined; Ft, filtered; B, bottled single-strength juice; C, concentrate; and CS, stored concentrate.

Figure 5B compares the concentrations of chlorogenic and caffeic acids during processing. It is evident that hydrolysis of chlorogenic acid, which will result in caffeic acid liberation, occurs during enzyme clarification. Apparently the commercial pectolytic preparation has esterase activity which will hydrolyze chlorogenic acid. Fining seems to stop chlorogenic conversion; presumably the enzymes responsible are removed or inactivated during this treatment. Chlorogenic acid content of apple juice as measured by HPLC has been proposed as one compositional factor to be used in evaluating apple juice for authenticity (9,10). In light of the extreme susceptibility of chlorogenic acid to the blanching and depectinization unit operations, such proposed standards need to be amended and used with caution.

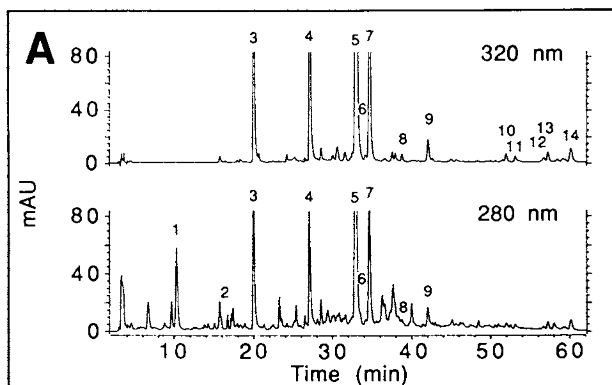
Figure 5C illustrates the effect of processing on the apple flavonols (quercetin glycosides) and dihydrochalcones (phloretin glycosides). While there is some degradation of these compounds during pressing, it is evident that they are not preferred substrates for PPO. They are relatively stable during subsequent unit operations. Because of this, these phenolic compounds would be a more reliable index for authenticity purposes than chlorogenic acid. Long-term concentrate storage results in flavonol loss of similar order to the cinnamics.

Figure 5D shows the effect of processing and storage on the procyanidin composition of apple juice. The procyanidins can serve as substrate for PPO. HTST treatment prevented their further deterioration by this route, and the compounds were reasonable stable through further processing unit operations. There was a dramatic loss of procyanidins during concentrate storage, no procyanidins being detected after nine months storage.

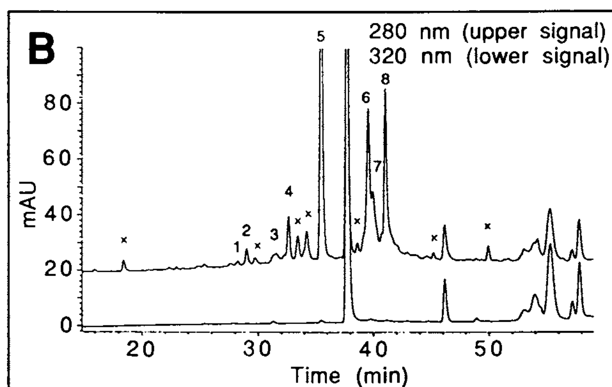
Pear Phenolics. The same HPLC analytical system gave good resolution of pear juice phenolics and enabled peak assignments and quantitation to be made in a similar manner to that described for apple phenolics (3,7). It was also necessary to isolate the procyanidins and catechins using the Sephadex clean-up procedure (4) in order to measure their concentration. Figure 6A shows the HPLC separation of cinnamics, flavonols, and arbutin in pear juice while Figure 6B is a HPLC chromatogram of the pear juice procyanidin fraction.

Figure 7A shows how the total cinnamics (chlorogenic acid and its isomers, p-coumaric and carreic acid derivaties) of d'Anjou pear juice are influenced by processing and storage. $SO_2$ addition was extremely effective in inhibiting PPO and preventing cinnamic destruction. There were higher levels of cinnamics in ripened than unripened pears; these compounds were afforded some protection by HTST treatment. There were considerable losses of cinnamics with storage of the concentrate.

The flavonols quercetin and isorhamnetic are reasonably stable through processing (Figure 7B). While they are not preferred substrates for PPO, they are protected by $SO_2$ as well as HTST treatment. There are higher levels in ripe than unripened fruit and there are considerable losses during storage of the concentrate.




Peaks: 1. arbutin, 2. HMF, 3. oxidized cinnamic, 4. oxidized cinnamic, 5. chlorogenic, 6. chlorogenic isomer, 7. caffeic, 8. coumarylquinic, 12., 13., 14. isorhamnetin glycosides




Peaks: 1. Procyanidin B3, 2. Procyanidin B1, 3. Procyanidin B4, 4. catechin, 5. Procyanidin B2, 6. trimer, 7. tetramer, 8. epicatechin, "x". unknown procyanidins

Figure 6. HPLC chromatograms of pear phenolics. A, Cinnamics, flavonols, and arbutin; B, procyanidins. (Reproduced from ref. 3. Copyright 1989 American Chemical Society.)

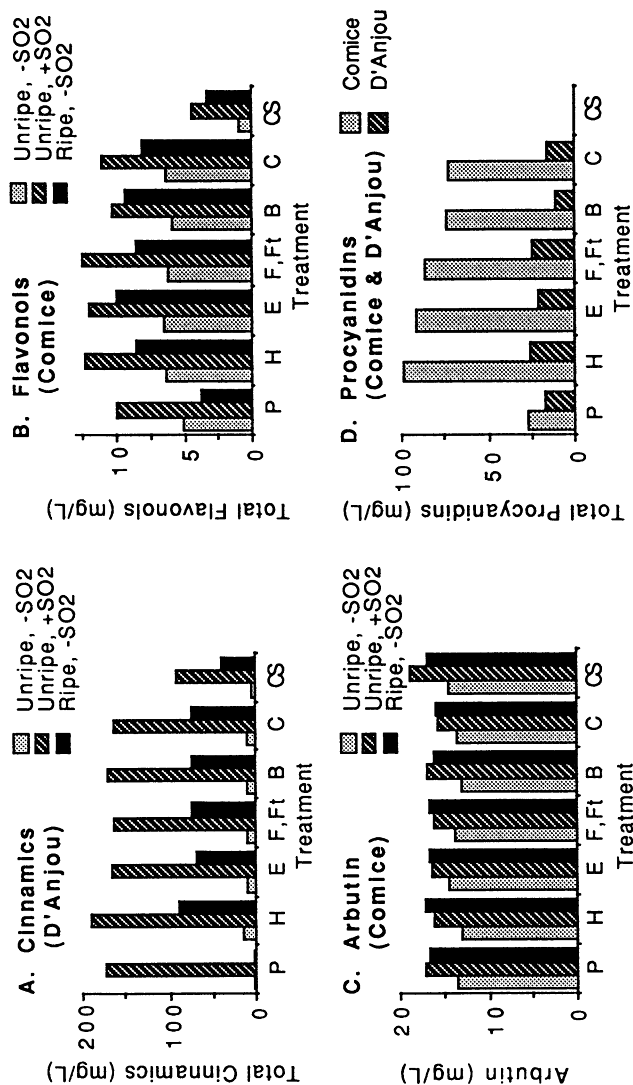



Figure 7. Effect of processing and storage on the phenolics of pear juice. Unit operations code: P, pressed juice; H, heat treatment; E, enzyme clarification; F, fined; Ft, filtered; B, bottled single-strength juice; C, concentrate; and CS, stored concentrate.

Arbutin, in contrast to the other phenolics, is very stable through storage and all processing unit operations (Figure 7C). Its stability along with its limited distribution in other fruits are characteristics that should promote its use as a reference marker for pear.

Procyanidins were not detected in pear juice unless they were treated with $SO_2$. Figure 7D compares the procyanidin levels in Comice and d'Anjou juices through processing and storage. Comice contained higher amounts of procyanidins than d'Anjou. HTST treatment had a protective effect, the procyanidins being reasonably stable through subsequent processing steps. They completely disappeared, however, after nine months storage as a concentrate.

White Grape Pehnolics. The same analytical system was again effective for the phenolic acids, flavonols and procyanidins of Thompson Seedless white grape juice. Grape differs from apple and pear in that the cinnamic acids are esterified with tartaric acid rtaher than quinic acid to give caftaric and coutaric derivatives. Figure 8A shows an HPLC chromatogram of the cinnamic acids and phenolics and Figure 8B shows a chromatogram of the procyanidin fraction.

Figure 9A shows the effect of processing and storage on the cinnamics of white grape juice. $SO_2$ protected cinnamics from oxidation. (HTST treatment was not done in the grape processing trials, so its effectiveness in inactivating PPO was not evaluated.) Fining, filtering, bottling and concentration had little effect on the cinnamics. There was a substantial loss of cinnamics during long-term storage of the concentrate.

The effect of processing and storage on the procyanidins of white grape juice is shown in Figure 9B. The most striking result is the complete disappearance of the procyanidins during long-term storage of the concentrate. $SO_2$ addition had a protective influence on the procyanidins while these compounds were relatively stable to the other processing operations.

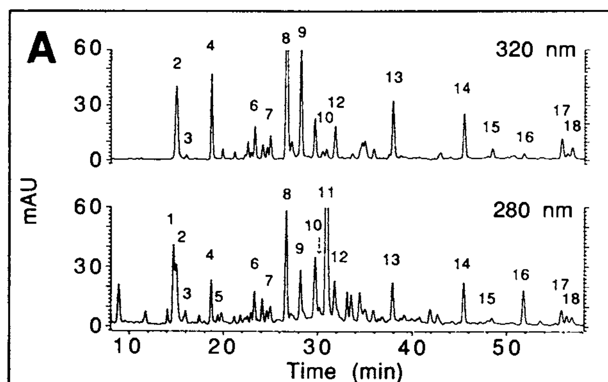
## Haze and Sediment Formation

Table III gives the protein content and percent haze readings for the single-strength apple juice and reconstituted apple juice concentrate samples. All of the samples showed high clarity as evidenced by the low haze readings, none having a propensity towards haze or sediment formation. A decrease in total soluble proteins was observed with increased storage time of apple fruit. Fining resulted in ca. 25-50% reduction in total soluble protein.

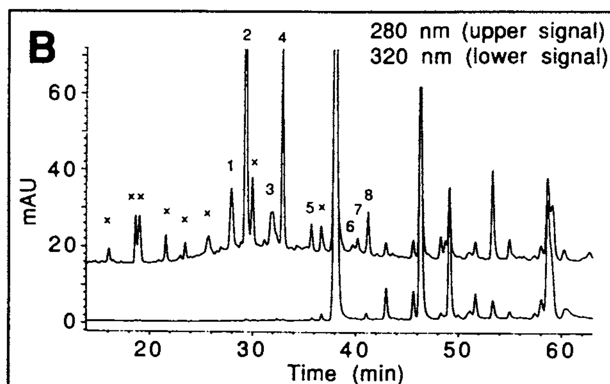
Polyacrylamide gel electrophoresis (PAGE) revealed that fining removed low molecular weight (<31,000) protein bands(5). Juice from long-term stored fruit had fewer bands (5). A heat stability test showed that juice from long-term stored fruit had more of a tendency to form haze than juice from short-term stored fruit. It was suspected that compounds other than or in addition to proteins were the likely cause of this haze (5). Another interesting observation was that new glycoproteins were detected in juices after clarification. The source of these bands was

Table III. Protein Content and Clarity of Apple Juice

| Sample | Short-term Storage | | Long-term Storage | |
|---|---|---|---|---|
| | Unfined | Fined | Unfined | Fined |
| | Total Soluble Protein (mg/L) | | | |
| Single Strength | 20.7 | 15.7 | 13.5 | 7.6 |
| Reconstituted | 19.9 | 14.7 | 15.1 | 10.2 |
| | Percent Haze | | | |
| Single Strength | 2.7 | 1.0 | 1.5 | 1.9 |
| Reconstituted | 0.5 | 0.3 | 0.3 | 0.2 |




Peaks: 1. tyrosine, 2. oxidized cinnamic, 3. gallic acid, 4. oxidized cinnamic, 5. HMF, 6. oxidized cinnamic, 7. cis-caftaric, 8. caftaric, 9. glutathionylcaftaric, 10. cis-coutaric, 11. tryptophan, 12. p-coutaric, 13. caffeic, 14. p-coumaric, 15. ferulic, 16. hydroxybenzoic ester, 17. rutin, 18. quercetin glycoside




Peaks: 1. Procyanidin B3, 2. Procyanidin B1, 3. Procyanidin B4, 4. catechin, 5. Procyanidin B2, 6. trimer, 7. tetramer, 8. epicatechin, "x". unknown procyanidins

Figure 8. HPLC chromatograms of Thompson Seedless grape juice phenolics. A, Flavonols and phenolic acids; B, procyanidins. (Reproduced from ref. 3. Copyright 1989 American Chemical Society.)

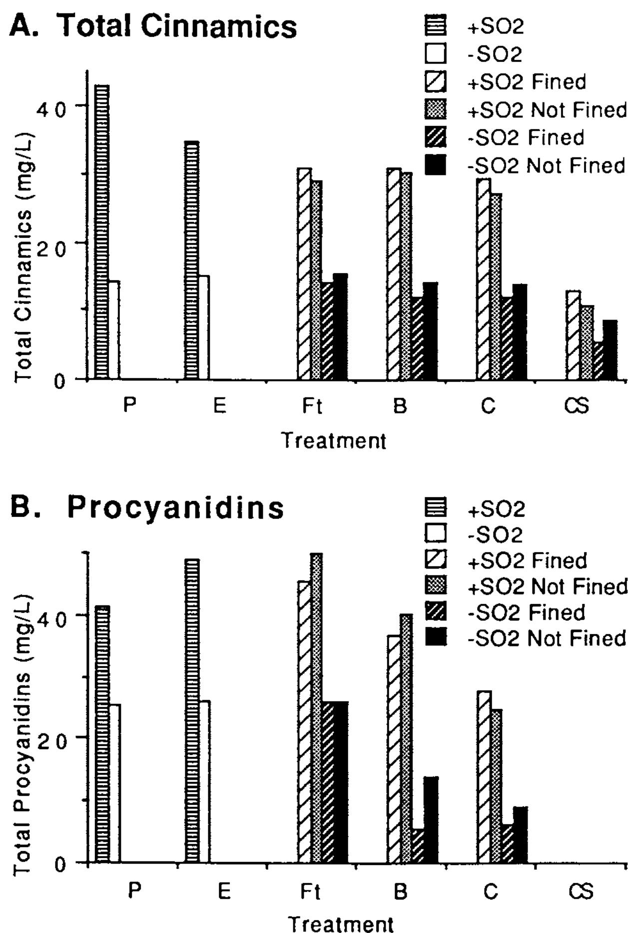



Figure 9. Effect of processing and storage on the phenolics of Thompson Seedless grape juice. Unit operations code: P, pressed juice; E, enzyme clarification; Ft, filtered; B, bottled single-strength juice; C, concentrate; and CS, stored concentrate.

shown to be the clarifying enzyme preparation which possessed pectolytic, arabinase and amylase activity. They were not removed with fining.

None of the bottled pear juices or reconstituted pear juice samples showed particular problems of haze or sediment formation (Table IV). $SO_2$ addition, which protected phenolics from oxidation, had no influence on the clarity of these samples. Juice from ripened d'Anjou and Comice fruit had slightly higher % haze readings than juice from unripe fruit. All of these samples were subjected to standard fining procedures which may account for their stability and clarity (6).

## Browning of Concentrates During Storage

Figure 10 shows a typical plot of the change in browning of Bartlett juice concentrates during storage at 25°C for 210 days. The plots are closer to zero order than to first order, but they show a faster rate during the latter part of the study. All juice concentrate samples showed similar behavior, the browning rate being faster at later stages. Table V shows the initial browning index, browning rates and the formol values for all concentrate samples. Initial browning values were not predictive of browning rate or final browning values. $SO_2$ addition greatly reduced initial browning indices, but it did not reduce the browning rates. Concentrates from ripe pear fruit tended to have higher initial browning indices and higher browning rates than those from unripe pears. There was a clear distinction between pear varieties with Bartlett having much higher initial browning values and browning rates than d'Anjou or Comice. Formol values were predictive of browning rates for the pear samples in that Bartlett had the highest quantities of free amino acids and highest browning rate, d'Anjou was intermediate and Comice had the lowest. This relationship held for the other juices except that while Thompson Seedless had the highest formol values, its browning rates were similar to Granny Smith apple and d'Anjou pear juice concentrates. This may be due to qualitative differences in the amino acid profile as individual amino acids differ in their browning rate (11). Analyses of the free amino acid composition of these samples are in progress.

Fining trials with apple and white grape juice reveal that fined juices have slightly lower initial browning indices than their unfined counterparts. The browning rates of the fined samples, however, were the same as those that were not fined. Long vs. short-term storage had no influence on either initial browning indices or browning rates.

These results suggest that processors should give attention to reduction of free amino acid levels as an effective means of reducing the browning rates of juice concentrates. Possibilities for consideration include varietal selection, post-harvest treatment, and use of processing adjuncts such as cation exchange resins (12).

## Conclusions

In this investigation we have tracked the compositional profiles of certain constituents in apple, pear and white grape juice and how they change (or do not change) during processing and storage. The sugars and nonvolatile acids are relatively stable while the phenolic profiles show considerable change, particularly as

Table IV. Haze and Clarity of Pear Juice

| | Bottled Single-strength | | Reconstituted Concentrate | |
|---|---|---|---|---|
| Sample | % Haze | Clarity[a] | % Haze | Clarity[a] |
| Bartlett | | | | |
| Unripe | 0 | - | 3.3 | +- |
| Ripe | 0 | - | 2.0 | +- |
| Comice | | | | |
| Unripe | 0 | - | 0.2 | - |
| Unripe, +$SO_2$ | 0 | - | 0.7 | +- |
| Ripe | 0 | - | 2.5 | +- |
| d'Anjou | | | | |
| Unripe | 0 | - | 0.8 | +- |
| Unripe, +$SO_2$ | 0 | - | 0.8 | +- |
| Ripe | 0 | - | 1.2 | +- |

[a]Visual clarity: -, clear; +-, trace of haze (only detected under strong light); +, slight haze

Table V. Initial Browning Indices, Rate Constants, and Formol Values of Juice Concentrates Stored for 210 Days at 25°

| Sample | Initial Browning Index (A 420 nm) | Rate Constant (Zero Order) | Formol Value (meq/100 mL) |
|---|---|---|---|
| Bartlett | | | |
| Unripe, fined | 0.582 | $26 \times 10^{-4}$ | 0.122 |
| Ripe, fined | 0.614 | $43 \times 10^{-4}$ | 0.110 |
| Comice | | | |
| Unripe, fined | 0.180 | $5 \times 10^{-4}$ | 0.039 |
| Unripe, fined, $+SO_2$ | 0.076 | $7 \times 10^{-4}$ | 0.036 |
| Ripe, fined | 0.251 | $5 \times 10^{-4}$ | 0.039 |
| d'Anjou | | | |
| Unripe, fined | 0.230 | $9 \times 10^{-4}$ | 0.076 |
| Unripe, fined, $+SO_2$ | 0.093 | $10 \times 10^{-4}$ | 0.094 |
| Ripe, fined | 0.264 | $10 \times 10^{-4}$ | 0.082 |
| White Grape | | | |
| Fined, $SO_2$ | 0.093 | $11 \times 10^{-4}$ | 0.197 |
| Fined | 0.133 | $10 \times 10^{-4}$ | 0.193 |
| Not Fined, $SO_2$ | 0.130 | $11 \times 10^{-4}$ | 0.206 |
| Not Fined | 0.172 | $10 \times 10^{-4}$ | 0.205 |
| Apple | | | |
| Short-store, Fined | 0.215 | $10 \times 10^{-4}$ | 0.070 |
| Long-stored, Fined | 0.222 | $11 \times 10^{-4}$ | |
| Short-store | 0.239 | $12 \times 10^{-4}$ | 0.072 |
| Long-store | 0.248 | $10 \times 10^{-4}$ | |

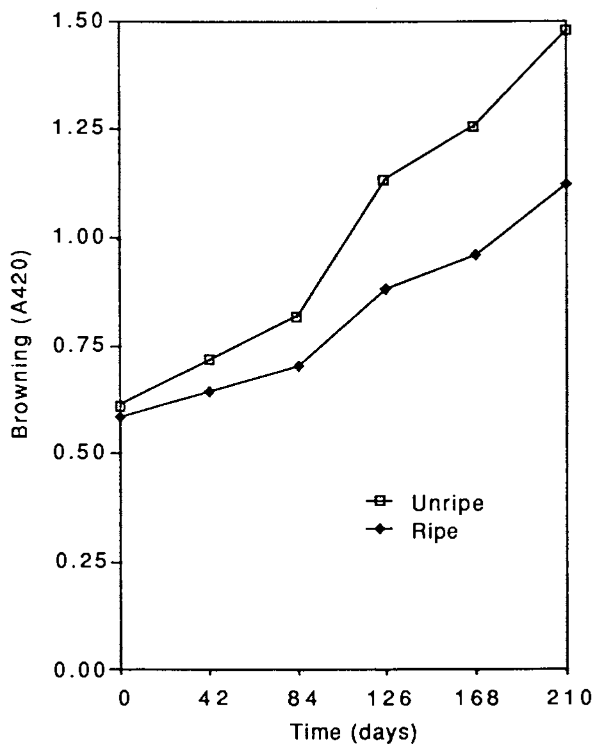



Figure 10. Browning ($A_{420\ nm}$) of Bartlett pear juice concentrates during storage at 25 °C for 210 days. Samples made from unripe and ripened pears from the same lot.

In Quality Factors of Fruits and Vegetables; Jen, J.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

influenced by HTST treatment, $SO_2$ addition, and storage of concentrate. It should be possible to exploit the complexity and dynamic character of the phenolics in investigations concerning authenticity and processing history. There is a need to expand the data base to include additional varieties and post-harvest practices. There is a particular need to give attention to such relatively new processing practices as enzymic liquefaction and diffusion extraction which are being widely adopted in commerce.

The formol index (total free amino acids) was the best predictor of browning rate of concentrates during storage for a given commodity. While haze and sediment formation were quality factors of major interest in this study, none of the juices or concentrates produced were particularly troublesome in this regard. The phenolic and protein profiles should still provide a reference with which unstable experimental or commercial samples can be compared in the future, however. In addition to color and appearance, quality factors of flavor, astringency, bitterness, viscosity and mouthfeel warrant investigation.

The results of this investigation, in addition to being useful to processors and users of juice concentrates, should be helpful to regulatory officials for making decisions concerning dietary recommendations and regulatory concerns.

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Chapter 22

# Chemical Changes in Citrus Juices During Concentration Processes


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This report discusses practical aspects of the effects of heating, evaporation, freeze concentration and reverse osmosis on certain juice constituents, most notably, the volatile flavor constituents. The commercial processes used to remove water from citrus juices have a requirement for thermal treatment of the feed stream to reduce microbial load and inactivate enzymes. While heating stabilizes juice to chemical changes caused by enzymes and microbes, it results in changes to volatile and non-volatile constituents. Also, oxygen reduction prior to heating and/or concentration is important to minimize changes in labile compounds. Most oxygen is removed by vacuum during juice concentration in the evaporator. However, elevated temperatures in the early stages of the evaporator result in some oxygen loss due to ascorbic acid oxidation and other thermally accelerated oxidation reactions.


Strong consumer demand for processed citrus juices is responsible for the very large quantities of concentrates produced and shipped worldwide. The major producers, Brazil and the United States, manufactured approximately 1.32 million metric tons (265 million gal.) of 65% soluble solids (65°B) orange juice concentrate during the 1987 season (1,2). This large amount represents a considerable commitment to concentration, since the final 65°B product has about a 7-fold increase in soluble solids, compared with the single-strength juice. Most of the concentration is performed by high temperature, short time vacuum-steam evaporation; however, recent design improvements in freeze concentration and reverse osmosis have helped to commercialize these technologies in the citrus industry. The following discussion will describe some of the primary juice quality changes resulting from these concentration processes.


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## Thermal Treatment

In order to preserve cloudiness and reduce the number of microorganisms which could cause spoilage, citrus juices are heated to temperatures above 90°C for varying lengths of time. The effect of this heating on certain juice constituents has been studied. Ting (3) reviewed thermal effects on nutrients in citrus juices and reported that the severe treatment of heating orange juice to boiling for 15 min resulted in only a 4% loss of ascorbic acid. Other authors also cited the thermal stability of this compound during processing of citrus juices (4).

Perhaps the most easily measured chemical change in citrus juice resulting from thermal treatment is loss of activity of pectinesterases, enzymes responsible for loss of juice turbidity or cloud. Commercial time-temperature conditions vary depending on juice pulp content (5), citrus cultivar, biological maturity, and whether heating is by heat exchanger to stabilize fresh juice or is part of the evaporation process (6). In practice, temperatures vary from 90 to 101°C for times of a few seconds to several minutes (6,7). If such adverse treatment is necessary to inactivate enzymes in juices containing heat-labile constituents, one might expect other chemical changes to occur.

Bitterness. Heating can affect the amount of perceived bitterness in some citrus juices, caused by the presence of the water-insoluble compound, limonin. The insoluble bitter form, limonin, is bound in the pulp and has a more soluble non-bitter hydroxyacid form. Heating of pulp-containing juice is thought to favor chemical equilibrium to the more soluble hydroxyacid, increasing concentration in the juice by extraction from the pulp (8).

Naringin is a bitter flavone glycoside present in grapefruit. Like limonin, it can cause the juice to be very bitter. Naringin is present in the membranes and tissues, is sparingly soluble in cold, but quite soluble in hot water (9). During heat processing, it is extracted into the juice from the insoluble solids and pulp, leading to increased bitterness.

## Evaporation

Vacuum evaporation to preserve vitamin C in citrus juice was the subject of a number of early studies (6). Generally, low temperature evaporation of non-heated fresh juice was performed to preserve volatile flavor constituents in the finished product. This technique has now been replaced in the citrus industry by high temperature short time commercial processes, including recovery and concentration of aroma volatiles, which otherwise would be lost during juice concentration (6,7). The most common type of citrus juice evaporator, manufactured by Gulf Machinery, Inc., Clearwater, FL, is referred to as a TASTE (Thermally Accelerated Short Time Evaporator) evaporator. The present process requires juice to be preheated before the first effect of a TASTE evaporator to temperatures as high as 101°C, and remain above 50°C for over 5 min (7). No doubt, such severe heat treatment under vacuum is responsible for quality and flavor changes comparing the fresh juice with the finished 65°B concentrate.

Aroma Changes. Varsel (6) described how the citrus industry practices recovery and use of volatile aromas as essence. However, due to loss of some volatiles during recovery and concentration of the essence, the final product essence does not contain all of the flavor notes of fresh juice. Citrus juice routinely is concentrated from single strength (10°B) to above 65°B. At the highest concentration factor, there is almost no aroma present which could serve to identify the fruit cultivar. In a typical 4-effect commercial citrus juice evaporator, the most volatile aromas and a high percentage of the terpenes have been removed at about 25% evaporation of the initial juice volume (10). Less than this volume results in low recovery of poorly volatile and azeotropic compounds; however, greater than 25% could mean longer exposure of heat sensitive compounds to higher temperatures.

Aroma Quantitation. The terpene fraction of the aroma is mostly S-(+)-limonene and can be used to give an indication of aroma stripping loss or efficiency during concentration. For example, Mannheim et al. (11) reported that at a temperature of 80°C in an evaporator, with 29% of the juice evaporated, the stripped juice contained only 9 ppm limonene of the original 110 ppm in the fresh juice. Here one might caution that measuring the limonene is simple and quantitative (12), but may not accurately represent separation and recovery of more highly volatile or less volatile aromas.

Quantitation and identification of aroma compounds in fruit juices has been a subject of many studies (13). For citrus, single strength juices from extractors have a range of limonene concentration from 0.02 to 0.05% by volume. However, the limonene content in juices reconstituted from evaporator pumpout is usually adjusted to less than 0.02% for best flavor quality. The content of 65°B evaporator pump-out is less than 0.003% limonene. Even though limonene can be reliably quantitated, other juice aroma constituents are probably more important to the flavor. However, quantifying these compounds for the purpose of studying processing changes or chemical changes in products has been at least as complex as identifying them.

Oxygen and Ascorbic Acid. Thermal abuse of juice in an evaporator is more severe than if the juice is pasteurized and cooled in a heat exchanger. Such abuse can result in noticeable chemical browning of the product stream. This browning in citrus juices has been, in part, linked to ascorbic acid loss for stored juices (14). Browning is more obvious for juices from lemon or white grapefruit, which do not benefit from masking pigments. Thermal treatment of grapefruit juice results in a predictable degree of browning, according to one study (15). In another report, the effects of heating during grapefruit juice concentration were related to loss of ascorbic acid and also shown to be predictable (16). An interesting study also suggested that the thermal history or degree of heating of orange juice might be predicted using a bacterial mutagenicity assay specific for some heat-induced browning products (17). However, large variations in some processing operations, or juice properties

(e.g. pulp content), may make application of prediction equations difficult.

## Oxygen and Ascorbic Acid Loss in TASTE Evaporators

Ascorbic acid degradation has both oxidative and non-oxidative roots. Chen (7) traced the temperature history of juice during concentration in a typical TASTE evaporator. Nagy and Smoot (18) gave extensive data on ascorbic acid loss for orange juice stored at various temperatures in hermetic containers. Calculated ascorbic acid loss based on temperature values of Chen and degradation rates from Nagy and Smoot are given in Table I. Thermally mediated ascorbic acid loss during evaporation would total < 0.02% of the ascorbic acid initially in the juice.

Table I. Anaerobic Destruction of Ascorbic Acid in Orange Juice from the Extractor to Various Stages in a TASTE Evaporator

| Stage | Average Temperature °C | Time in Stage Sec | Reaction* Constant (k) %/Sec | Ascorbic** Acid Loss mg/100 ml |
|---|---|---|---|---|
| Extractor | 25 | 40 | $2.2 \times 10^{-7}$ | $5.3 \times 10^{-6}$ |
| 1 | 46 | 40 | $3.0 \times 10^{-6}$ | $7.2 \times 10^{-5}$ |
| Preheater | 86 | 10 | $1.8 \times 10^{-4}$ | $1.1 \times 10^{-3}$ |
| 2 | 88 | 45 | $2.2 \times 10^{-4}$ | $5.9 \times 10^{-3}$ |
| 3 | 76 | 45 | $6.8 \times 10^{-5}$ | $1.8 \times 10^{-3}$ |
| 4 | 63 | 65 | $1.8 \times 10^{-5}$ | $7.1 \times 10^{-4}$ |
| 5 | 48 | 70 | $3.6 \times 10^{-6}$ | $1.5 \times 10^{-4}$ |
| 6 | 42 | 75 | $1.9 \times 10^{-6}$ | $8.4 \times 10^{-5}$ |
| 7 | 42 | 80 | $1.9 \times 10^{-6}$ | $9.0 \times 10^{-5}$ |
| | | | | 0.01 mg/100 ml |

*Based on Arrhenius equation $\ln k = 18.28 - 7709 \cdot 1/T$

**Based on an initial ascorbic acid concentration of 60 mg/100 ml

The oxygen concentration in juice at various stages of a TASTE evaporator is shown in Figure 1. Stage 3 readings identified a vacuum leak. There was no measurable oxygen beyond the 5th stage. Reaction constants for oxidative ascorbic acid loss at various temperatures were determined by Sadler (19). Temperature values of Chen (7) and kinetic solubility values of Sadler (20) were used to calculate oxidation losses in each evaporator stage (Table II). Although oxygen was flashed from the system early in evaporation, oxidative ascorbic acid losses (0.37 mg/100 ml) were over 30 times greater than non-oxidative degradation. This is consistent with previous research which indicated oxidative ascorbic acid losses in citrus ranged from 10 (21) to 1000 (22) times faster than destruction through the anaerobic pathway. Even if all dissolved oxygen was devoted to ascorbic acid degradation, only about 4 mg of ascorbic acid would be lost.

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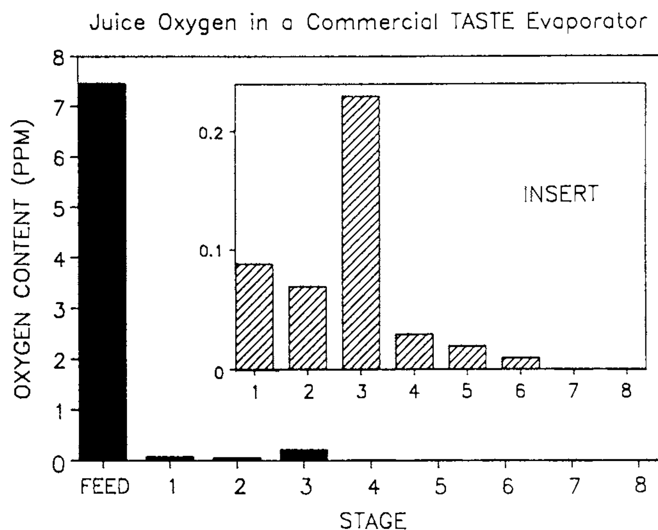


Figure 1. Oxygen content in the feed juice and various stages (1-8) of a TASTE citrus juice evaporator.

Table II. Values for Aerobic Destruction of Ascorbic Acid in Orange Juice from the Extractor to Various Stages in a TASTE Evaporator

| Stage | Avg. Temp. °C | Time in Stage (Sec) | Reaction* Constant (k) %/Sec | Ascorbic** Acid Loss mg/100 ml | Oxygen Content Entering Stage ppm |
|---|---|---|---|---|---|
| Extractor | 25 | 40 | $5.1 \times 10^{-4}$ | 0.01 | 7.47 |
| 1 | 46 | 40 | $2.8 \times 10^{-3}$ | 0.07 | 7.42 |
| Preheater | 86 | 10 | $4.1 \times 10^{-2}$ | 0.25 | 7.27 |
| 2 | 88 | 45 | $4.6 \times 10^{-2}$ | | 6.82 |
| 3 | 76 | 45 | $2.1 \times 10^{-2}$ | | 0.09 |
| 4 | 63 | 65 | $9.2 \times 10^{-3}$ | 0.04 | 0.07 |
| 5 | 48 | 70 | $3.1 \times 10^{-3}$ | | 0.03 |
| 6 | 42 | 75 | $2.0 \times 10^{-3}$ | | 0.02 |
| 7 | 42 | 80 | $2.0 \times 10^{-3}$ | | 0.01 |
| | | | | 0.37 mg/100 ml | |

*Based on Arrhenius equation $\ln k = 18.28 - 7709 \cdot 1/T$

**Based on 60 mg/100 ml initial ascorbic acid concentration.

## Freeze Concentration

Compared with heat concentration, the superior flavor quality of fruit juices concentrated by freeze concentration has been known for many years (23). Stahl (24) stated for citrus, "juice concentrated by this method possesses a richer fruit flavor than that previously obtained by any other known process, because no volatile flavors or aromas are lost and the chemical changes liable to occur during concentration are reduced to a minimum." More recent reviews of this water removal technique applied to fruit juices concluded the aroma, flavor and nutrient content of products were of superior quality (25,26). The main problem of early freeze concentration designs was loss of soluble sugars during ice removal, resulting in increased cost of concentrated product. This problem has been solved with the commercialization of a multistage process meeting requirements of flavor retention as well as minimal loss of soluble sugars and other juice constituents (27,28). However, high viscosities of the concentrated products at low temperatures limit the degree of concentration and the amount of pulp and insoluble solids which can be present.

To reduce viscosities and favor more efficient water removal at high concentrations, juice pulp reduction by centrifugation is performed prior to freeze concentration of citrus juices. However, this process needs careful control to prevent enzymatic or chemical

changes to the juice prior to centrifugation. Also, some important flavor compounds may be bound to or associated with the pulp fraction (29). This might necessitate cautious handling of the separated pulp fraction, particularly if it is to be blended back into a finished product.

Since initial flavor is preserved, the most important consideration for freeze concentration is that feed juices be of very high quality. For example, juice from immature or overmature, or a few rotten fruit, may contain off-flavors which will still be present in the finished product. Also, juice handling practices prior to actual concentration will affect final product quality. For example, holding raw juice for a short time will allow destabilization of the cloud by citrus pectinesterase. This process is not reversible by mechanical homogenization or other treatment.

Orange Juice. In a study of some parameters important to freeze concentration of orange juice, horticultural factors related to the fruit, juice handling and the extent of thermal treatment were more important to product quality than the concentration process (30). It is possible to freeze concentrate fresh, unpasteurized juice, followed by thermal treatment to inactivate enzymes. One could expect that chemical and physical changes to the product would be more apparent after concentration than if heating occurred prior to concentration. Increasing temperature from 80 to 111°C did significantly alter the proportion of reducing sugars and sucrose in freeze concentrate (30). However, only slight changes were measured in common juice quality parameters resulting from thermal processing of freeze concentrate at temperatures of 80, 97 and 111°C (Table III).

Table III. Quality Parameters of Fresh Orange Juice Which was Freeze Concentrated (FCNP), Then Pasteurized for 6 Sec at 80 (FC80), 97 (FC97) and 111°C (FC111)

| | FCNP | FC80 | FC97 | FC111 |
|---|---|---|---|---|
| °Brix | 13.3 | 13.3 | 13.3 | 13.3 |
| Acid (%) | 0.85 | 0.85 | 0.85 | 0.85 |
| °B/acid | 15.7 | 15.7 | 15.7 | 15.7 |
| Oil (%) | 0.020 | 0.018 | 0.017 | 0.016 |
| Vitamin C (mg/100 ml) | 43 | 44 | 43 | 41 |
| Color (CR) | 21.7 | 21.2 | 20.8 | 22.5 |
| (CY) | 64.6 | 64.2 | 64.6 | 65.3 |
| (N) | 33.2 | 33.1 | 33.1 | 33.4 |
| Pulp (%) | 1 | 1 | 1 | 1 |
| Enzyme (PEU) | 0.5 | 0.1 | 0 | 0 |
| Viscosity (mPa · s) | 3 | 3 | 4 | 4 |

Data compiled from reference 30.

Orange juice quality parameters may not show obvious change resulting from heat treatment; but sensory changes are more readily

detected. Flavor panelists asked to rank the concentrates listed in Table III according to degree of fresh juice or processed flavor were capable of identifying the sample with the most severe heat treatment (111°C), but could not distinguish between concentrates listed in Table IV heated to 80 and 97°C (30).

Table IV. Mean and Standard Deviations for Sensory Ranking Fresh Concentrated Orange Juice Which was Pasteurized for 6 Sec at 80 (FC80), 97 (FC97) and 111°C (FC111)

| Ranking | Sample | Evaluations | Mean | Std. Dev. |
|---|---|---|---|---|
| Most fresh | FC80 | 15 | 1.5 c | 0.64 |
| | FC97 | 15 | 1.7 c | 0.70 |
| | FC111 | 15 | 2.8 d | 0.41 |
| Most processed | FC111 | 15 | 1.3 e | 0.72 |
| | FC97 | 15 | 2.3 f | 0.62 |
| | FC80 | 15 | 2.3 f | 0.71 |

Means with similar letters are not significantly different. Data compiled from reference 30.

Volatile flavor constituents in freeze concentrated orange juice have been studied and gas liquid chromatographic profiles and data are published. Significant studies by researchers from the Procter and Gamble Co. validate the premise that orange juice concentrated by freeze concentration has exceptional aroma and flavor qualities (31-33). Use of GLC techniques for aroma evaluation of freeze concentrated juices may also be used to indicate something of a juice's processing history. For example, losses of the most volatile juice aromas (alcohol, acetaldehyde) may occur during pasteurization or handling, affecting the proportions between compounds with high and low volatilities (30). On the other hand, a very high concentration of alcohol in aroma fractions condensed from juices could be indicative of yeast growth prior to pasteurization.

Grapefruit Juice. Similar to orange juice, freeze concentration of grapefruit juice results in a product with important aroma and flavor properties preserved. Strobel (34) reported that such a concentrate would contain at least 65% of the total volatile aromas present in the fresh juice. These volatile aromas would also need to be in recommended proportions in order for the reconstituted, blended products to be most like the natural juice. Thermal processes to inactivate enzymes prior to freeze concentration of grapefruit juice may also cause changes in the volatile oils and aroma fractions. Too much peel oil (> 0.023%) in grapefruit juice may not be perceived as acceptable quality; however, steam infusion heating and vacuum cooling/stripping to reduce it to an acceptable amount (< 0.012%), also resulted in a corresponding decrease of

other aromas (35). Even though grapefruit has different sensory properties than orange juice, the requirement for highest quality feed juice to produce highest quality freeze concentrate holds true.

## Reverse Osmosis

Membrane concentration of liquid foods is common in the food industry. This technology will expand to additional applications with development of more heat stable, chemically inert membranes with high water flux properties. Applications using cellulose acetate reverse osmosis membranes to concentrate citrus juices allowed good retention of important water soluble juice constituents, i.e., sugars, acids, minerals; however, there was poor recovery of some flavor compounds significant for juice quality (36,37). Recent membrane processes for fruit juice concentration have effectively dealt with improving retention of aroma compounds and increasing flux rates (38,39).

Pulp-Serum Separation. As for freeze concentration, high viscosity contributes to limiting the degree of concentration which can be achieved by membrane concentration. Since the insoluble solids present in citrus juices contribute significantly to viscosity, one consideration for process development is to separate the pulp from the clear serum, prior to concentration. Because consumers recognize that citrus juices are cloudy and contain pulpy material, it becomes a requirement to blend the separated pulp back with the product concentrate (31).

Pulp-serum separation followed by pulp addition after concentration is not as simple as it may appear. First, while higher flux and concentration factors of the serum portion may be achieved, the soluble solids and water content of the separated pulp is in equilibrium with the single strength feed juice. Thus, dilution of the concentrate will occur when the pulp is mixed, depending on the amount added. Juice handling time and number of steps will also increase. Under commercial conditions, requirements to handle large volumes may then allow microbial or enzymatic changes to occur in the juice or pulp before and during concentration. Examples of such changes could be cloud destabilization by pectinesterase or production of off-flavors by microbial growth. The latter may be important, since membrane concentration processes do not have the benefit of vacuum aroma stripping as does evaporation.

Another question to be considered is how and when does one remove the pulp? Before enzyme stabilization? Or after? If the pulp is removed first, it is more difficult to heat stabilize the pectinesterase bound in the pulp, requiring high temperatures and long hold times (5), not to mention engineering difficulties of handling and heating pulp. If pulp is removed after heating, removal to yield a serum is more difficult and may involve more handling and longer process times. In either case, flavor and quality properties of the pulp fraction can deteriorate.

Finally, there is evidence that desirable flavor constituents are bound to or associated with the pulp fraction in the juice (29,40). This implies that care must be taken to prevent changes to

Table V. Properties of Feed, Concentrate (Conc) and Permeate (Perm) Streams for Orange, Grapefruit and Lemon Juices Concentrated by Reverse Osmosis

| | | Orange | Grapefruit | Lemon |
|---|---|---|---|---|
| Concentration (°B) | feed | 11.98 | 8.68 | 7.70 |
| | conc | 25.26 | 25.06 | 22.52 |
| | perm | 0 | 0 | 0 |
| Hexose (%) | feed | 10.68 | 7.94 | 1.38 |
| | conc* | 10.80 | 7.15 | 1.38 |
| | perm | 0.03 | 0.05 | 0.05 |
| Acid (%) | feed | 0.89 | 0.90 | 4.63 |
| | conc | 1.86 | 2.66 | 13.30 |
| | perm | 0 | 0 | 0 |
| Vitamin C (mg/100 ml) | feed | 43 | 46 | 36 |
| | conc* | 43 | 45 | 36 |
| | perm | 0 | 0 | 0 |
| Volatile oil (%) (as limonene) | feed | 0.023 | 0.007 | 0.035 |
| | conc* | 0.019 | 0.005 | 0.024 |
| | perm | 0 | 0 | 0 |
| Pectin (%) | feed | 0.033 | 0.022 | 0.05 |
| | conc* | 0.020 | 0.026 | 0.05 |
| | perm | 0 | 0 | 0 |
| Pulp (% v/v) | feed | 10 | 7 | 9 |
| | conc* | 8 | 5 | 8 |
| Viscosity (cs at 30°C) | feed | 1.9 | 1.5 | 1.8 |
| | conc | 3.9 | 2.7 | 6.6 |

*Values measured after dilution to single strength (feed) °B. Data compiled from reference 42.

these labile compounds during processing and holding for blending to the concentrated serum. Also, the contribution of flavor from the pulp to the blended mixture must be considered, since the flavor spectrum of the concentrated serum-pulp product will be dependent on how much pulp is added.

Juice Properties. Reverse osmosis concentration of orange juice has been reported on at least a small commercial scale (41). For present technology considering preconcentration without the alternative of pulp-serum separation, chemical differences between concentrates and feed streams are slight. Juice and concentrate properties listed in Table V verify that the process is mild. Also, juice aroma and mineral constituents retention by commercial membranes has been shown to be very good for orange, grapefruit and lemon juice concentrated in a pilot plant study (42). The potential for using membranes to recover and concentrate citrus essences, oils, flavor chemicals and other by-products has also been a subject of study (43,44). When one considers the advanced state of membrane technology and pool of available information, the logical conclusion is that large-scale commercial concentration of citrus juices and products will be a reality.

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## Chapter 23

# Chemical Changes in Aseptically Processed Kiwi Fruit Nectars

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Changes in chemical constituents of kiwifruit (Actinidia chinensis, Blanch, Hayward cultivar) during post-harvest cold storage and ripening under controlled conditions were investigated. The fruit was harvested at the mature green stage with soluble solid content at 8.0° Brix. Ripening the fruit at 20°C under a stream of water-vapor saturated air containing 5 ppm ethylene gas resulted in rapid softening in texture and rising of soluble solids to 14.5° Brix in 4 to 5 days. The increase in soluble solids was accompanied by increase in fructose, glucose and decrease in starch content. The volatile compounds in ripe kiwifruit were identified by the gas chromatography-mass spectroscopic methods (GCMS).

Kiwifruit (Actinidia chinensis) is becoming an important fruit crop in California. In 1987, more than 30,000 tons of kiwifruit were produced in the State. About 20% of the crop are not suitable for marketing as fresh fruit because of the irregular shape, odd size, defects and soft texture. It becomes very important to find methods for preservation and utilization of the crop. One of the approaches is to process the kiwifruit into juice and nectar.

The fruit juice industry is undergoing a period of rapid change in the technology used for processing and packaging of shelf-stable juices. The change in technology started in 1981 with the approval by the Food and Drug Administration (FDA) of hydrogen peroxide for the sterilization of packaging materials used in aseptic processing systems. Since that time, flexible multilayer cartons are partially replacing the traditional can and glass containers. Presently, increasing amount of apple, citrus, cranberry, pineapple and mixed fruit jices are processed by the aseptic packaging tehnique in 250 mL containers as single-serving juice products (1).

The advantages of the high-temperature short-time aseptic canning process on quality retention in heat-processed products have been discussed by Luh and York (2). The importance of ripening process on quality of kiwifruit has been reported by

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Matsumoto et al. (3). Container and sterilization technique in aseptic packaging of foods in plastic or paper-based containers were fully discussed by Toledo (4) and Ito et al. (5).

This paper presents the changes in chemical constituents of kiwifruit during post-harvest cold storage and ripening under controlled conditions. Kiwifruit at optimum canning ripeness were processed by the aseptic canning process with sucrose or high fructose corn syrup as sweeteners. The changes in starch, sugars, volatiles, chlorophylls and organic acids in the fruit during post-harvest ripening or after processing are reported.

## Experimental Materials and Methods

Kiwifruit. One hundred kg of kiwifruit (Hayward variety) were harvested at the mature green stage with the soluble solids at 8.0° Brix, and stored at 1°C under 90% relative humidity. The average weight per fruit was 81.6 g. The average pressure test (0.794 cm plunger) was 7.5 ± 0.6 kgf. The total solids content was 16.5%.

Ripening. Twelve kg of randomly selected kiwifruit were placed in glass containers in a room kept at 20°C. A stream of water-vapor saturated air containing 5 ppm ethylene gas was passed over the fruit at a rate of 50 ml per minute. The control sample was treated under the same condition except that no ethylene gas was used in the aeration process. Samples of approximately 2 kg each were taken at designated time intervals for physical and chemical tests.

Texture. Firmness of the kiwifruit was measured in a 10 x 10 mm peeled area on both cheeks. A U. C. Davis fruit pressure tester with a 0.794 cm plunger was used in the study. The average value of twelve determinations is reported.

Sample preparation. Kiwifruit purees were prepared from the hand peeled fruit in a 1°C room after removal of seeds anmd cores. The product was homogenized in a Waring blender, and frozen immediately in sealed glass jars at -26°C. The frozen purees were thawed and mixed thoroughly before analysis. For the nectars, four representative cans were tested for each sample and the average values are reported.

pH and titratable acidity. An Altex Ø 70 pH meter with glass electrode was used to determine the pH values of the samples. Ten-g samples of the kiwifruit puree were diluted with 100 ml distilled water and titrated to pH 8.0 with 0.1 N NaOH. The results are expressed as % citric acid.

Total Solids. The AOAC vacuum oven drying method (6) was used. Five grams of sample were weighed accurately into aluminum dishes using Sartorius #2400 analytical balance. The aluminum dish containing diatomaceous earth was dried at 110°C in an oven, cooled in desiccator and weighed prior to adding the sample. The loss in weight after vacuum drying at 70°C for six hours was determined. The average of three determinations is reported.

Soluble Solids. A Zeiss Opton refractometer was used to determine the soluble solid content of the juice at 20°C. The results are reported in degrees Brix at 20°C.

Starch. The Anthrone reagent and iodine colorimetric methods described by McCready et al. (7) were employed to determine the starch and amylose contents respectively. A Perkin Elmer's Coleman 575 spectrometer was used to measure amylose at 660 nm, and starch at 630 nm.

Ascorbic Acid. The 2,6-dichlorophenol indophenol colorimetric method (11) was used to determine the ascorbic acid content of the samples.

Color. A Hunter color difference meter, Model D25D2 was used to evaluate the color of the canned products. A yellowish green porcelain plate, No. C 2 1063 (L = 78.2; a = -2.2; b = +21.7) was used as reference. The Hunter reading was made on the sample in a cylindrical plastic port 5.6 cm in diameter and 3 cm deep. The bottom part of the port was made of colorless plastic plate of 1.5 mm thickness. A light-trap can coated inside with black coating was used to exclude interfering light from the sample port. The illuminated area was ellipitical (4.1 x 4.4 cm).

Consistency. Consistency of the nectars were measured with a Brookfield Synchro-Lectric viscometer, Model RVT at 25°C.

Isolation of Volatile Constituents. The kiwifruit harvested at 8° Brix soluble solids were ripened at 20°C under 90% relative humidity in the presence of 5 ppm ethylene gas for 5 days, reaching a soluble solids of 14.5° Brix. The peeled fruit were blended at 1°C gently in a Waring blender. An aliquot of 1.2 kg of blended pulp was diluted with 700 mL distilled water in a 3-L three neck flask and distilled at 25-30°C under vacuum (1 mm Hg) for 40 hours, yielding about 500 mL of distillate that was collected in two liquid nitrogen traps. A total of 3.6 kg of kiwifruit pulp was distilled in three batches. The distillates were combined and frozen immediately until use. The combined distillate was extracted in 250-mL batches for 20 hr with 60 mL Freon 11 (bp 23.8°C). The Freon 11 was distilled through a glass distillation column, packed with Fenske helices, prior to use. Each extract was concentrated to approximately to 200 micro L, using a Vigreux column (16 cm), and a maximum temperature of 30°C.

Gas Chromatography. A Hewlett-Packard 5880A gas chromatographic unit with a flame ionization detector, equipped with a 60 m x 0.25 mm i.d. DB-WAX capillary column (J and W Scientific; bonded polyethylene glycol phase) was employed. The column temperature was programmed as follows: 30°C hold 2 min to 38°C at 1°C/min, then to 180°C at 2°C/min. Hydrogen carrier gas was used at a flow rate of 1.5 mL/min (30°C). The injector and detector were maintained at 225°C. A modified injection splitter was used at a split ratio of 1:30.

Gas Chromatography-Mass Spectrometry. A VG Trio-2 mass spectrometer was directly coupled with the Hewlett-Packard 589 R gas chromatograph, equipped with a 30 m x 0.32 mm DB-WAX capillary column (J and W. Scientific; bonded polyethylene glycol phase). The carrier gas was He at 2.3 ml/min. The injector and transfer temperature was programmed as follows: 30°C (2 min isothermal), to 38°C at 1°C/min, then to 180°C at 2°C/min. The instrument was operated in the electron-impact mode at an ionization voltage of 70eV. Quantitative analysis by peak area, plots of chromatograms, and library search results were obtained.

Sugars. The high performance liquid chromatography (HPLC) method described by Hunt et al. (8) was used to determine the sugars in the kiwifruit and nectars with some modifications (21). A Waters Association Chromatograph equipped with a Model 6000-A solvent delivery system, a Model R401 refractometer detector, a U6K universal injector column was a 30 cm x 4 mm i.d. stainless steel tube packed with u-bondapak-carbohydrate (Waters Associates). The precolumn was packed with CO-PELL PAC (Whatman). The eluent was acetonitril and distilled water (85/15, v/v).

Five grams of the sample were refluxed with 45 ml of 90% (v/v) ethanol containing a small amount of calcium carbonate for 1 hr at 80°C on a water bath. The mixture was filtered through Whitman filter paper into a round bottom flask, and the residue was recovered by washing with 80% ethanol. The filtrate was concentrated to about 5 ml under vacuum at 30°C in a Rotarvapor-R unit, and diluted to 10 ml in a volumetric flask. Finally the solution was filtered through a 0.5 micrometer celotate filter (Millipore Corp.), using a Swinnex syringefilter. The injection volume was 10 microliter in all cases. Sugars in the sample were quantified by comparing peak areas of the samples to those of the sugar standards.

Chlorophyll. The AOAC spectrophotometric method (9) was used for determination of chlorophyll. A Perkin-Elmer model Coleman 575 spectrophotometer was used to measure the absorbance at 660 and 642.5 nm respectively. The results were reported as chlorophyll, chlorophyll a and chlorophyll b.

Organic Acids. The HPLC method for determination of organic acids described by Gancedo and Luh (10) was used.

Aseptic Canning. Eight-hundred kg of kiwifruit harvested at 8.0° Brix soluble solids were received from the Alcop Farms, Chico, CA. They were kept at 1°C under 90 relative humidity for two months and then ripened under 90% relative humidity in the presence of 5 ppm ethylene gas at 20°C for five days. The product was sorted, washed with chlorinated water at 5 ppm free chlorine, and stored at 1°C overnight. A Rietz disintegrator with a 1.25 cm screen was used to break up the fruit at 900 rpm. The puree was passed through a Brown juice extractor with a 0.033-inch (0.838 mm) screen to remove skin and seeds. The back pressure in the Brown juice extractor was set at 4.5 kgf. The juice was pumped through a swept-film heat exchanger with hot water at 91°C as heating medium, and held for 70 sec in a 14 meter long, 2.29 cm ID stainless steel tube,

followed by cooling in a second swept-film heat exchanger to room temperature (22°C). Kiwifruit nectars containing 30, 40, and 50% pulp were prepared using either sucrose or high fructose corn syrup as sweeteners. The final concentration of the nectars were at 10.5 to 11.5° Brix. The products were aseptically canned in the Dole aseptic canning line through heat sterilization at 91°C in a swept-film heat exchanger, held for 15 seconds, cooled to room temperature, and canned aseptically in 202 x 314 (6 oz) cans. The lids were sterilized by heating wtih hot air at 177°C for 3 minutes before entering the aseptic sealer. Heat sterilized nitrogen gas was used to keep a positive pressure in the sealer.

## Results and Discussion

It was necessary to harvest kiwifruit at the mature stage (8.0° Brix), and ripen them under controlled conditions to achieve best sensory quality. When properly handled during harvest and post-harvest storage under refrigeration at 90% relative humidity, the fruit can be kept at 1°C for 5 or more months provided that the storage room is free from ethylene gas by use of potassium manganate or proper ventilation with devices to remove ethylene gas (12). The standards for grade of kiwifruit have been published by the United States Department of Agriculture in 1982 (13). The minimum soluble solids content of kiwifruit at harvest time was set at 6.5° Brix. At this stage, the fruits are mature and suitable for cold storage for shipping to distant market under refrigerated storage (14).

Seasonal Changes in Fresh Weight and Ascorbic Acid. The changes in fresh weight and ascorbic acid of Hayward cultivar kitifruit have been studied in detail by Okuse et al. (15). The composition changes in the developing kiwifruit after full bloom. Starch was the dominant carbohydrate stored in the capillary tissue and becomes partially hydrolyzed as the fruit reach maturity. Glucose was rich in the green immature fruit, but the level decreased while starch accumulate rapidly during late July and August. As starch hydrolysis began, glucose level increased rapidly by harvest. Fructose increased gradually from the youngest stage until harvest.

Firmness, Soluble Solids and Acidity Changes. During the post-harvest ripening period, the changes in firmness, soluble solids, pH and acidity of kiwifruit at 20°C in the presence of 5 ppm ethylene gas are shown in Table I.

Table I. Changes in Firmness, Soluble Solids and Acidity of Kiwifruit when Ripening at 20°C with 5 ppm Ethylene

| | Days | | | | | | L.S.D. |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | (p=.05) |
| Firmness, kgf | 7.5 | 3.4 | 1.8 | 1.4 | 1.2 | 0.45 | 0.1 |
| ° Brix | 7.2 | 9.7 | 12.0 | 13.2 | 13.8 | 14.5 | 0.3 |
| pH | 3.28 | 3.32 | 3.34 | 3.40 | 3.42 | 3.42 | 0.1 |
| Acidity, as citric, % | 1.45 | 1.40 | 1.38 | 1.37 | 1.34 | 1.28 | 0.02 |

Firmness, one of the indicators for ripeness level was influenced greatly by the presence of 5 ppm ethylene at 20°C. A rapid softening in texture was observed after ripening for 1 day. Very little change was observed in the control sample which was kept at 1°C without ethylene gas. It has been reported that kiwifruit is a climacteric fruit through the study of its physiological changes during growth (16) and post-harvest ripening (17). Ethylene was shown to be produced naturally in plant tissue from glucose, linoleic acid and methionine (18). It acts as a plant hormone which controls the ripening process. There are two concepts about fruit ripening. First, ripening occurs due to the increase in cell permeability which leads to the random mixing of enzymes and substrates present in the tissue. Secondly, the ripening process is at the final stage of differentiation which is under genetic control. It involves the programmed synthesis of specific enzymes required for ripening. The influence of ethylene gas on the ripening of kiwifruit was clearly observed.

The sensory quality of kiwifruit was greatly improved, resulting from the softening of the texture, decrease in acidity, and increase in soluble solid content due to the conversion of starch into sugars. In addition to these changes, there were enzymatic reactions which cause formation of more volatiles that improve the aroma of the fruit.

Starch. Starch is an important polysaccharide present in fruits such as apple, banana, mango and others. Wright and Heatherbell (19) reported that kiwifruit contains 5-8% starch, and Pratt and Reid (17) reported that young kiwifruit has a high starch content. The changes in starch during ripening at 20°C in the presence of 5 ppm ethylene are presented in Table II.

Table II. Changes in Starch and Amylopectin During Post-harvest Ripening of Kiwifruit at 20°C

| Component | Initial | Days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Starch, % (fwb) | 5.56 | 4.12 | 2.40 | 1.34 | 0.84 | 0.65 |
| Amylopectin, % (fwb) | 2.72 | 2.09 | 1.22 | 0.60 | 0.29 | 0.24 |

It was observed that rapid loss of starch and amylopectin occurred during the first three days of ripening. The differences were significant at the 95% probability level.

At the beginning, the amylose content was about 50% of the total starch. It was maintained at that level during the first two days of ripening, but decreased to approximately to 40% at later dates. In the control sample which was kept at 1°C without ethylene gas, only a very small decrease in starch content was observed. The explanation is that at lower temperature, the enzyme amylases are less active. Formation of sugars from starch is one of the important biochemical changes in the ripening process.

Sugars. Fructose, glucose and sucrose are present in kiwifruit as evidenced by the HPLC method. The changes in relative amounts during ripening are presented in Table III.

Table III. Changes in Sugar Content of Ripening Kiwifruit During Ripening at 20°C with 5 ppm Ethylene

| Component | Initial | Days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Fructose, % | 2.98 | 2.80 | 3.70 | 4.30 | 4.60 | 4.95 |
| Glucose, % | 2.78 | 2.80 | 3.68 | 4.28 | 4.71 | 5.04 |
| Sucrose, % | 0.52 | 1.95 | 2.35 | 1.82 | 1.60 | 1.40 |
| Total | 6.28 | 7.55 | 9.73 | 10.40 | 10.91 | 11.39 |

The total sugar content increases almost in parallel with the increase in soluble solids during ripening. Fructose and glucose did not increase during the first day of ripening but gradually increased thereafter. Sucrose content increased rapidly during the first two days of ripening, but decreased thereafter. In the control sample which was kept at 1°C in the absence of ethylene, there was no significant increase of sugars during the 5 day storage period.

Heatherbell (20) reported that the levels of fructose, glucose and sucrose were 33.2%, 49.3% and 17.5% respectively in mature fresh kiwifruit. In the present study, fructose and glucose were present at almost identical level throughout the experiment. However, the sucrose level in the ethylene treated samples increased in the first two days of ripening and then decreased gradually thereafter. It is possible that sucrose synthesis occurred in the ethylene-treated sample in which starch was acted on by the enzyme phosphorylase. In the later stage, the enzyme invertase can convert the sucrose into glucose and fructose through enzymic hydrolysis.

Chlorophylls. The changes in chlorophyll content during ripening of kiwifruit at 20°C are presented in Table IV.

Table IV. Chlorophyll Content of Kiwifruit During Ripening at 20°C, mg/100 g

| | Days | | | | | | L.S.D. |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | (p=.05) |
| Chlorophyll *a* | 3.40 | 3.31 | 3.32 | 3.40 | 3.39 | 2.60 | .2 |
| *b* | 2.95 | 2.90 | 2.93 | 2.99 | 2.93 | 1.70 | .06 |
| Total | 6.35 | 6.21 | 6.25 | 6.39 | 6.32 | 4.30 | |
| a/b | 1.15 | 1.14 | 1.13 | 1.14 | 1.40 | 1.53 | |

No significant change in chlorophyll *a* and *b* was observed during the first 4 days of ripening. On the fifth day, some changes in pigments was observed. Thgere was no change in chlorophylls in the control sample which was kept at 1°C.

The chlorophyll pigments are not stable to heat. The green color disappeared after the canning process largely due to the breakdown of chlorophylls into pheophytins. This was also evidenced by the large change in $a_L$ value from -8.1 to +0.3 (see Table VIII).

Volatile Constituents of Ripe Kiwifruit. The volatile constituents present in ripe kiwifruit are presented in Figure 1 and Table V. Through gas chromatography and gas chromatography-mass spectrometry, 39 volatile constituents were identified in the fruit. These include 13 esters, 13 carbonyls, 9 alcohols and 4 hydrocarbons. The more abundant volatiles are: (E)-2-hexenal (peak #28), (E)-2-hexenol (peak #39), ethyl butanoate (peak #13), 1-hexanol (peak #35), hexanal (peak #15), and methyl butanoate (peak #9). They contribute to 93.26% of the total volatiles, largely as unsaturated carbonyls, unsaturated alcohols, saturated alcohol and esters. All the other 33 volatiles are present in small amounts. The most abundant volatile is (E)-2-hexenal. Young et al. (22) reported on volatile aroma constituents of kiwifruit grown in New Zealand. They used vacuum steam distillation and freeze concentration to collect the volatiles. The concentrated distillate was analyzed by gas chromatography, gas chromatography-mass spectrometry and reaction gas chromatography. According to them, apart from methyl benzoate, all the aromatic components identified were alkyl and alkenyl esters, alcohols, aldehydes and ketones. The most abundant component was trans hex-2-enal. Odor evaluation of the components at the exit port of gas chromatograph indicated that ethyl butanoate, hexanal and trans hex-2-enal are important contributors to the aroma of kiwifruit. The flavor of processed kiwifruit, particularly where processing has involved heat treatment, bears little resemblance to that of the fresh fruit. Instead, it is similar to that of the cooked green European gooseberry (*Ribes grossularia L*). Flavor changes also occur during production of kiwifruit juice, even under conditions where heat treatment has been avoided.

The method used in isolating the volatiles from kiwifruit is an important factor causing the difference in results. Young and Paterson (23) applied quantitative analysis to study the effects of harvest maturity, ripeness and storage on kiwifruit aroma. Their study was very comprehensive and conclusive. They reported 26 aroma compounds in kiwifruit and concluded that increasing ripeness is associated with a rapid increase in the levels of aroma volatiles, especially esters, while increasing storage time prior to ripening is accompanied by a decrease in the amount of aroma volatiles. They reported the presence of ethanol and limonene in kiwifruit. These two compounds were not found in California grown kiwifruit as shown in Table V. It is possible that the presence of ethanol may be related to the presence of some moldy kiwifruit which are contaminated with ethanol due to mold growth in the fruit. The methods of extraction, the solvent used in isolating the volatiles, the equipment in detecting the volatiles, and the ripeness level of the fruit may all influence the results on the volatile study. By and large, the results from New Zealand are very informative and useful in the study of sensory quality of kiwifruit.

Takeoka et al. (24) reported in 1986 that there were 52 volatiles present in kiwifruit, including 11 carbonyls, 9 alcohols, 16 esters, 11 hydrocarbons and one miscellaneous components. They suggested that peak no. 2 was tetrachloromethane which was derived from the solvent used in the extraction. It is possible that the presence of toluene (peak #12), p-xylene (peak #20), m-xylene (peak

**Figure 1.** Capillary gas chromatogram of kiwifruit volatiles.

Table V. Volatile Constituents of Ripe Kiwifruit

| Peak no. | Constituent | Kovats Index DB-WAX | Peak Area, Rel. Amt., % |
|---|---|---|---|
| 3 | Ethyl acetate | 890 | 0.72 |
| 4 | Methyl propanoate | 909 | 0.03 |
| 5 | Methyl 2-methylpropanoate | 926 | 0.02 |
| 6 | Ethyl propanoate | 961 | 0.19 |
| 7 | Ethyl 2-methylpropanoate | 969 | 0.04 |
| 8 | Pentanal | 978 | 0.03 |
| 9 | Methyl butanoate | 987 | 2.54 |
| 10 | α-Pinene | 1015 | tr |
| 11 | 1-Penten-3-one | 1020 | 0.07 |
| 13 | Ethyl butanoate | 1040 | 3.52 |
| 14 | Ethyl 2-methylbutanoate | 1056 | 0.01 |
| 15 | Hexanal | 1081 | 2.78 |
| 16 | Methyl pentanoate | 1087 | 0.01 |
| 17 | β-Pinene | 1095 | 0.01 |
| 18 | Ethylbenzene | 1119 | 0.03 |
| 19 | (E)-2-Pentenal | 1124 | 0.09 |
| 22 | (E)-3-Hexenal | 1139 | 0.21 |
| 24 | (Z)-3-Hexenal | 1144 | 0.04 |
| 25 | 1-Penten-3-ol | 1170 | 0.07 |
| 27 | (Z)-2-Hexenal | 1196 | 0.87 |
| 28 | (E)-2-Hexenal | 1210 | 78.00 |
| 29 | Ethyl hexanoate | 1236 | 0.03 |
| 31 | 1-Pentanol | 1261 | 0.02 |
| 32 | p-Cymene | 1263 | 0.01 |
| 33 | (E)-2-Heptenal | 1317 | 0.03 |
| 34 | (Z)-2-Pentenol | 1329 | 0.04 |
| 35 | 1-Hexanol | 1363 | 3.40 |
| 36 | (E)-3-Hexenol | 1370 | 0.32 |
| 37 | (Z)-3-Hexenol | 1389 | 0.17 |
| 38 | 2,4-Hexadienal | 1393 | 0.01 |
| 39 | (E)-2-Hexenol | 1413 | 5.80 |
| 40 | (Z)-2-Hexenol | 1423 | 0.02 |
| 41 | (E,Z)-2,4-Heptadienal | 1461 | 0.03 |
| 42 | 1-Heptanol | 1465 | 0.01 |
| 43 | (E,E)-2,4-Heptadienal | 1486 | 0.04 |
| 45 | Benzaldehyde | 1512 | 0.02 |
| 46 | Methyl furoate | 1575 | 0.03 |
| 48 | Methyl benzoate | 1613 | 0.02 |
| 49 | Ethyl furoate | 1621 | 0.01 |

#21), o-xylene (peak #26), styrene (peak #30) and naphathelene (peak #51) may have been derived from sources other than the kiwifruit. Further research on this subject will be of great value as to whether the aromatic hydrocarbons are really components of the kiwifruit.

Canned Kiwifruit Nectars. The pH, acidity, Brix and ascorbic acid content of aseptically processed kiwifruit nectars after storage at 20°C for two months are presented in Table VI.

Table VI. pH, Acidity, °Brix and Ascorbic Acid Content of Canned Kiwifruit Nectars

| Nectar | Kiwi-fruit, % | Sweetener | pH | Total Acidity, % | °Brix | Ascorbic Acid, mg/100 g |
|---|---|---|---|---|---|---|
| 1 | 50 | HFCS | 3.45 | 0.681 | 10.5 | 25.5 |
| 2 | 40 | HFCS | 3.45 | 0.545 | 11.1 | 21.6 |
| 3 | 30 | HFCS | 3.50 | 0.388 | 10.9 | 14.4 |
| 4 | 50 | Sucrose | 3.45 | 0.661 | 11.5 | 24.2 |
| 5 | 40 | Sucrose | 3.45 | 0.519 | 11.6 | 20.0 |
| 6 | 30 | Sucrose | 3.45 | 0.393 | 11.7 | 16.1 |
| L.S.D. (p=.05) | | | N.S. | 0.045 | 0.9 | 1.9 |

The sensory quality of fruit nectars depends largely on the pH value, acidity, and sugar content of the fruit at processing time. Control of the ripeness and adjustment of sweetness in relation to the acidity are two major procedures in order to produce high quality nectars. The kiwifruit itself is rather strong in flavor, thus the adding of water and sugars to lower down the acidity and to improve the sweetness taste will improve the sensory appeal. For most of the fruit nectars, the fruit content is now 45% (Standard of identity). The former standard was 50% fruit in the nectar.

Ascorbic acid retention in the nectars was improved by the aseptic canning process. Previous work on kiwifruit nectar made by the hot-fill method reported by Wildman and Luh (21) revealed a lower ascorbic acid retention. Thus processing method is important to retention of ascorbic acid in the canned product.

Consistency. The Brookfield viscosimeter was used to evaluate the consistency of the canned nectars at 25°C. The log-log plots of the Brookfield readings of the nectars in centipoises vs the speed of spindle No. 2 yields a linear relationship. The speeds used were 5, 10, 20 and 50 rpm. The results are tabulated in Table VII. The Brookfield values decreased as the spindle speed increased. The phenomenon may be explained by the orientation of the water-insoluble particles as the spindle speed increases. The higher speed of the rotation pushes the particles toward the circumference resulting in lower Brookfield readings. Results indicate the percent fruit pulp in the nectar was an important factor influencing the consistency of the product. If we use the No. 2 spindle rotating at 20 rpm, the corresponding values of the nectars in cps can be used as an objective measure of the viscosity of the product. The sweeteners used here did not influence the consistency as much as the fruit pulp percentage.

Table VII. Consistency of canned kiwifruit nectars at 25°C Brookfield Readings in cps

| Nectar | Kiwifruit | rpm | | | |
|---|---|---|---|---|---|
| | | 5 | 10 | 20 | 50 |
| 1 | 50 | 706.7 | 404.0 | 330.6 | 209.0 |
| 2 | 40 | 392.0 | 225.2 | 164.6 | 95.6 |
| 3 | 30 | 152.0 | 101.2 | 78.0 | 43.2 |
| 4 | 50 | 848.0 | 508.0 | 312.0 | 206.4 |
| 5 | 40 | 365.6 | 213.2 | 148.0 | 95.4 |
| 6 | 30 | 176.0 | 108.0 | 77.4 | 43.4 |
| L.S.D. (p=.05) | | 51.0 | 45.0 | 32.0 | 19.0 |

Color. The Hunter color difference meter, Model D25D2 was used to evaluate the color of the canned nectars. The results are shown in Table VIII.

Table VIII. Hunter Color Difference Meter Readings of Canned Kiwifruit Nectars

| Nectar | Kiwifruit, % | Sweetener | L | $a_L$ | $b_L$ |
|---|---|---|---|---|---|
| 1 | 50 | HFCS | 41.1 | +0.3 | +17.3 |
| 2 | 40 | HFCS | 39.6 | -0.2 | +16.2 |
| 3 | 30 | HFCS | 37.5 | -0.6 | +14.2 |
| 4 | 50 | Sucrose | 40.7 | +0.1 | +17.3 |
| 5 | 40 | Sucrose | 39.3 | -0.3 | +15.9 |
| 6 | 30 | Sucrose | 37.2 | -0.7 | +14.1 |
| Kiwifruit puree | 100 | None | 35.9 | -8.1 | +15.6 |
| L.S.D. (p=.05) | | | 1.5 | 0.2 | 0.8 |

Organic Acids in Kiwifruit Nectar. The sensory quality of kiwifruit changes greatly with the ripening process in which the organic acid was metabolized thus increase the pH value of the fruit. According to Okuse et al. (15), the most abundant organic acid was quinic acid during the earlier part of the growing season. After ripening the relative amounts of the organic acids differ greatly from those present in the mature but under-ripe fruit. In the present study it was found that the major organic acids present in the ripe kiwifruits were citric, quinic and malic acids as analyzed by the HPLC method. The acid contents of the aseptically canned nectar containing 50% kiwifruit pulp were as follows: citric acid, 0.295%, quinic acid, 0.247%, and malic acid, 0.150%. These three acids contribute to the acidity of the kiwifruit.

Conclusions. The chemical changes in kiwfruit during post-harvest and ripening can greatly influence the sensory quality of the fresh and canned product. The changes in firmness, starch, sugars, organic acids and volatile compounds during fruit ripening are reported in this paper. Aseptic canning of kiwifruit nectars at 30, 40, and 50% fruit levels was accomplished using high fructose corn syrup or sucrose as sweeteners. Proper control of the harvesting, storage and ripening process are the key steps for successful processing fo the fruit. Kiwifruit harvested at 6.5 to 8.0% soluble solid content can be stored under refrigeration at 1°C under 90% relative humidity for storage up to 5 or 6 months if ethylene gas is removed from the storage room. The stored kiwifruit must be ripened to optimal level prior to processing. The chlorophyll pigments were changed into pheophytins resulting in loss in green color after septic canning.

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# Chapter 24

# Quality Improvement of Candied Fruits

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A survey of candied fruit products in Taiwan revealed significant percentages of samples containing exceeding allowable concentrations of preservatives. Laboratory tests indicated that low concentrations (<0.05%) of preservatives in high sugar content candied fruits was effective in controlling microorganisms. Development of mold on candied fruits was delayed by high sugar concentrations, replacing air with nitrogen in the package, and using packaging materials with low water vapor and oxygen transmission rates. Fruit firmness was closely linked to sugar content and influenced by storage time and temperature. Discoloration of candied fruits in storage was reduced by vacuum or nitrogen packing, using packaging material with low oxygen and water vapor transmission, and low temperature. Absorption of sorbate as a preservative was decreased by high sugar levels in candied fruits and tended to increase with high temperatures and low sorbate concentrations.

Candied fruits are traditional Chinese foods with characteristic flavors. Taiwan produces many fruits and vegetables which are suitable for candied products, such as mei(wume or Japanese apricot), kumquat, dates, tomatoes and pineapples. In general, candied fruits consist of 65-70 % sugar with water activity ( Aw ) values of 0.6 - 0.8, to control microbial growth. However, molds are often found in candied fruits due to the warm and humid weather of Taiwan. This problem is currently addressed by adding more sugar or preservatives.

In recent years, consumer awareness of the adverse effect of high sugar and preservatives in candied fruits has resulted in a marked decrease in consumption of these products. Two approaches are being followed to reverse this trend. One is the improvement of the quality of candied fruits by using new processing techniques such as vacuum syruping or suitable packaging. The other is to develop new

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low sugar products supplemented with refrigeration, preservatives and improved packaging.

Chen et. al. (1) studied the processing, packaging and storage of low-sugar candied fruits. They found that cold storage at 2-5°C, low pH and the addition of preservatives could prevent microbial spoilage during five months storage. With $CO_2$ pack, vacuum pack or the addition of an oxygen absorbent to inhibit the growth of aerobic microorganisms, candied plums had a shelf-life of seven months.

Low-sugar candied fruits, with 35-55 % sugar, have Aw values ranging from 0.89 to 0.96. Huang and Wang (2) studied the effects of sodium benzoate and potassium sorbate on the growth of *Aspergillus* F-14 isolated from candied fruits. To inhibit the growth of *Aspergillus* F-14 at pH 4.0, sodium benzoate concentrations of 0.15, 0.14 and 0.12 % at Aws of 0.96, 0.93 and 0.89, respectively were required. If the pH value was adjusted to 3.0, reduced benzoate concentrations of 0.04, 0.04 and 0.035% could be used. Similarly, potassium sorbate concentrations of 0.16, 0.16 and 0.15 % at pH 4.0 and concentrations of 0.06, 0.05 and 0.04 % at pH 3.0 could be employed.

This investigation measured the quality and sanitation of candied fruits randomly bought in the market. Major contaminating microorganisms were isolated and identified from candied kumquats, wumei, etc. Oxygen requirement and the effects of Aw, pH and preservatives on growth of those microorganisms were determined. In addition, the effects of packaging materials such as PET/SARAN/CPP(a laminated film of polyethylene terephthalate, a copolymer of vinylidene chloride and vinyl chloride made by Dow Chemical Co. and cast polypropylene; Sun A Co. Taiwan), NY/PE/EVA (a laminated film of polyamide of Dupont Co., polyethylene and ethylene vinyl acetate; Sun A Co.), KOP/PE/EVA ( a laminated film of SARAN-coated oriented polypropylene, polyethylene and ethylene vinyl acetate; Sun A Co.), OPP/CPP ( a laminated film of oriented polypropylene and cast polypropylene; Sun A Co.) and CPP/PE/CPP ( a laminated film of oriented polypropylene, polyethylene and cast polypropylene; Sun A Co.) and packing methods such as the use of vacuum, $N_2$ and $CO_2$ on the quality of candied fruits were studied.

## Manufacture of Candied Fruits

Candied fruit differs from fruit candy only in the ratio of fruit to sugar; and it differs from canned fruit only in the ratio of fruit to water (3). Here, kumquats were used as an example for manufacturing of candied fruits (4). Fresh fruits of proper ripeness were washed after harvest and sorted. Overripe with an undesirable soft texture and unripe material which lacked flavor were not used. The peel of each kumquat fruit was scored with four slight cuts to speed up the soaking process. To avoid shrinkage, fruits were first soaked in 30° Brix sugar solution and cooked for 5 min. The cooked kumquats were cooled immediately and allowed to stand overnight. The next day, kumquats were removed and the sugar solution was raised to 40° Brix. The fruits were cooked in 40° Brix solution for 2 min, cooled rapidly and allowed to stand overnight. This procedure was repeated with 10° Brix increases in the sugar solution until the sugar concentration reached 70° Brix. To dry the fruits, the kumquats were carefully placed on trays and dried in an oven at 60°C for 8 hrs. After cooling, they were packed in bags and stored in boxes.

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Inspection of Candied Fruits sold in Markets

The quality and sanitation of candied fruits were inspected in seventy two samples randomly obtained from supermarkets, grocery stores and traditional markets in Taiwan. Water contents were found to range from 11 % to 56 %, Aw values were from 0.54 to 0.89, total soluble solids from 25 to 85 %, acidity from 0.3 % to 3.5 %, and pH from 2.5 to 4.3. Their benzoate contents was found to be from 0 to 3.55 g/kg, sorbate from 0 to 0.41 g/kg, and saccharin from 0 to 11.6 g/kg.

According to Chinese National Health Administration regulation, every kilogram of candied fruits with water content above 25 % may contain not more than one gram of preservatives (5). The results of the survey showed that 40 % of samples contained excessive preservatives ( Table I ). Among the samples containing excessive preservatives two-thirds had a water content below 25 %. More than half of the samples with permissible levels of preservatives were in products purchased from the grocery stores. Among the samples with a permissible amount of preservatives, half contained only 5 g or less of preservatives per kg of candied fruits. This indicates that the low concentration (<0.05%) of preservatives in high sugar content candied fruits is effective in controlling microorganisms and that the excessive preservative content of most commercial products is unnecessary.

Table I. Survey of Preservatives Added to Candied Fruits Bought in the Market

| Samples Exceeding Legal Concentrations of Preservatives | | | | |
|---|---|---|---|---|
| Non-compliance Category | All Sources 29/72 (40%) | Supermarkets 17/29 (59%) | Grocery Stores 7/29 (24%) | Markets 5/29 (17%) |
| Non Allowed | 19/29 (66%) | 12/19 (63%) | 5/19 (26%) | 2/19 (11%) |
| Exceeding Allowed Level | 10/29 (34%) | 5/10 (50%) | 2/10 (20%) | 3/10 (30%) |
| **Samples Not Exceeding Legal Concentrations of Preservatives** | | | | |
| Preservative Conc. Group | All Sources 43/72 (60%) | Supermarkets 13/43 (30%) | Grocery Stores 24/43 (56%) | Markets 6/43 (14%) |
| 0.07-0.1% | 11/43 (26%) | 3/11 (27%) | 4/11 (36%) | 4/11 (36%) |
| 0.05-0.07% | 12/43 (28%) | 7/12 (59%) | 4/12 (33%) | 1/12 ( 8%) |
| < 0.05% | 20/43 (46%) | 3/20 (15%) | 16/20 (80%) | 1/20 ( 5%) |

The saccharin content in candied fruits sold in market was also surveyed (Table II). According to Chinese National Health Administration regulations, candied fruits with water content below 25 % may contain up to two grams of saccharin and/or cyclamate per kilogram of product. This survey showed that 70 % of the samples had excessive amounts of these sweeteners. These artificial sweeteners were found in about four-fifths of the samples with a water content above 25 %.

Properties of Packaging Materials and Mold Growth on Packaged Candied Fruits

The water vapor and oxygen transmission rates of single films of va-

rious packaging materials were investigated. Among them, SARAN has the lowest vapor and oxygen transmission rates and KOP ranks second. PE, PP and EVA have very high oxygen transmission rates while NY and EVA have high vapor transmission rates. The vapor and oxygen transmission rates of laminated packaging materials such as PET/SARAN/CPP and NY/PE/EVA make them good vapor and oxygen barriers, whereas the most commonly used OPP/CPP and OPP/PE/CPP for candied fruits have very high oxygen transmission rates (6).

Table II. Survey of Saccharin Added to Candied Fruits Bought in the Market

| Samples Exceeding Legal Concentrations of Saccharin | | | | |
|---|---|---|---|---|
| Non-compliance Category | All Sources 50/72 (70%) | Supermarkets 22/50 (44%) | Grocery Stores 20/50 (40%) | Markets 8/50 (16%) |
| Non Allowed | 41/50 (82%) | 15/41 (37%) | 18/41 (44%) | 8/41 (19%) |
| Exceeding Allowed Level | 9/50 (18%) | 7/9 (78%) | 2/9 (22%) | 0/9 ( 0%) |
| **Samples Not Exceeding Legal Concentrations of Saccharin** | | | | |
| Compliance Category | All Sources 22/72 (30%) | Supermarkets 8/22 (36%) | Grocery Stores 10/22 (46%) | Markets 4/22 (18%) |
| No Saccharin | 15/22 (68%) | 3/15 (20%) | 9/15 (60%) | 3/15 (20%) |
| Legally Added Level | 7/22 (32%) | 5/7 (71%) | 1/7 (14%) | 1/7 (14%) |

Mold growth on candied fruits depends on the sugar content, pH, oxygen concentration and Aw. Using NY/PE/EVA as packaging material, mold growth on 42° Brix candied kumquats reached 53 % within 10 days of storage. Using PET/SARAN/CPP, mold growth on the same candied kumquats was only 47 % after 20 days of storage. For 47°Brix candied kumquats, mold growth was present on 36 % of the samples packed in NY/PE/EVA and 20 % packed in PET/SARAN/CPP in 10 days (6). No mold growth was observed for candied dates and wumei due to their high sugar content (70°Brix) and low Aw (0.6). Karel (7) reported mold growth was inhibited at Aw 0.65 or below.

In this study candied kumquats packaged in OPP/CPP, either without excluding air or in $N_2$, showed mold growth regardless of the sugar content ( 50 or 65° Brix ) of the product. Similar mold growth was observed in candied kumquats packaged in KOP/PE/EVA with air present but no mold was found after 4 months storage in those packaged in KOP/PE/EVA with $N_2$ (Table III). For 55° Brix candied wumei packaged in KOP/PE/EVA without excluding air, mold was found in 30 days. No mold was found in 65° Brix products after four months storage.

As previously reported (5), a direct relationship between the sugar content of candied fruits and soaking solutions was observed. The sugar content of candied fruits correlated positively with the sugar contents of soaking solutions while correlated negatively with the Aw and water content. In storage studies using PET/SARAN/CPP, NY /PE/EVA, KOP/PE/EVA and OPP/CPP, candied fruits packed with $CO_2$, $N_2$ or vacuum and stored at room temperature or at 5°C showed that there

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were no significant difference in water content, Aw, pH, acidity and total soluble solids changes among the samples during the four months storage (8).

Table III. Effect of Packaging Materials and Methods on Mold Growth in Candied Kumquat and Wumei with Different Sugar Contents

| Brix | Plastic Film | Pack Method | Aw | Mold Growth (%) 1 | 3 | 5 | 7 | 10 | 15 | 30 | 60 | 90 | 110(days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kumquat | | | | | | | | | | | | | |
| 72 | A* | air | 0.72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 65 | A | air | 0.83 | 0 | 0 | 0 | 0 | 23 | 23 | 23 | 23 | 23 | 23 |
| 65 | A | $N_2$ | 0.83 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 65 | B | air | 0.83 | 0 | 0 | 0 | 14 | 29 | 29 | 29 | 29 | 29 | 29 |
| 65 | B | $N_2$ | 0.83 | 0 | 0 | 0 | 0 | 14 | 23 | 23 | 23 | 23 | 23 |
| 60 | A | air | 0.87 | 0 | 0 | 0 | 42 | 67 | 72 | 77 | 77 | 77 | 77 |
| 50 | A | air | 0.92 | 0 | 0 | 0 | 50 | 70 | 100 | 100 | 100 | 100 | 100 |
| 50 | A | $N_2$ | 0.92 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | B | $N_2$ | 0.92 | 0 | 0 | 0 | 30 | 70 | 95 | 100 | 100 | 100 | 100 |
| 40 | A | air | 0.95 | 0 | 0 | 68 | 82 | 100 | 100 | 100 | 100 | 100 | 100 |
| Wumei | | | | | | | | | | | | | |
| 55 | A | air | 0.89 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 10 | 10 | 10 |
| 65 | A | air | 0.84 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* A: KOP/PE/EVA B: OPP/CPP

## Quality Parameters of Candied Fruits

Heating during processing is the most significant factor influencing the firmness of candied fruits. Sugar, water and dryness of candied fruits are also important factors. Higher sugar content of candied kumquats was associated with more firmness, while long storage time lead to less firmness. In general, candied fruits were firmer before storage than after storage. The firmness of candied dates decreased from 1000 g w/v to 400 g w/v during three months storage, and further decreased to 350 g w/v in the fourth month (6).

Under low temperature storage conditions the pattern was different. During the first 15 days of storage at 5° C, the firmness increased by 25 % and then leveled off. After three months storage, the products stored at 5° C was 2-fold firmer than that of those stored at room temperature (8).

Browning of candied fruits occurred during storage. Color difference meter measurements ( NIPPON DENSHOKU CO. ), showed little change in L values and a slight increase in a values during storage, while the change rates of b values in all samples were three thimes greater than those of a values. Vacuum packed candied fruits did not show much color change. The degree of discoloration of candied fruits with PET/SARAN/CPP was lower than that with NY/PE/EVA. Color change during storage at low temperature was less than at room temperature. Only at room temperature, the degree of discoloration of candied fruits packaged with KOP/PE/EVA was less than that with OPP/CPP. $N_2$ pack treatment enhanced these differences (8).

Isolation, Identification and control of Contaminating Microorganisms in Candied Fruits

Two different types of *Aspergillus*, one of *Penicillium* and two kinds of yeast were isolated from candied kumquats and wumei (9). Huang and Wang (2) found *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp., *Fusarium* sp. and *Saccharomyces* sp. in similar products from several candied fruit factories. This suggests unsatisfactory sanitary conditions exist in many candied fruit processing facilities.

The minimum oxygen concentrations required for the growth of *Aspergillus* and *Penicillium* were 3.84 % and 5.2 %, respectively. When the oxygen concentration in the headspace of bags of candied fruits reached 3.62 %, mold could grow. The oxygen concentration in the headspace of packages using various packaging materials such as PET/SARAN/CPP, NY/PE/EVA, KOP/PE/EVA and OPP/PE/CPP, decreased at different rates during storage. The OPP/PE/CPP bags had the highest oxygen concentration in the headspace, while PET/SARAN/CPP and KOP/PE/EVA packed with $N_2$ had a very low oxygen content throughout 110 days of storage (9). Apparently OPP/PE/CPP is not a good packaging material for candied fruits.

Sorbic acid is a preservative with low mammalian toxicity which can inhibit the growth of bacteria, yeast and mold (10). Different concentrations of potassium sorbate prepared in PDA media can inhibit mold growth (11). At pH 5.5, 0.15 % sorbate inhibited the growth of *Aspergillus* while concentrations of 0.04 % and 0.03 % sorbate inhibited growth of *Penicillium* and yeast, respectively. When the pH of the media was lowered from 5.5 to 3.0, less sorbate was needed to inhibit the growth of *Aspergillus*, *Penicillium* and yeast ( 0.05, 0.02 and 0.015 %, respectively). If sorbate was added in syrup of candied kumquats and wumei, only 0.02 % sorbate was needed to inhibit microbial growth.

With the development of low sugar candied fruits, the addition of legal preservatives becomes one means of inhibiting of microorganisms. The effect of sugar content on the absorption of sorbate by candied wumei is shown in Figure 1. The higher the sugar content of the syrup, the lower the absorption of sorbate by the fruit. The absorption rate of sorbate by candied kumquats tended to increase with decreasing sorbate concentration. The absorption rate was lower at low syruping temperature (5°C) than at room temperature (Table IV).

Table IV. Effect of Different Potassium Sorbate Concentrations on Absorption of Sorbate by Candied kumquat of 50° Brix

| Conc. of K sorbate (%) | | Absorption (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 | 7 | 10 | 14 days |
| 0.02 | 24°C | 19.1 | 27.2 | 36.2 | 48.9 | 77.7 | 56.5 | 57.0 |
| | 5°C | 12.3 | 19.6 | 26.3 | 31.4 | 35.8 | 39.2 | 42.3 |
| 0.05 | 24°C | 17.6 | 26.3 | 33.7 | 42.5 | 49.3 | 51.7 | 52.3 |
| | 5°C | 11.2 | 18.3 | 21.6 | 26.0 | 31.8 | 36.3 | 39.6 |
| 0.10 | 24°C | 17.1 | 24.7 | 29.6 | 36.7 | 45.2 | 48.3 | 49.6 |
| | 5°C | 10.4 | 18.6 | 21.4 | 24.9 | 29.3 | 33.4 | 36.2 |

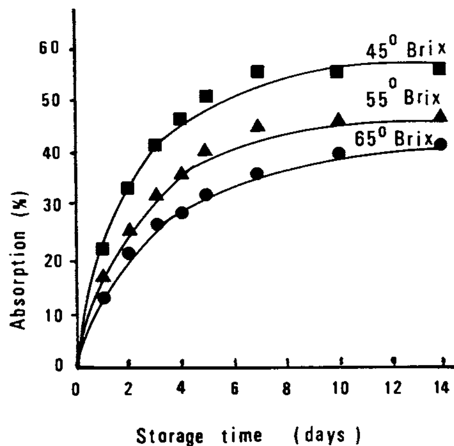


Figure 1. Effect of sugar content on absorption of potassium sorbate by wumei.

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# Chapter 25

# Irradiation Processing of Fruits and Vegetables

## Status and Prospects

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Ionizing radiation can be effectively applied to fruits and vegetables for: (a) disinfestation as a quarantine treatment at 0.15 kGy or above, a process more efficacious than thermal, chemical or cold treatment; (b) delaying ripening in selected fruits, and inhibiting sprouting in tubers and bulbs at 0.02 to 0.75 kGy; (c) decontamination of vegetable seasonings at 10 to 30 kGy; and (d) product improvement in dried products such as dried vegetables and beans at 0.30 to 5.0 kGy. Between 1963 and 1986, the U.S. FDA has cleared six food groups for irradiation. To date, 36 countries have cleared 42 foods or food groups for irradiation with 19 countries commercially irradiating 20 food items. The prospects for commercial radiation processing of selected fruit and vegetable products are promising. Irradiation in a combined process for synergistic effects should be explored.

All fresh fruits and vegetables are perishable and have a limited marketable life due to post-harvest insect infestation, microbial infection, physiological changes and breakdown, and environmental factors such as storage temperature and humidity. Various food processing and preservation techniques such as dehydration, canning, freezing or pickling can extend the marketable life of these commodities but often change their sensory and nutrient qualities. This is because of the rather drastic applications of heat, cold or acidity to the fruits and vegetables.

Since fresh fruits and vegetables are an enjoyable part of our diet, extending their marketable life and expanding their markets to different parts of the world are always the goals of the food industry. Proper chilling and packaging between harvest and the markets are effective ways of achieving these goals. For some export markets, insect-infested products would require quarantine treatments prior to shipment. Additional means to slow down the

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ripening and senescence of the climacteric group of fruits and vegetables during shipment and storage are also desirable. Some of these techniques include chemical fumigation and atmospheric modification respectively.

Coming into play is the possibility of application of low doses of ionizing radiation to fruits and vegetables. The technology of radiation preservation of foods has been studied for more than three decades around the world. A great deal of research findings suggest that the technology is simple, versatile, and efficacious when compared with some of the established food preservation techniques. This chapter will attempt to summarize several beneficial applications of irradiation to fruits and vegetables, the processing variables and chemical factors that should be considered, the current status of the technology with respect to the food industry, the market and the consumer, and the technical and commercial prospects of using this technology on fruits and vegetables in the foreseeable future.

## Technical Efficacies of Irradiation

Technical efficacy of any food processing technology suggests that the process is capable of achieving a certain desirable technical effect, such as killing some or all of a microbial population, or inactivating insects in various stages. In addition, the process should have a high efficiency to achieve that technical effect. The combination of effectiveness and efficiency makes a process efficacious. Examples will show that food irradiation is efficacious as well as versatile.

Radiation Disinfestation. For fresh fruits and vegetables infested by insects, ionizing radiation, often in the form of gamma-radiation, can inactivate various stages of insects and serve as a quarantine treatment when the commodities are to be shipped from an infested area to non-infested market places. Fruit fly eggs in papayas, oranges and grapefruits, seed weevils in mangoes, and codling moths in apples and cherries can be inactivated with an applied doses of 0.15-0.35 kGy[a] (1). The qualities of these irradiated fruits are completely retained (2, 3). The time required to treat Hawaii grown papayas by irradiation is about 10 to 20 minutes as compared to more than one hour by the double-dip hot water treatment and 6-9 hours by the vapor heat treatment. The enzymes for ripening the papayas are sometimes inactivated in the double-dip hot water treatment (42°C, 30 min., 49°C, 20 min.) causing the fruit not to ripen normally.

Shelf Life Extension. Applying radiation doses of 0.02-0.15 kGy will inhibit the sprouting of potatoes, onions and garlic for 6 months or longer, while doses of 0.12-0.75 kGy will delay the ripening of tropical fruits such as papayas, mangoes and some varieties of bananas. Some delay in senescence was found in

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(a) Units of absorbed dose: 1 Gray = 100 rad = 1 J/kg
1 kGy = 100 krad = 1 kJ/kg

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papayas, apricot and sweet cherries at doses varying from 0.75 to 2.0 or 3.0 kGy (4). However, it is believed that doses of 2.0 to 3.0 kGy would exceed the tolerance doses for apricots and sweet cherries and might cause softening of the fruit and thus make the process technically impractical.

Decontamination. Vegetable seasonings are often contaminated with various pathogens and other unwanted organisms. Spices and vegetable seasonings are routinely fumigated with ethylene oxide, a toxic, carcinogenic and explosive gas that could be hazardous to the operators and consumers. Irradiation at 10 to 20 kGy could be an effective substitute for fumigation. (In April, 1986, the U.S. Food and Drug Administration raised the allowable upper dose for decontamination of spices and seasonings from 10 to 30 kGy, although spices currently being irradiated in the United States receive less than 20 kGy.)

Product Improvement. In the 1960s, Lipton Co., Inc. in the United States found that irradiating dehydrated vegetables could reduce the cooking time of dry soup mixes several folds, e.g. from 10 to 3 minutes. The dose required varied from 0.30 to 5.0 kGy dependent upon the types of vegetables. The decrease in time for rehydration and cooking was probably due to some depolymerization by irradiation, thus softening of the vegetables.

A study of irradiated soy beans at the University of Hawaii (5) showed that an optimal dose of 2.5 kGy on germinating soy beans with subsequent drying substantially reduced the gas producing factors (oligosaccharides) in the beans, and also made the beans cook faster and taste better.

## Irradiation Processing Variables

In order to irradiate fruits and vegetables for useful purposes mentioned above, several variables should be considered and carefully controlled:

Host Tolerance to Irradiation Treatment. Every fruit and vegetable responds differently to ionizing radiation because of its inherent chemistry and biochemistry. One of the first variables to be determined should be the tolerance dose, which can be defined as the "maximum dose below which a fruit or vegetable exhibits no external or internal symptoms of injuries or changes." If a fruit or vegetable is irradiated above its tolerance dose, symptoms of phytotoxicity such as off-aroma, off-flavor, softening, scalding, or decrease in nutrient content could occur. Of course other processes such as thermal process could cause similar changes. In fact, the general observation has been that among various processes, the responses of a host (food) to irradiation and thermal processing are more similar to each other than are other processes.

Variation of cultivar. Response to irradiation may differ due to variation of cultivar of a fruit or vegetable. An example is banana. Delay in ripening occurs in some varieties of bananas after irradiation. In others, no changes in ripening occur. Yet in still

others, ripening is accelerated after irradiation. Generally, only the ripening of climacteric fruits and vegetables would be affected by irradiation. The non-climacteric group is not affected.

Maturation at Time of Treatment. The general rule for irradiating fruits and vegetables is that the more ripe they are, the better they can tolerate irradiation. Often if a fruit is harvested too early, its full flavor may not develop later and it may be prone to radiation injuries. For climacteric fruits and vegetables, the irradiation must be done prior to the onset of the climacteric process in order to delay ripening. For disinfestation of papayas, irradiation is effective at all stages of ripeness beyond the mature-green stage. The double-dip hot water treatment, however, requires that the papaya be less than one-quarter ripe at the time of treatment. The irradiation process therefore allows the fruits to ripen to an optimal degree on the tree before harvest and treatment, resulting in higher quality fruits for the consumer.

Pretreatment and Conditioning. Since radiation doses for insect disinfestation have little or no effect on the fungi on fruits and vegetables, fungicidal or thermal treatment prior to irradiation is often beneficial and necessary to control fungal diseases.

Sometimes irradiated chilled fruits can be damaged due to moisture condensation during irradiation. To avoid moisture condensation, temperature of such fruits should be allowed to rise pass the dew point of the ambient air prior to irradiation.

For sprout inhibition of onions and potatoes by irradiation, conditioning of bulbs and tubers at optimal temperatures and relative humidities and for a time period until they are at the dormant stage would make the irradiation process more effective (6).

White potatoes must be held after harvesting for a period sufficient to enable the healing of "wounds" resulting from harvesting handling. Onions must be irradiated promptly after harvesting and prior to significant internal bud growth in order to minimize the black internal "spot" that results from the radiation-caused death of this tissue.

Post-Irradiation Storage. For sprout inhibition of bulbs and tubers by irradiation, proper post-irradiation storage is also important (6) If irradiated potatoes are for culinary use or chipping, it is not necessary to store them at low temperature. However, low temperature (5-10°C) and a relative humidity of 95% or higher are recommended if an extended storage period after irradiation is anticipated. For irradiated onions, storage at low temperature is recommended, but high relative humidity was found to contribute to rotting. For both irradiated potatoes and onions, good ventilation in the storage area is also necessary.

Tropical fruits such as papayas tend to become more sensitive to low temperature storage, e.g., 7°C, after irradiation. They would keep better if stored at a few degrees higher, e.g., 10°C.

Packaging and Transport. A very challenging task for irradiated fruits and vegetables is to find suitable individual and bulk packaging to maximize their qualities and marketable life after

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irradiation. For example, irradiation of strawberries at 2.0 kGy effectively controls several fungi, but the fruits suffer from transport damage due to abrasion, decreasing the amount of marketable fruits (7).

A recent trend in marketing fresh fruits is to wrap each fruit individually. It should improve the fruit's appearance and minimize abrasion between fruits. However, this would require finding a film with the optimal permeability for air, water vapor, ethylene and carbon dioxide between the fruit and the surrounding atmosphere so as to slow down the fruit's respiration rate, and to maintain the proper relative humidity. If irradiation and a fungicidal agent can be incorporated into the process and packaging, it should further extend the shelf-life of the fruit substantially.

## Chemical and Quality Factors

To determine the applicability and effectiveness in using ionizing radiation to preserve fresh fruits and vegetables, the following factors should be evaluated:

Phytotoxicity/Tolerance. These two terms express opposite results of radiation treatment. If a fruit or vegetable irradiated at a certain dose level exhibit no detectable or measurable external or internal changes or injuries, then the fruit or vegetable is said to be tolerant to irradiation at that dose. The maximum dose a fruit or vegetable can tolerate before phytotoxicity occurs should be determined. Age or ripeness of the commodity, its temperature, and the condition of the atmosphere at the time of irradiation should be reported with the dose applied.

Quality Indices. Measuring the aroma, flavor, color and texture of irradiated fruit or vegetable against the non-irradiated controls will reveal whether or not these qualities are affected by irradiation and at what does level.

## Current Status

Food irradiation has been defined in the United States as a food additive according to the 1958 Miller amendment of the Federal Food, Drug, and Cosmetic Act. Each food item allowed for commercial irradiation must first be petitioned to the FDA for clearance on the basis of data and evidence supporting technical effects, safety and sometimes user interest. A regulation permitting the use is issued by the FDA, and in some cases, by other government agencies.

Update on the U.S. and World Situation. A limited number of food products have been cleared since the early 1960s by the U.S. Food and Drug Administration for commercial irradiation and marketing. Table I lists these products (8). These clearances represent several applications of food irradiation with disinfestation the leading potential application.

Table I. Clearance of food products in the U.S.A. for commercial irradiation

| Product | Purpose | Dose, kGy | Date of Clearance |
|---|---|---|---|
| Wheat and wheat flour | Disinfestation | 0.20-0.50 | August 1963 |
| Potatoes | Sprout inhibition | 0.15 | June 1964 |
| Enzymes (Dried) | Decontamination and disinfestation | 10 | June 1985 |
| Pork | Disinfestation of parasites | 0.30-1.0 | July 1985 |
| Fresh foods | Disinfestation and Delay of maturation | 1.0 | April 1986 |
| Spices and Seasonings | Decontamination | 30[a] | April 1986 |

(a) Clearance for 10 kGy in July 1983.

Currently in the United States, some spices such as black peppers, ground paprika and some blend of spices are irradiated for the wholesale market and for some speciality foods such as pastrami. The purpose is decontamination at a dose range of 10 kGy and higher. These spices and seasonings have been irradiated in the past few years at commercial irradiation plants in New Jersey, California and elsewhere. The quantities irradiated in the past two years are estimated to be around 1,000 to 2,000 metric tons per year.

On the international scene, 36 countries have approved 42 foods or food groups to be irradiated for various purposes for commercial marketing, most of which on an unconditional basis, with a few in several countries for test marketing only. Nineteen countries are currently irradiating some 20 food items commercially: Belgium, Brazil, Chile, China, Cuba, Denmark, Finland, France, German Democratic Republic, Hungary, Israel, Japan, Republic of Korea, Netherlands, Norway, South Africa, the United States, USSR, and Yugoslavia. Ten other countries are either constructing or in an advance stage of planning commercial or large-scale demonstration irradiators for treating food and non-food items: Australia, Bangladesh, Canada, Cote D'Ivoire, Italy, New Zealand, Pakistan, Poland, Thailand, and Vietnam (9).

Table II shows the number of countries that have approved the irradiation of fruits and vegetables for three different applications, and the number of countries currently irradiating fruits and vegetables on a commercial scale.

Industry Interest and Consumer Acceptance. To advance the use for food irradiation, interest for whatever prupose and application must be shown by the food industry which is a major beneficiary of all the research that has been carried out worldwide. However, over the years, the food industry in the United States has not taken a very active role in food irradiation research and development. There may be several reasons for this:

o Needs for irradiation -

The needs for irradiation have not always been clearly identified or well defined. There are other processing technology available to manufacture and provide the consumer with a wide variety of processed foods.

o Concerns for large capital investment -

By most estimates, a commercial irradiator to be built in the United States would cost three to four million dollars. The economic feasibility may or may not exist if a company does not have a large enough throughput to keep the irradiator busy.

o Logistics of using irradiation -

Even if several companies pool their resources together and build an irradiator, the logistics of trucking products to and from the irradiator plant would present problems in manpower allocations and plant operations. Optimal and efficient scheduling is possible with computers, but company management might still worry about the products not being kept under the best conditions.

o Consumer acceptance -

All segments of the food industry in the United States are concerned about consumer acceptance of irradiated foods. The fact remains that publicity over the past four decades about negative aspects of nuclear energy such as nuclear weapons, nuclear reactor leakage tends to overshadow the benefits and safety of food irradiation by a large margin. Anti-nuclear activists, including those who oppose food irradiation, fuel the situation by spreading a great deal of misinformation to the public. Inadequate public education plus the misinformation on food irradiation cause the industry to hesitate in considering the use of this technology.

Table II. Number of Countries Approved and Using Irradiation of Fruits and Vegetables for Three Applications[a]

| Countries Approved | Countries Using | Disinfestation | Shelf-Life Extension | Decontamination |
|---|---|---|---|---|
| 29 | | 13 | 26 | 20 |
| | 19 | 1 | 7 | 13 |

(a) Some countries approved or are using only one application while some others approved or are using more than one applications (9).

## Outlook and Conclusion

A large volume of research data amassed over the past thirty years has demonstrated that radiation preservation and processing of foods at low (up to 1.0 kGy) and medium (up to 10 kGy) dose is a simple, versatile and efficacious technology. For fruits and vegetables, the most technically feasible and promising applications include: (a) disinfestation of fruits and vegetables to maintain product quality and as a quarantine treatment at low doses (0.15 kGy or higher), a process more efficacious than thermal, chemical or cold

treatment; (b) shelf-life extension of selected fruits through delaying of their ripening, and of bulbs and tubers such as onions, garlic and potatoes through sprout inhibition at low doses (0.02 to 0.75 kGy); and (c) decontamination of dried vegetable seasonings at medium to high doses (10-20 kGy). By combining irradiation with other processing and packaging technology to synergize the above applications, the efficacy and processing costs can be further optimized.

History has shown that a new food processing technology such as canning, freezing and microwave heating have taken many years to be accepted by the consumer and to be established in the industry. Radiation technology for food preservation could take even longer than other established processing technology because of its unique relationship with nuclear energy and the controversy generated therein.

While 42 foods have been cleared by 36 countries for irradiation to date and 19 of these countries are irradiating 20 food items commercially, food irradiation around the world still faces a number of uphill hurdles which must be overcome before it can gain a foothold in the consumer market. These include: (a) clear indication of industry interest and need in using a particular application; (b) positive demonstration of economic feasibility; (c) proper selection and sizing of irradiation plant facility and optimal logistics of irradiation processing; (d) favorable majority consumer acceptance of irradiated food products; and (e) early establishment of international trade agreements among countries on the import and export of irradiated products.

Commercializing food irradiation has worldwide applications. Some fruits and vegetables may be better preserved, supplies are increased, and export markets can be expanded, all of which should benefit the consumer and improve the economy of every country concerned. To take advantage of this technology and to reach the goals, the challenge ahead would be to launch an effective consumer education program about the purposes, safety and benefits of food irradiation and to increase its commercial applications gradually and selectively through the joint efforts of the industry, government and researchers.

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## Chapter 26

# Effects of Gamma Irradiation on Chemical and Sensory Evaluation of Cabernet Sauvignon Wine

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Cabernet Sauvignon wines received gamma irradiation doses of 0, 0.6, 1.2 or 2.4 KGy and were stored at 21°C for up to 18 months. As radiation dose and storage time increased, total anthocyanin concentration decreased, while color density, hue and color age increased. Acetaldehyde concentration increased with increasing radiation dose and decreased as storage time increased. Sensory evaluation indicated no difference in color or astringency, but off-flavors were detected in wines given a 2.4 KGy dose. Use of gamma irradiation to rapid age Cabernet Sauvignon wines did not appear to be feasible.

Wine aging involves a complex and little understood series of chemical reactions which are perceived by the consumer as an increase in flavor complexity, a shift from a bright red or red-purple associated with young red wines to a tawny red hue associated with well-aged red wines, and decrease in astringency. A major objective of most wine irradiation studies was to determine if irradiation can increase the rate of aging of wines. Wine irradiation experiments were reviewed by Singleton (1) and Mellinger (2). Gamma irradiation of wines was reported to increase acetaldehyde concentrations (3-7) and decrease color in red (5,7,8) and white (5,9) wines. Sensory evaluation of wines irradiated with 0, 1, 5 or 10 KGy showed a decrease in grape flavor and overall quality at the higher doses, while there were no differences in bottle bouquet, hotness, bitterness or astringency (5). Singleton (5) suggested that wine irradiation for rapid aging was worth investigation at doses of 10 KGy and probably less than 5 KGy. Therefore, this study was conducted to determine the effects of

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gamma irradiation on the color and acetaldehyde concentration of Cabernet Sauvignon wine, to ascertain the role of storage time, and to determine the effect of irradiation on sensory attributes.

## Materials and Methods

Thirty-eight liters of 1985 Washington state Cabernet Sauvignon wine were obtained from each of two commercial wineries 8 months after crush. The wines had completed malolactic fermentation and were aged several months in oak barrels. Wines were fined with gelatin at the rate of 120 mg/l, stored in 17 l glass carboys at 10°C for one week, cold stabilized at 1-3°C for 12 days, filtered through 1 µm and 0.45 µm filters (Gelman, Ann Arbor, MI) in series and bottled in 375 ml glass screwcap wine bottles. The bottled wine was transported to the University of Washington, Seattle for irradiation with a cobalt-60 research irradiator M. K. II (Brookhaven National Laboratory, NY). Dose levels used were 0, 0.6, 1.2 and 2.4 KGy. The dose rate of the irradiator was 206 Gy/hr. The wine was stored at 10°C for one month prior to initial chemical analyses and was then stored for up to 18 months at 21°C.

Color was determined by the method of Somers and Evans (10). All absorbance measurements were conducted using a Beckman (Irvine, CA) DU-6 spectrophotometer. Absorbance of the wine was measured at 420 and 520 nm with a 1 mm length path cuvette. Thirty µl of 20% sodium metabisulfite solution were added to another 2 ml of wine. Samples were mixed by inversion and allowed to stand at 20°C for 45 min and absorbance measured at 520 nm. One hundred µl of wine were added to 10 ml of 1 M HCl, mixed and held at 10°C for 3 hr and absorbance measured at 520 nm. All absorbance values were adjusted to a 10 mm length path and corrected for dilution. Color expressions were calculated as follows:

(1) Color density (absorbance units) = $Abs_{420} + Abs_{520}$

(2) Color hue = $[Abs_{520(HCl)} - (5/3)Abs_{520(SO2)}]$

(3) Total anthocyanins (mg/l) = $20\ [Abs_{520(SO2)} - (5/3)Abs_{520(SO2)}]$

(4) Features of chemical age

(a) Age I = $Abs_{520(SO2)}/Abs_{520(CH3CHO)}$

(b) Age II = $Abs_{520(SO2)}/Abs_{520(HCl)}$

Acetaldehyde was determined as described by A.O.A.C. (11). All analyses were triplicated.

Nine months after irradiation, sensory evaluations were held at the WSU Irrigated Agricultural Research and Extension Center in Prosser, Washington. The room was air conditioned to remove odors and provide a comfortable temperature and humidity. The 9 panelists were commercial winemakers from Washington and experienced in the evaluation of red wine quality.

Aroma, color and flavor were evaluated separately and in that order. For aroma and flavor evaluation, red background room lights were used to mask any color differences. Color was evaluated using

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Sylvania cool white fluorescent room lights. For aroma and flavor evaluations, samples from winery "B" were presented first as a set, followed by samples from winery "A". Each set consisted of a sample from each of the four radiation levels presented in random order. For color evaluation the eight samples were presented simultaneously. For each evaluation, 20 ml of wine were presented in a tulip-shaped glass covered with a watch glass.

The wine was scored using a modification of the descriptive analysis outlined by Stone et al. (12). Each major category of color, odor and taste was evaluated separately. For each attribute, a horizontal scale of 10 cm was provided with at least three labeled anchor points, one at each end and in the middle. Stage of color development was rated using descriptors: red purple, ruby-red, ruby, ruby with amber highlights, amber and brown. Bouquet and flavor character were scored from 0 to 10 where 0 represents off character; 5, average and 10, superior. Intensity of color, bouquet, flavor undesirable odors, undesirable flavor and astringency were scored where 0 represents weak; 5, moderate and 10, strong. After evaluating the wine, the taster marked a cross representing the magnitude of the attribute in question. The scale was assumed to be linear and a numerical value was assigned based on the length of the line. The line was measured to the nearest 0.1 cm and the results were analyzed statistically using an analysis of variance.

## Results

As red wines age, the monomeric forms of anthocyanins undergo condensation reactions with other anthocyanin or tannin molecules to form polymeric pigments. The polymeric pigments are less sensitive to bisulfite bleaching and changes in pH (10). The color analysis method of Somers and Evans (10) as described above takes advantage of these differences to follow color changes as wines age.

Radiation dose and storage time at 21°C affected all color attributes measured, Table 1. Total anthocyanins decreased and color density, hue, color age I and color age II increased with increasing storage time, Figures 1-5. Storage time had a greater effect on wine color than radiation dose, Table 1. Wines receiving a radiation dose of 2.4 KGy had lower total anthocyanin concentrations and color density compared to lower radiation doses, Figures 1-2. Higher hue, chemical age I and chemical age II values for wines receiving a dose of 2.4 KGy would indicate an older color age than wines receiving lower doses, Figures 3-5. Wines from the four irradiation treatments aged at the same rate as indicated by a lack of interactive effects on color measurements between irradiation dose x storage time. Therefore, once the initial effects of irradiation occurred, the color aging process was similar for the four doses. As reported by others (3-7), acetaldehyde concentrations increased in direct proportion to radiation dose, Figure 6. Acetaldehyde decreased as storage time increased and after 18 months at 21°C there were no differences in acetaldehyde concentration between the four radiation doses.

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Table I. Degrees of freedom, 'F-values' and error mean squares for repetitions and main effects and interactions of wine source, radiation dose and storage time at 21°C on the color of Cabernet Sauvignon wine

| Source | df | Antho-cyanin conc. | Color density | Color hue | Color Age I | Color Age II | Acet-aldehyde |
|---|---|---|---|---|---|---|---|
| Total | 94 | - | - | - | - | - | - |
| Repetition | 2 | <1 | <1 | <1 | 1.6 | 3.9 | 3.0 |
| Wine Source (WS) | 1 | 51***[z] | 228*** | 228*** | 41*** | 188*** | 130*** |
| Radiation Dose (RD) | 3 | 15*** | 13*** | 13*** | 6.1** | 15*** | 75*** |
| Linear | (1) | 3.3 | 9** | <1 | 2.2 | 1.5 | 28*** |
| Quadratic | (1) | <1 | <1 | <1 | <1 | <1 | <1 |
| Cubic | (1) | <1 | <1 | <1 | <1 | <1 | <1 |
| Storage Time (ST) | 3 | 336*** | 21*** | 981*** | 213*** | 727*** | 118*** |
| Linear | (1) | 436*** | 11*** | 697*** | 374*** | 551*** | 46*** |
| Quadratic | (1) | 61*** | <1 | 7** | 37*** | 4.4* | 6.5* |
| Cubic | (1) | <1 | <1 | <1 | 2.9 | <1 | 2.7 |
| WS X RD | 3 | 2.2 | 1.4 | 1.2 | <1 | 2.9* | 3.9* |
| WS X ST | 3 | 2.4 | 2.4 | 7.4*** | <1 | 3.3* | 6.2*** |
| ST X RD | 9 | 1.2 | 1.0 | 1.8 | 1.1 | 2.0 | 13*** |
| WS X RD X ST | 9 | <1 | 1.4 | 1.3 | <1 | 2.7* | 1.6 |
| Error Mean Square | 62 | 754 | 0.28 | 0.0002 | 0.003 | 0.0008 | 6.32 |

[z] *,**,*** represent $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

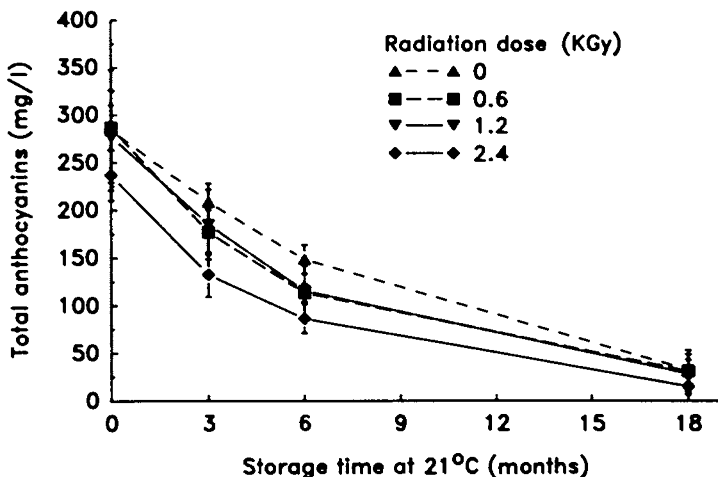


Figure 1. Change in total anthocyanin concentration due to radiation dose and storage time. Bars represent the 95% confidence limit for each point which represent the mean of two wine sources and three repetitions.

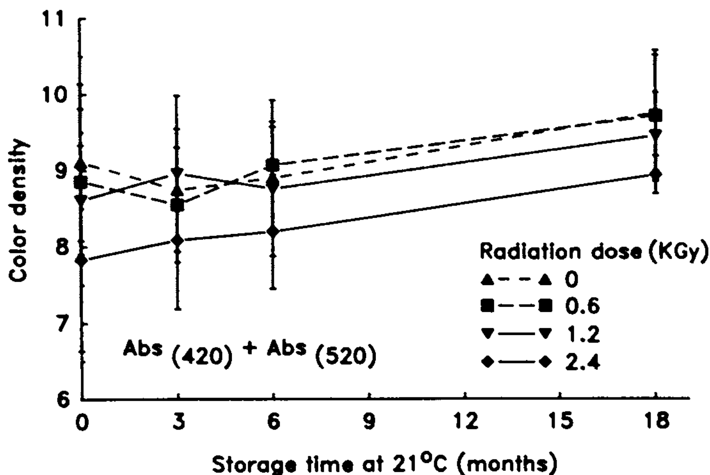


Figure 2. Change in color density due to radiation dose and storage time. Bars represent the 95% confidence limit for each point which represent the mean of two wine sources and three repetitions.

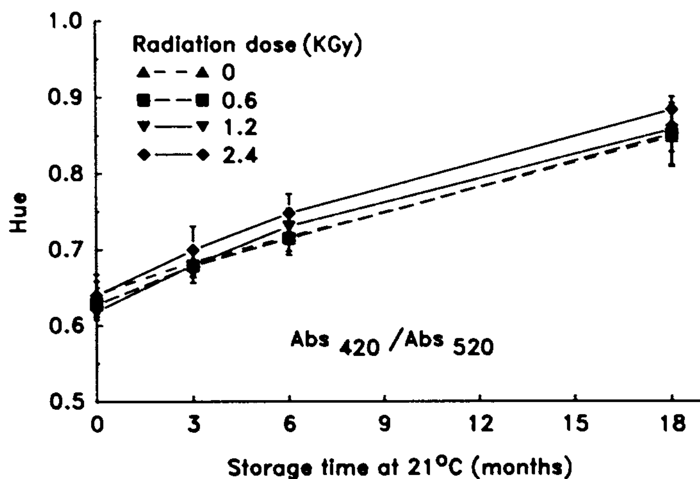


Figure 3. Change in hue due to radiation dose and storage time. Bars represent the 95% confidence limit for each point which represent the mean of two wine sources and three repetitions.

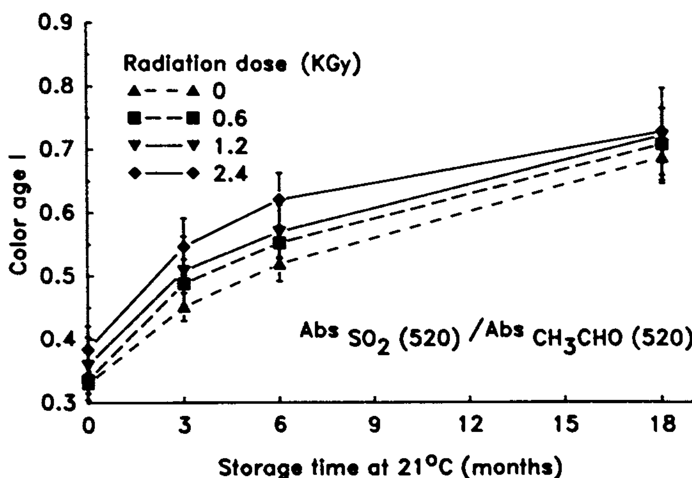


Figure 4. Change in color age I due to radiation dose and storage time. Bars represent the 95% confidence limit for each point which represent the mean of two wine sources and three repetitions.

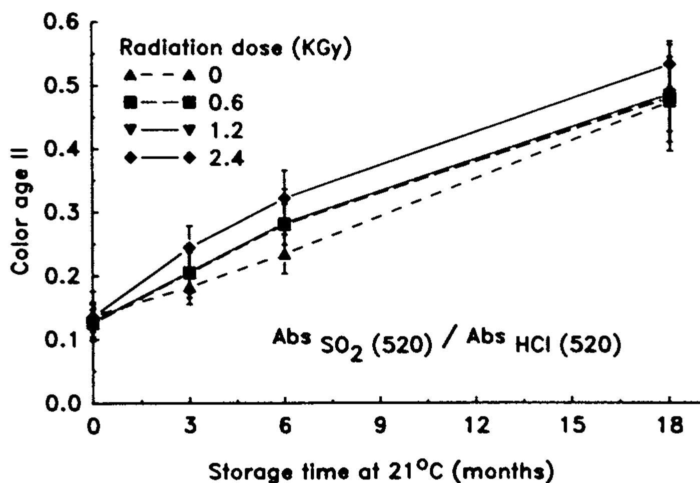


Figure 5. Change in color age II due to radiation dose and storage time. Bars represent the 95% confidence limit for each point which represent the mean of two wine sources and three repetitions.

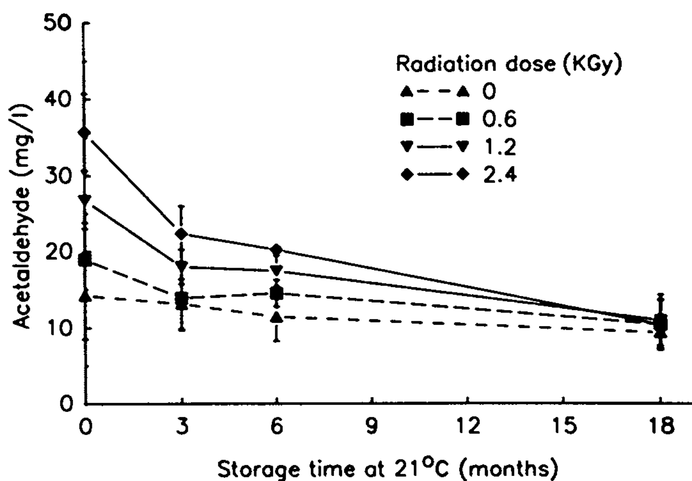


Figure 6. Change in acetaldehyde due to radiation dose and storage time. Bars represent the 95% confidence limit for each point which represent the mean of two wine sources and three repetitions.

Sensory panelists were unable to detect any differences in color between the control wines and irradiated wines. Wine receiving the 2.4 KGy dose was of poor quality compared to other wines based on ratings for bouquet, character, undesirable odors, flavor character and undesirable flavor. The wines receiving 0.6 or 1.2 KGy were similar to the controls (non-irridiated).

## Discussion

Hypothetically, direct and indirect products of gamma radiation are capable of reproducing the chemical reactions occurring in tannin-anthocyanin polymerization as proposed by Timberlake and Bridle (13). Hydrogen peroxide is a product of water radiolysis (14). Ethanol reacts with hydrogen peroxide to form acetaldehyde (15). Similar results were obtained when hydrogen peroxide was added to carbonyl compounds (16). Radiation interactions remove the hydroxyl group from the carbonium ion either directly by scission or indirectly by hydrogen ion interaction. Chemical groups attached to a conjugated ring, such as the methanol group at position 8 of the carbonium ion, are more susceptible to attack than the bonds within the aromatic ring since the conjugated system degrades energy without concentrating it in one bond (17). Finally, the reactive carbonium ion combines with an anthocyanin to produce a tannin-anthocyanin dimer. This scenario is similar to that occurring during normal wine aging.

Although irradiation of red wine increased the chemical color "age" of the Cabernet Sauvignon wines, sensory evaluations found no perceivable difference in color due to irradiation at the dose rates used in this study. Additionally, irradiation did not decrease astringency of the wines. Due to the adverse effect of the 2.4 KGy dose on flavor and aroma attributes, use of higher dose rates to rapid age Cabernet Sauvignon wines does not appear to be feasible.

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# Chapter 27

# Ultrafiltration of Fruit Juices with Metallic Membranes

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Fruit juice production has been greatly simplified by the use of the Ultrapress process. This process utilizes formed-in-place metallic membranes on sintered stainless steel tubes for simultaneous pressing and ultrafiltration of fruit purees. The fruit purees are enzymatically depectinized and pumped directly through the metallic membrane system in a single pass to obtain highly clarified juice which can be aseptically packaged. Insignificant changes in the volatile composition of apples were noted using the Ultrapress process.

The fruit juice industry is constantly seeking improved methods of obtaining highly clarified juices. Most of the research effort has been directed to the clarification of pressed juice by ultrafiltration (UF) membranes, although some effort has been made to obtain clarified juices by centrifugation and/or filtration of enzymatically liquified fruit pulps. Hollow fiber or thin-channel polymeric membrane systems were the first to be used in the ultrafiltration of fruit juices (1), but membrane fouling with increased feed concentration or as a result of compression effects as transmembrane pressure increased resulted in very low flux rates (2, 3). Large pressure drops across hollow fiber or thin-channel UF membranes cannot be achieved. Furthermore, virtually no suspended solids can be tolerated, since blockage of the flow channels would readily occur.

Open tubular UF designs have been more successfully applied to juice clarification, since solutions high in suspended solids and viscosity can be accomodated due to large, circular flow channels. The disadvantage to open tubular designs is the low membrane packing density and increased tube support needed for higher

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pressure operation (4). However, if open tubular systems can be operated in a single pass the best possible average flux can be obtained, since all increments of the membrane area are exposed to the minimum possible concentration. Also, if very high solids could be accomodated by an open tubular UF system operated in a single pass, the need for conventional fruit pressing would be eliminated. Instead, a process for clarifying liquified fruit pulp directly would be possible.

The concept of producing clarified fruit juice by a single pass of enzyme-treated fruit puree through a tubular, metallic ultrafiltration membrane system was recently described by Thomas et al. (5, 6). The process is known commercially as the Ultrapress process, since pressing and ultrafiltration are accomplished simultaneously (7). The metallic membrane ultrafiltration system is composed of sintered stainless steel tubes of varying diameters with membranes formed-in-place within the porous matrix of the tubes by deposition of various metallic oxides. Metallic oxides in combination with polymers are also possible.

Earlier studies (5) utilizing 5/8-inch diameter tubes showed that high juice recoveries were possible, but only with extremely large pressure drops. With 5/8-inch diameter tubes pressure drops increased dramatically as juice recovery increased and were in the range of 700-800 psi at 85% juice recovery. In the single pass design viscosity effects are greatest near the end of the tube, thus most of the pressure drop will occur in this region. Large, dramatic pressure drops near the end of the system created instabilities which made operational control difficult.

Subsequent studies (6) produced a mathematical model of the process which defined the importance of tube diameter on pressure drop. The design flow in a channel was found to be proportional to the 2.0 to 2.5 power of the tube diameter. Thus, a prototype utilizing 1 1/4-inch diameter tubes was constructed and operated stably at 85% juice yields with much lower pressure drops (250-350 psi). This same study (6) also addressed optimization of viscosity reduction of the puree and membrane flux utilizing commercial liquefaction enzymes. Viscosity reduction was readily obtained with even small amounts of liquefaction enzymes, and further increases in enzyme concentration did not appreciably affect viscosity reduction. However, steady state flux was proportional to the level of enzyme used up to 0.044%. Membrane flux correlated very well, as expected (3), with reduction of total pectin. It was evident that enzyme pretreatment should be further developed with the goal of enhancing flux rather than reducing viscosity, especially since increased tube diameter could be used to overcome pressure drops imparted by the viscosity of the retentate at high juice recoveries.

The previous studies also suggested that the Ultrapress process offered the potential for higher quality juice by reducing process time and also the potential for microbiologically stable juice, which could also provide an improved product by eliminating

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heat pasteurization. Further optimization of enzyme pretreatment, determination of microbial rejections by the membrane, evaluation of the overall quality of the product, and potential new applications of the Ultrapress system are current objectives in the further development of this process.

## Materials and Methods

Enzyme Treatment. Apple puree was produced as described in previous studies (5, 6). Two commercially available enzymes were added at varying concentrations to the purees, which were stirred constantly at 35°C for 1 hr for all enzyme treatments. Rohapect 7016, a liquefaction enzyme preparation (Rohm Tech, Inc., Malden, MA), was added at a concentration of 0.044% (w/w) to serve as the basis for comparison to increasing amounts of a pectinase preparation, Rohapect DIL (Rohm Tech, Inc., Malden, MA). The liquefaction enzyme was successively reduced by one-half in other treatments as the amount of pectinase was increased by approximately 50%, starting with an initial pectinase concentration of 0.049% (w/w). After the 1 hr enzyme treatments, the viscosity of the purees were measured and they were ultrafiltered with metallic membranes as described by Thomas et al. (6). Steady state fluxes were measured after 30 min of operation and reported as gallons/ft$^2$/day (GFD).

Microbial Challenges. Metallic UF membranes were as described by Thomas et al. (6) and were challenged with microbial cultures prepared as indicated by Barefoot et al. (8). Challenge cultures were *Pseudomonas diminuta* ATCC 19146 (American Type Culture Collection, Rockville, MD), *Bacillus coagulans* 43P, *Saccharomyces cerevisiae* NRRL Y-2034 (9), baker's yeast kindly provided by Dixie Yeast Corp., Gastonia, NC, or *Penicillium roqueforti* blue mold powder (Dairyland Food Laboratories, Waukesha, WI). Cultures were added to either apple puree or peptone (0.1% aqueous).

For microbial challenges and juice production, the system was operated in a 50 L recirculating mode as previously described (8). In summary, feeds consisting of apple puree or aqueous peptone were mixed with microbial suspensions in a temperature-controlled steam-jacketed kettle. Feeds were pumped from the kettle by a centrifugal suction booster pump feeding a diaphragm pressurizing pump. Flow was regulated by a manual pressure control valve and monitored by a pressure gauge at the inlet. Concentrated feeds were recirculated to the kettle. Permeates passed from the shell through a steam or permeate outlet to two-way valves that diverted flow to a controlled atmosphere glove box for aseptic collection or back to the feed kettle. Metallic UF membranes were sterilized as described by Barefoot et al. (8) and connected via sterile permeate lines to the glove box. Permeate (usually 150 mL) was collected in sterile containers and transported to the laboratory for microbiological analysis. Populations of challenge microorganisms in feed and permeate samples were determined by viable counts according to

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standard methods (10). Apple juice was stored at 20-25°C and observed for turbidity indicative of microbial growth.

Volatile Analysis. Volatile profiles of enzyme treated apple puree and ultrafiltered juice were obtained by gas liquid chromatography. A Hewlett Packard 5880-A gas chromatograph equipped with a flame ionization detector, a 5880-A series level four computerized data system and a DB-5 (30 m x 0.25 mm I.D.) fused silica capillary column (J&W Scientific, Folsom, CA) were used. Puree was centrifuged at 10,000 x g and the supernatant was injected onto the column (direct aqueous injection). Ultrafiltered juice was injected directly with no sample preparation. Sample size in all cases was 5µL split 15:1 with helium as the carrier gas at a flow rate of 1 ml/min. The oven temperature was held at 60°C for the initial seven minutes of the injection time. It was then increased to 200°C at a rate of 5°C/min and was held there for twenty five minutes. Total volatiles were obtained by summation of integrated peak areas on the chromatograms. Percent areas of each peak were also calculated.

## Results and Discussion

With the objective of enhancing membrane flux by enzymatic degradation of pectin in the puree, the liquefaction enzymes normally used in the process were reduced while a commercial pectinase preparation was used in increasing amounts as a replacement (Table I).

Table I. Cost Comparison of Commercial Liquefaction Enzyme Versus Replacement with Commercial Pectinase

| Enzyme Treatment | | Conc. (%) | Cost ($)/gal[1] | |
|---|---|---|---|---|
| A | (Rohapect 7016[2]) | 0.044 | - | 0.054 |
| B | (Rohapect 7016) | 0.022 | 0.027 | 0.041 |
| | (Rohapect DIL[3]) | 0.049 | 0.014 | |
| C | (Rohapect 7016) | 0.011 | 0.014 | 0.034 |
| | (Rohapect DIL) | 0.074 | 0.020 | |
| D | (Rohapect 7016) | 0.006 | 0.007 | 0.031 |
| | (Rohapect EIL) | 0.098 | 0.024 | |

[1]Based on 200 gal/ton on 85% juice yield

[2]A commercial liquefaction enzyme

[3]A commercial pectinase

The commercial liquefaction enzyme typically used (Rhoapect 7016) contained predominantly cellulase and hemicellulase activity but with some pectinase activity. The use of more pectinase and less liquefaction enzyme represented a more favorable treatment economically and was actually more effective in reducing viscosity (Table II). Also, as predicted, the steady state flux of the membranes was higher when pectinase predominated.

To assess microbial rejections, sterile membranes were challenged with P. diminuta, B. coagulans, S. cerevisiae, and P. roqueforti spores suspended in apple puree or 0.1% peptone. Reductions achieved by ultrafiltration of cells in peptone at normal operating pressure (300 psi) were 5.1, 6.0, 7.6, and >9.0 logs for smallest to largest organisms (Table III).

Membranes typically retained more cells from apple puree than from peptone at normal operating pressures. Retention of cells from apple puree was more than 1.5 logs greater than retention of cells from peptone by the same membrane (data not shown). The acid tolerant microflora in apple pulp averages approximately $10^5$ colony forming units per gram and consists primarily of fungi; 30 to 40% of the microbial population is mold and 60 to 70% is yeast (11). Thus, rejection of yeast and mold spores as shown here demonstrates that organisms most commonly found in apple puree that proliferate in and spoil juice would be retained completely by the metallic membranes. These results were verified in a straightforward manner by aseptically bottling apple juice from uninoculated apple puree in the glove box. The juice was shelf stable for over a year. These microbial rejections and the fact that the metallic membrane and their stainless steel supports are inherently thermostable demonstrate that a commercially sterile product can be produced by the Ultrapress process.

Fruit juice quality was assessed by a direct comparison of the volatile composition of the clarified, Ultrapress juice with that of juice obtained from apple puree by centrifugation. A direct aqueous injection technique was employed for this comparison. The volatile profile for clarified juice from McIntosh apples was nearly identical to that of the centrifuged juice obtained from the purees of the same apples with the exception of volatiles with retention times of 2 to 3 minutes (Figures 1 and 2). A reduction of 50 to 80% was observed for these volatiles from the Ultrapress juice, which included four peaks representing approximately 1.5% of the total volatiles. However, notable differences in peak areas were not observed for peaks with retention times of 6 min or greater. Saper et al. (12) identified the volatile components of McIntosh apples that were responsible for the characteristic aroma of the ripe fruit. These included ethyl acetate, ethanol, ethyl proprionate, ethyl butyrate, ethyl 2-methylbutyrate, hexanal, 2-methylbutanol, trans-2-hexenal, and hexenol. These compounds had retention times of 8 min or greater on a GC column with operating conditions similar to that used in this study. Poll (13) showed the rapid accumulation of these same volatiles in McIntosh apples at the stage considered "ripe for eating," which had the highest fruit aroma score.

Table II. Percent Viscosity Reduction and Steady State Flux with Various Enzyme Treatments

| Enzyme Treatment[1] | Viscosity Reduction[2] (%) | Steady State Flux (GFD) |
|---|---|---|
| A | 40 | 30.6 |
| B | 37 | 24.7 |
| C | 53 | 34.0 |
| D | 74 | 37.0 |

[1]Enzyme treatment as described in Table I

[2]After 60 minutes of exposure

Table III. Logarithmic Reduction Values for Microorganisms in Peptone Achieved by Metallic Ultrafiltration Membranes

| Operating pressure[a] (psig) | LRV[b] for | | | |
|---|---|---|---|---|
| | *P. diminuta* | *P. roqueforti* | *B. coagulans* | *S. cerevisiae* |
| 325-400 | 5.1 | 6.0 | 7.6 | >9.0 |
| 575-650 | 3.6 | NA | 6.1 | >9.0 |

[a]Samples were taken at 5 min intervals after 10 min operation at each pressure. The system was operated for 100 min at 300-400 psig and 30 min at 575 to 675 psig.

[b]Logarithmic reduction values (LRV) were calculated by the formula: LRV = $\text{Log}_{10}$ CFU/mL Feed - $\text{Log}_{10}$ CFU/mL permeate.

Although the peaks in Figures 1 and 2 were not identified, it was apparent that the Ultrapress process caused insignificant changes in the volatile composition of the apple, indicating that fruit juices with aroma profiles nearly identical to that of the fresh fruit can be obtained.

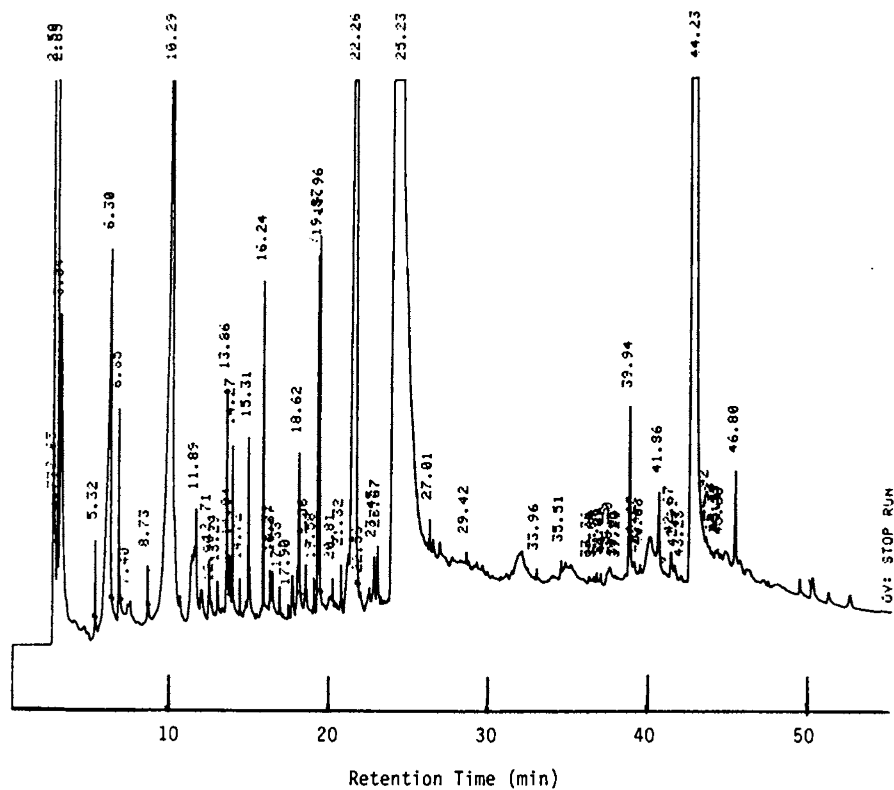


Figure 1. Volatile profile of McIntosh apple puree.

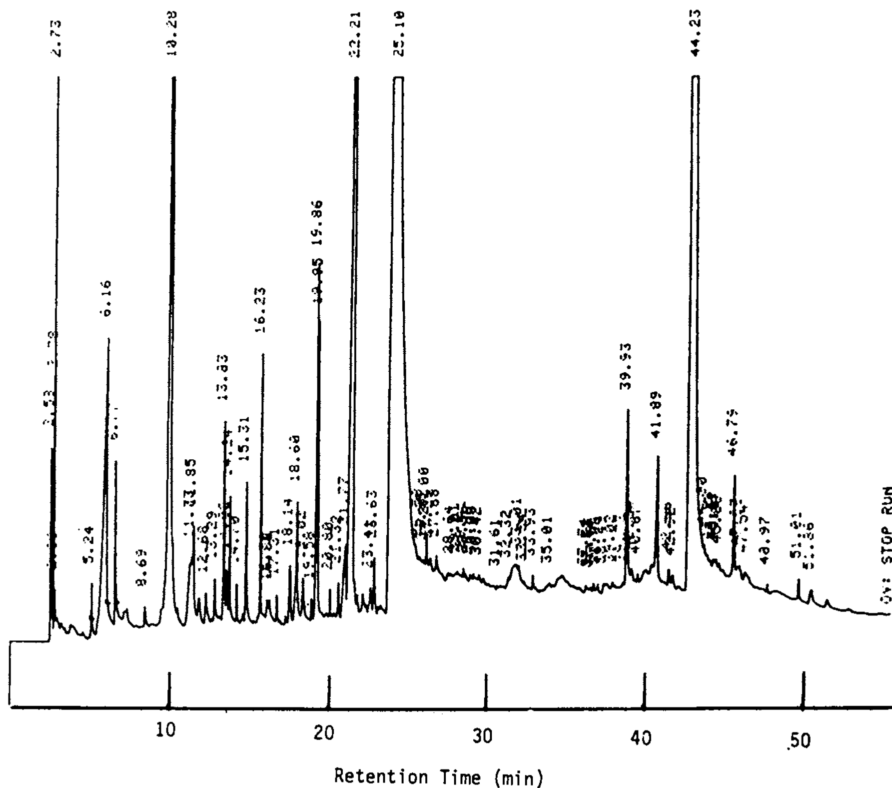


Figure 2. Volatile profile of apple juice manufactured by the Ultrapress process using McIntosh apples.

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Chapter 28

# Microfiltration of Enzyme-Treated Apricot Puree

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Apricot was used as a model for studies of fruit puree clarification. Various puree concentrations were treated for 30 min at 50° C with 0.4 cc/L, each, of commercial cellulase and pectinase enzyme and clarified by filtration through a 0.45μm pore size ceramic microfilter. Sparkling clear apricot juice was produced at flux rates from 90-190 L/m²h. Above 13° starting Brix, juice flux showed little increase with increasing starting Brix. Dissolved solids flux increased substantially with increasing starting Brix. Permeate remained clear and retained most of its flavor and aroma when concentrated by vacuum evaporation to 58° Brix. There appears to be some retention of enzymes by the filter. Retained enzymes were successfully utilized in a 4 h trial in which untreated puree was continuously added to retentate, in amounts equal to permeate removed, after startup on enzyme-treated puree.

Fruit crops typically have a short season and inelastic demand. As a consequence, many processors convert part of the annual harvest to concentrated purees for later utilization. This, in effect, reduces plant size needed to accommodate the crop and better utilizes the plant by extending the operating season.

Concentrated purees typically utilize excess fruit and are also an outlet for wholesome but off-grade product. Their principal uses have been in nectars, baby foods, preserves, and sauces, such as Bar-B-Que sauce. This is a limited market; but the high consistency and pulpy nature of purees make them unsuitable for many other uses, such as in clear beverages or soft drinks. A high Brix concentrate of clear juice might be utilized as a natural additive for flavoring, sweetening, or increasing solids content of a number of bever-

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ages or liquid foods ranging from carbonated drinks to school lunch supplements.

Apricot is very representative of the fruits just described. It has a very short harvest season and storage life, and thus the freshmarket is very limited. Because of its short season, a processor will usually convert part of the crop to puree, at 30-32° Brix, for later reprocessing. Consequently, apricot was chosen as a model for our research with the goal of increasing the value of the crop by expanding the market for its concentrated puree. It was felt that this could be accomplished by converting the puree to clarified juice.

The addition of pectic and cellulolytic enzymes to aid juice clarification and increase yield has been a relatively common industrial practice for several years (1-3). The enzymes are used to free more juice by cell lysis and to increase filtration efficiency by viscosity reduction.

In recent years, developing technology has led to the use of ultrafiltration and microfiltration membranes for juice clarification (1,4-13). The use of membranes has several advantages. Lower labor costs may be possible due to automation possibilities of membrane filtration (8). Filter aids such as diatomaceous earth (DE) aren't needed (9) so that product that would have been discarded with DE is saved, and DE acquisition and disposal costs are eliminated. Enzymes may be rejected by ultrafiltration membranes (4,12) causing the ultrafiltration equipment to act as an enzyme reactor (12), although some odor-active volatiles may be retained, resulting in some loss or change in flavor (8).

Membranes have been successfully used to clarify both untreated and enzyme-treated juices. Considerable literature exists regarding ultrafiltration of single strength apple juice. Flux rates as high as about 80-115 $L/m^2h$ have been reported for enzyme-treated, preclarified juice (8,9) and about 50-60 $L/m^2h$ for enzyme treated puree. Preclarification is generally thought necessary for effective ultrafiltration.

Microfiltration involves a larger pore size than ultrafiltration, typically 0.2-0.5 µm compared to 0.001-0.02 µm, which should result in increased permeation while maintaining many of the advantages of ultrafiltration. Although microfiltration has been suggested for clarification (5,6,12,13), virtually no literature exists regarding microfiltration of fruit purees, particularly at concentrations higher than single strength.

Ceramic microfilters initially had problems with inconsistent quality, but improved production methods have solved these problems. They are expected to show substantial sales growth as they generally have longer operating lifetimes and are better able to withstand severe operating and cleaning conditions than other types of membranes(14-15). Ceramic filters are expensive, but technology to produce these filters is new and still evolving. Consequently, it is expected that the price of these filters may be reduced substantially in the near future as production technology is improved or economies of scale are realized.

The purpose of this study was to examine the efficacy of a process to produce clear juice from a model concentrate, apricot, utilizing enzyme treatment and microfiltration. Specific goals were to determine (a) if effective flux rates could be achieved, and (b) if clarified juice of acceptable quality could be produced.

Materials and Methods

Commercial apricot concentrate (30-32° Brix), in aseptic drums from the 1986 and 1987 pack, was used immediately after opening the drums or subdivided into 19 L containers and stored frozen until used. Puree was prepared by diluting concentrate with distilled water to a desired brix. No differences in filtration were noted between purees made from frozen or unfrozen concentrate.

Enzymes. The enzymes used were Genencor brand Cellulase 150L and Pectinol 80SB. These are commercial grade rather than purified chemicals and have activities beyond those for which they are named. For example, the Cellulase may also contain substantial pentosanase or glucanase activity. Preliminary laboratory experiments established that a combination of 0.4 cc/L, each, Cellulase 150L and Pectinol 80SB, was a suitable enzyme treatment. All enzyme treatments in this report used this enzyme level.

Apparatus and Definitions. The apparatus used for this investigation consisted of a puree/retentate reservoir, a feed pump, a heat exchanger, and a microfilter module connected in series to form a recirculation loop. At startup puree and enzyme was added to the reservoir and the mixture recirculated between the heat exchanger and the reservoir, bypassing the microfilter via a secondary loop. After enzyme treatment, puree was diverted to the microfilter where permeate was withdrawn and retentate continuously recirculated back to the reservoir. Filter pressure was adjusted by a valve on the retentate return line. Filter inlet and outlet pressure was measured by pressure transducers, inlet temperature by a thermocouple, and inlet flow rate by a paddle wheel flowmeter (Model FP5205, Omega Engineering, Inc., Stanford, CT). Data were collected by a CR-7 data logger (Campbell Scientific, Inc., Logan, UT). Flux was measured by graduated cylinder and stop watch.

A sanitary design, positive displacement pump (Model D025, Waukesha Foundry Co., Inc., Waukesha WI) with 1.5 Hp adjustable drive was used as the feed pump. Flow rate to the filter varied between 10-42 L/min.

The microfilter module consisted of a stainless-steel sanitary housing enclosing a single porous ceramic ($Al_2O_3$) microfilter element. Both Ceraflow (Norton Co., Worchester, MA) and Membralox (Ceraver Co., Tarbes, France; Aluminum Co. of America, U. S. representative) filters were used.

The Norton filters included an original design (tube) filter element and an improved design (lumen) filter element. Overall dimensions of both filter elements were 2.1 cm diameter by 40.2 cm length. The tube element consisted of a bundle of 28 porous tubes and the lumen element consisted of a porous cylinder with 19 tubular channels or lumens extending the length of the cylinder. The filter membrane comprised of the inner surface of the tubes or lumens and had a smaller pore diameter than the non-membrane portions of the filter. Inside diameter of both tubes and lumens was 2.8 mm resulting in a total membrane area 0.091-$m^2$ (1 $ft^2$) for the tube filter and 0.060-$m^2$ (0.65 $ft^2$)for the lumen filter. Membrane pore diameter was 0.45 µm for both filters.

The Ceraver filter element consisted of a hexagonal rod about 40 cm wide by about 81.5 cm long containing 19 lumens. Lumen diameter was about 6 mm resulting in a total membrane area of 0.29-$m^2$ (3.14 $ft^2$). Membrane pore diameter was either 0.2 or 0.8 μm.

The Ceraver and original design Norton filter elements were the same as those used in commercial scale filter modules. The improved design Norton element was the same as a commercial element except for being about half the length. Commercial scale modules house a number of these filter elements in parallel. Thus the filters used in this investigation were essentially single-element versions of the multi-element commercial filters.

In this report, transfilter flow refers to the linear velocity of retentate over the membrane surface (retentate velocity through a filter tube or lumen). Filter pressure was the gage pressure of the retentate at the filter inlet. The permeate side of the filter was maintained at atmospheric pressure for all experiments. Pressure drop was the difference between retentate pressures at the filter inlet and outlet. Flux was permeation rate per unit area of membrane.

Permeate was vacuum concentrated in the laboratory using a Büchi (Brinkmann Instruments Co., Westbury, N.Y.) Rotavapor RE120 evaporator and model 165 vacuum controller. In the pilot plant, a pilot scale, conventional single stage vacuum evaporator, fabricated to Western Regional Research Center specifications was used.

Analyses. Qualitative tests were performed for the presence of pectinase in permeate. Since pectinase was a smaller molecule, it was assumed that the absence of pectinase in the permeate would also indicate the absence of cellulase. A substrate solution of 0.1% pectin in distilled water was prepared by gentle overnight agitation. A permeate sample was prepared by adding 1 cc of permeate and 1 cc of distilled water to 8 cc of pectin solution in a test tube. A control sample was similarly prepared by adding 2 cc distilled water to 8 cc pectin solution. Samples were incubated at 50° C for 15 min then visually examined. Pectinase causes a precipitate to form so a precipitate in the unknown sample or difference in turbidity between control and unknown would indicate the presence of pectinase.

Brix was measured by a Bausch and Lomb refractometer.

Quality of clarified juice was evaluated informally by assembling a panel of 5 associates. Samples of clarified juice, concentrated clarified juice, and concentrated puree, from which the clarified juice was made, were adjusted to 13° Brix and the samples identified to panel members. Panel members were then asked to compare flavor and aroma of all clarified juice samples to puree samples and to compare flavor, aroma, and color of samples from concentrated, clarified juice to samples from never-concentrated, clarified juice.

Experimental. Treated puree was prepared by adding 25-35 L of diluted concentrate to the reservoir and heating to 50° C, the optimum enzyme reaction temperature. Cellulase and pectinase were then added to the reservoir. The mixture was continuously recirculated at 50° C (recirculation provided efficient mixing) and held at that temperature for 30 min. All subsequent filtration was done at a controlled temperature of 50° C.

Filtration was done in either a permeate return or batch mode. In permeate return mode, permeate was recombined with retentate and recirculated. This mode was used to observe the effect on flux of increasing filter pressure (a pressure scan) or increasing transfilter flow (a flow scan).

A pressure scan was made by holding transfilter flow constant and making a series of trials at differing filter pressures. In similar manner, a flow scan was made by holding filter pressure constant and making a series of trials at different transfilter flows. All pressure and flow scans were 60 min in duration, sufficient to establish flux change.

Batch mode was used in determining representative flux and clarified juice yield for various starting Brix levels. Concentrate was diluted to a desired starting Brix (13-24°) and either enzyme treated or left as untreated control. Permeate was removed and its volume recorded, resulting in decreasing retentate volume and increasing retentate viscosity. Pressure and flow were held constant until retentate thickened to the point where flow had to be reduced to maintain constant pressure. For yield determination, trials were arbitrarily stopped when transfilter flow fell below 0.5 m/s, the point where it became impractical to operate the apparatus. Permeate volume as a percentage of starting puree volume was calculated as yield of clarified juice.

Top-off batch mode was intended to take advantage of apparent membrane enzyme rejection. A trial was begun in regular batch mode with the puree reservoir filled with treated puree. Permeate was removed as usual, but additional untreated puree was added to the retentate in the reservoir, at periodic intervals, in amounts equal to the permeate volume removed, thus maintaining a constant retentate volume and utilizing retained enzymes to treat the makeup puree.

Fifteen Brix permeate, accumulated from several filtration trials, was vacuum concentrated to 68.4° Brix in the laboratory evaporator, using a bath temperature of 50° C and an absolute pressure of 10 kPa, and to 55.8° Brix in the pilot plant evaporator, using an absolute pressure of 8.2 kPa and a steam jacket temperature of 35°C.

The filter was cleaned in place after each trial using either a variation of a technique recommended by Norton or a technique recommended by Ceraver. In batch tests using the Norton filter, retentate was diluted at the end of each trial with distilled water to less than single strength, otherwise there was danger of retentate clogging the filter tubes or lumens upon shutdown. The system was then flushed, the permeate valve closed, and cleaning solution recirculated through the filter. With the permeate valve closed, pressure was uniform on the permeate (permeate pressure was the average between retentate inlet and exit pressure). As a result of retentate pressure drop through the filter, permeation occured at the high pressure end and backflushing at the low pressure end. Cleaning occured by crossflow the length of the filter surface and by backflushing in the low pressure region. In the modified Norton technique, three separate solutions were successively recirculated through the filter. The first solution contained 19.5 ml of Micro brand detergent/L of distilled water. The second solution was 0.1*N*

KOH, and the third was 0.1*N* $HNO_3$. Each solution was recirculated for 30 min at a filter pressure of about 100 kPa and at 70°C. The system was then thoroughly flushed with distilled water. The Ceraver technique was similar, also involving three cleaning solutions, but somewhat more severe. First a solution of 200 ppm free chlorine (from NaOCl) in distilled water was recirculated at room temperature for 10 min. This was followed by a 2 wt % NaOH solution and finally a 2 wt % $HNO_3$ solution. Both solutions were circulated at 70° C: the NaOH solution for 15 min at a low filter pressure followed by 5 min at about 100 kPa, and the acid solution for 20 min at about 100 kPa. Between each cleaning solution, a distilled water rinse was recirculated at room temperature for 10 min.

Thoroughness of the cleaning was tested by filtering distilled water at a temperature of 21-24° C, a transfilter velocity of 3.4 m/s, and a filter pressure of 75 kPa. Permeation rate was noted 1 min after filtration was begun and compared to that recorded when the filter was clean. If rate was not within 92% of the clean rate, the filter was recleaned. Filters were always restored to this rate after 1-3 cleaning cycles.

## Results and Discussion

Flux Values and Operating Conditions. Figure 1 shows the change in juice flux with time for batch trials using untreated and enzyme-treated puree at different brix levels. Puree was filtered at 200 kPa filter pressure for 13° starting Brix trials, at 230 kPa for 17° starting Brix trials and at 300 kPa for 20° starting Brix trials; all trials were run at 3 m/s transfilter flow, using the Norton 0.45µm-28 tube filter. These filter pressures were at or near the minimum possible for untreated puree in the equipment when using 3 m/s transfilter flow. From an initial value of about 80-90 $L/m^2h$, flux of untreated puree typically decreased with time. In contrast, treated puree typically had initial flux values of about 90-115 $L/m^2h$; and, surprisingly, flux generally exhibited substantial increases with time. End-of-run values as high as 190 $L/m^2h$ were achieved for some trials after 3-5 h operation.

In qualitative tests, no pectinase was detected in permeate from some runs and detected at what appeared to be low levels in permeate from other runs. Presumably, this indicates pectinase, and therefore cellulase, were concentrated to some extent in the retentate and this may be related to the increase in flux rate with filtering time. Other factors involved may be longer contact time with enzymes, general cell rupture and size reduction caused by pumping shear, or other undetermined factors.

Table I lists the change in juice flux for treated and untreated purees at different starting Brix levels using the Norton tube filter. Puree at 13-15° starting Brix was filtered at 200 kPa filter pressure, 17° starting Brix at 230 kPa, and 20-24° starting Brix at 300 kpa. All trials were at 3 m/s transfilter flow. Although the number of trials were limited, some trends were indicated. At 13° starting Brix (single strength), there was no flux improvement for treated puree over untreated puree. However, at 15-20° starting Brix, flux was increasingly improved by enzyme treatment when compared to untreated puree. Above 20° starting Brix, untreated puree was too thick to filter in the equipment. At comparable filtration

times, treated purees above 13° starting Brix had higher flux rates than treated puree at 13° starting Brix, although single strength puree did tend to achieve high rates after longer filtration times. In comparing treated purees in trials above 13° starting Brix, there was little increase in flux rate with increasing starting Brix, although 20° starting Brix did achieve the highest flux rates. On the basis of juice flux alone there seemed to be little advantage to use a puree higher than 15° starting Brix. But the soluble solids in the juice were increased in proportion to the increase in the starting Brix. Consequently, within the range of flux values of Table 1, soluble solids passing the membrane increased with increasing starting Brix. This provides a strong economic incentive to filter at as high a starting Brix as practical.

Table I. Change in juice flux with filtration time for treated and untreated apricot puree at different starting Brix

| Starting | | Filtration time, min | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Brix | Sample | 5 | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 |
| | | Juice flux, L/m²h | | | | | | | | |
| 13 | Untreated | 93 | 98 | 104 | 104 | | | | | |
| 13 | Treated | 92 | 94 | 96 | 98 | 98 | 104 | 108 | 116 | 132 |
| 13 | Treated | 98 | 99 | 105 | 113 | 120 | 127 | 152 | 188 | |
| 13 | Treated | 92 | 93 | 99 | 107 | 116 | 132 | 180 | | |
| 15 | Untreated | 88 | 84 | 84 | 86 | 85 | 80 | | | |
| 15 | Treated | 101 | 110 | 123 | 127 | 149 | 172 | | | |
| 17 | Untreated | 81 | 74 | 69 | 66 | 66 | | | | |
| 17 | Treated | 105 | 113 | 120 | 136 | 146 | 161 | | | |
| 20 | Untreated | 82 | 69 | 66 | 66 | 66 | | | | |
| 20 | Treated | 116 | 124 | 127 | 141 | 152 | | | | |
| 22 | Treated | 110 | 116 | 124 | 136 | 146 | | | | |
| 24 | Treated | 107 | 110 | 116 | 127 | 136 | | | | |

Norton has recently changed their filter design, replacing the tube filter with the lumen filter. The tube filter, at least in the laboratory model, was fragile and breakage limited the number of trials. The lumen filter proved to be a much more rugged design, and may also be lower cost. Flux rates appeared to be lower for the lumen filter, but difficult to estimate because of the limited number of trials. For treated, 15° starting Brix puree, at conditions similar to those of Table 1, flux increased from an initial value of about 77-86 $L/m^2h$ to as high as 91-114 $L/m^2h$ after 180 min filtering. Thus, increasing flux rate with filter time was common to both filter designs. From a filtration standpoint, only the thickness of porous membrane backing material was changed by the design, consequently, only flux rate would be expected to be affected by the design change.

Although most treated-puree trials showed a flux increase with filtering time, a few trials, regardless of filter, experienced an unexplained decrease with time. Decrease in flux was usually about 15-25% below starting flux after about 3 h filtering and greater with longer filtering time. These results are currently being investigated and may be related to filter cleaning.

As expected, retentate thickened as clarified juice (permeate) was removed by filtration. To maintain constant filter pressure, retentate flow had to be decreased. Under these conditions, a test was arbitrarily stopped when transfilter flow fell below 0.5 m/s and the collected clarified juice was taken as the yield from a particular starting Brix. Figure 2 shows the yield based on starting Brix, under these transfilter flow conditions, for both treated and untreated puree. The tube filter was used with the filter pressures listed for Table 1. Yield from both treated and untreated puree decreased with increasing starting Brix, with treated puree varying from about 90% yield for 13° Brix to a low of about 40% for 24° Brix. Untreated puree varied from about 50% to about 20%.

Higher yields might be obtained by maintaining flow and letting pressure increase, but several factors would need to be considered. Flux had a relatively flat response to increasing pressure (see subsequent discussion). Associated with increased pressure would be increased capital and operating costs, increased danger of pulp plugging the filter lumens, and increased difficulty and time of filter cleaning.

Figure 3 show the results of a flow scan at 250 kPa filter pressure, using the tube filter, and a pressure scan at 3 m/s, using the lumen filter. All trials were in the laminar flow range. Increased transfilter flow produced a uniform flux increase over the range tested. In contrast, increased filter pressure produced a relatively flat response, with the highest flux obtained at 200 kPa. Increased pressure may result in a denser boundary layer on the membrane surface counteracting the increased driving force. Each filter installation probably has an optimum where increased flux resulting from increased transfilter flow is balanced by costs of increased filter pressure and pumping.

Pressure drop increased with increased retentate viscosity and increased transfilter flow. Under conditions of about 200 kPa filter pressure and 3 m/s transfilter flow, and depending on starting Brix and running time, treated puree generally had a pressure drop of 30-80 kPa over the 40 cm length of a Norton filter. Similar untreated purees, run at a filter pressure of about 300 kPa and a transfilter flow of 3 m/s had a pressure drop of 90-275 kPa, again depending upon starting Brix and running time.

In single tests of each Ceraver filter, the filters produced results reasonably similar to those from the Norton filters. Flux rates were somewhat lower but in the same range. The larger lumen diameter of the Ceraver filters resulted in less pressure drop per unit filter length.

Filters from both manufacturers were somewhat difficult to clean, sometimes requiring multiple cleanings and use of the more severe Ceraver cleaning technique. Increased filter pressure seemed to increase cleaning difficulty, as did the amount of use a filter had experienced. These items were not specifically addressed in the tests.

Clarified Juice. Sparkling clear juice was produced with flavor and aroma judged to compare well with the original reconstituted puree in informal taste tests. When blended in other beverages, it imparted a desirable apricot flavor or background. Depending on the end use of the clear juice, any enzymes present in the permeate may need to be heat inactivated.

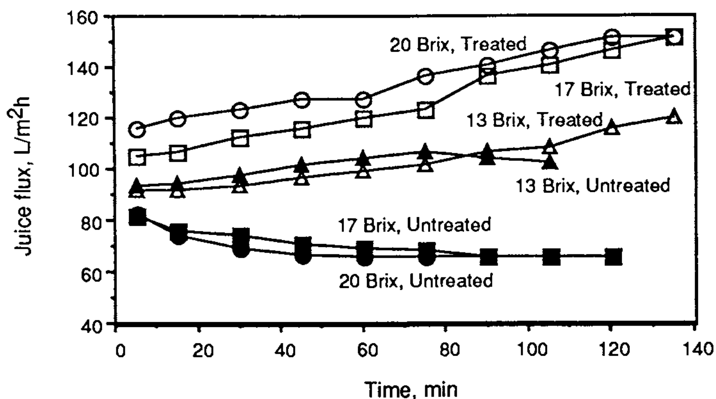


Figure 1. Change in juice flux with time, in batch trials, for enzyme treated and untreated puree at several different starting Brix levels. All samples filtered at 50 C, with same starting Brix level samples filtered at same pressure and transfilter flow.

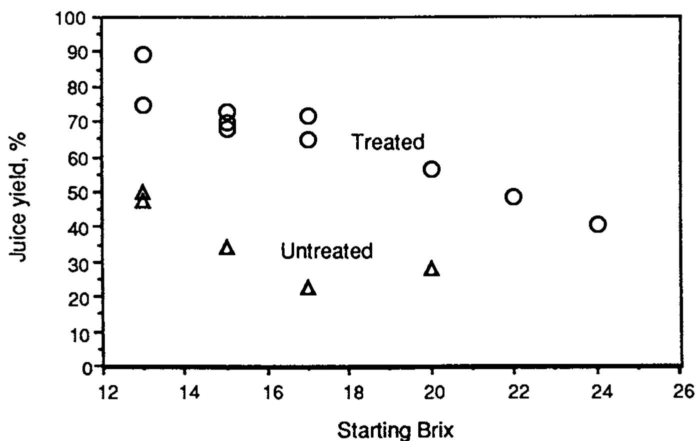


Figure 2. Clarified juice yield, in batch trials, from enzyme treated and untreated apricot puree as a function of puree starting Brix. Same starting Brix level samples filtered at same pressure and transfilter flow.

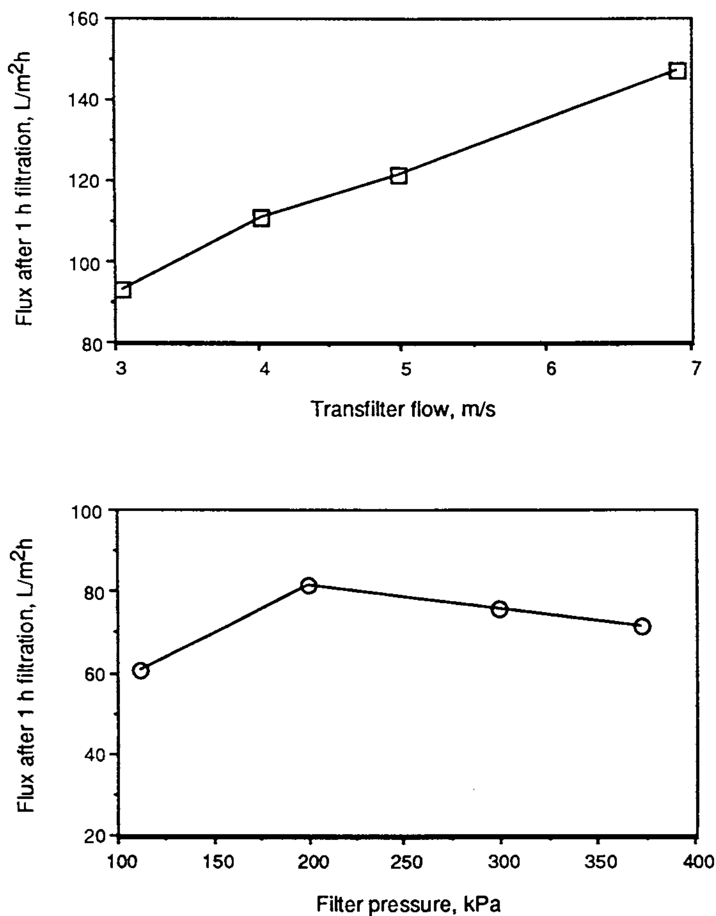


Figure 3. Effects of transfilter flow and filter pressure on juice flux. Pressure held constant at 250 kPa in upper figure and transfilter flow held constant at 3 m/s in lower figure.

Clear juice was concentrated by vacuum evaporation to as high as 68.4° Brix in laboratory tests and to 55.8° Brix in a pilot scale vacuum evaporator. Concentrate remained clear, but at 68.4° Brix some darkening and loss of flavor was observed.

Top-off Batch Operation. Retention of enzymes, at least for part of a run, suggests the filter system might be operated as a bioreactor, utilizing retained enzymes to treat fresh incoming puree. Figure 4 shows the results of a test where untreated puree was added to treated retentate in amounts equal to permeate removed. In this test, puree was enzyme treated as usual and then filtered for 30 min before addition of untreated puree was begun. The test was run for 4.25 h, during which an amount of untreated puree equal to about 80% of starting puree volume was added to the retentate. The Ratio curve indicates the nominal concentration of puree. Permeate removal resulted in a 1.88 fold reduction in total puree volume by the end of the trial. Flux experienced no drop off with the addition of untreated puree and actually increased about 20% over the course of the test.

The test was terminated upon feed exhaustion, but indications were that the test could have continued for considerably more time, perhaps as long as 8 h. Some thickening of retentate was noted, and this would have eventually affected operation. However, this would have occurred at a slower rate than in a strictly batch operation since fresh puree was continually diluting the retentate. While the enzyme savings are evident for this mode of operation, its greatest utility may be in enabling more efficient filtration at higher Brix. Thickening of retentate increasingly limits operation at higher Brix. The continual dilution provided by the addition of fresh puree would retard this thickening. The run might be finished off with lower Brix puree as retentate approaches an unacceptable level of thickening, resulting in an average brix higher than that feasible with straight batch operation.

## Conclusions

These tests have demonstrated the technical feasibility of producing sparkling-clear apricot juice from enzyme-treated concentrate using microfiltration with ceramic membranes. Informal judging indicated juice flavor and aroma was similar to original concentrate. Treated puree could be filtered at levels at least as high as 24° starting Brix, but 15-17° starting Brix may be a more practical operating range. Flux rates were in the range of 80-190 L/m$^2$h, with the unusual feature of flux increasing with time in batch tests. A starting enzyme level 0.44 cc/L,each, of commercial cellulase and pectinase was used for treatment of the puree. The enzymes were retained, at least in part, in the retentate and their increasing concentration may have been responsible for the increasing flux rates over the course of a test. Furthermore, retained enzymes enabled the use of top-off batch operation, which is a continuous mode of operation after initial batch start up. However, it is yet to be determined whether some make up enzyme might be needed and the feasible operating time for this mode. Clarified juice could be concentrated at least as high as 56° Brix without apparent loss of quality for blending in other foods and beverages.

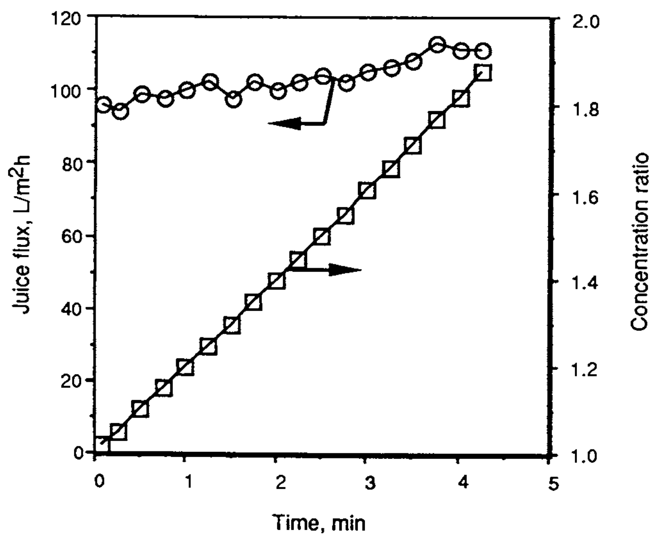


Figure 4. Change in juice flux and retentate concentration ratio (total puree volume/retentate volume) with time for a top-off batch trial. Enzyme treated puree used for startup then untreated puree added to retentate in amounts equal to permeate removed.

These results, based on single-element filters, provide an indication of the region of performance that might be expected for the process on a commercial scale. However, commercial filters are multi-element modules normally operated in series. Consequently, these results are not directly suitable for scaleup.

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# Chapter 29

# Recovery and Utilization of Byproducts from Citrus Processing Wastes

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Growth and microbial production of by-products offers a practical alternative use of citrus processing waste. The unique physico-chemical properties of citrus peel and pulp residue provides an underutilized resource. Reports of fermentation byproducts of microbial growth on dried peel or by submerged culture include citric acid, glutamic acid, riboflavin, and cobalamin. Single cell protein constitutes the bulk of research in value added processing of citrus waste. Typically, the protein content of single cell biomass can be increased five fold through fermentation processes. Hydrolytic enzymes, such as polygalacturonase, pectinesterase, cellulase, lyase, and xylanase not only represents a fermentation resource, but these enzymes are also responsible for increases in the amount of fermentable sugars. Soluble sugars can be used as substrate for other processes and can increase the yield of endogenous products not easily recovered from the cell structure.

Utilization of many agroindustrial wastes to produce value added products is receiving critical evaluation. The high content of carbohydrate relative to content of protein presents a challenge to develop higher value products economically. Currently, if fruit and vegetable processing waste cannot be dried or utilized fresh for cattle feed, it must be disposed at land fill sites. Disposal of fruit and vegetable processing waste then has a negative impact on the economic value as well as the environment.

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Citrus production accounts for nearly half of the total fruit and vegetable production. According to agricultural statistics, 13.8 and 15.2 million metric tons of citrus and non-citrus fruit, respectively, were produced in the U.S. in 1987/88; of this amount, 9.3 million metric tons were processed (1). If 50% of the weight is waste (2), then this equates to about 4.9 million metric tons of citrus waste in 1987/88. Thus, a large concentration of fruit and vegetable processing waste is localized within the citrus industry and provides an excellent model for production of value added products by further processing of fruit and vegetable wastes.

Citrus processing wastes are high in carbohydrate relative to the protein content. The proximate composition of dried lemon waste is 68% carbohydrate, 18.5% fiber, 7% protein, 3.6% ash, 3.5% lipid, and 0.3% soluble reducing sugars (3). The carbohydrate composition of Pineapple orange and Marsh grapefruit is given in Table I. The distribution between hemicellulose, cellulose, pectin, and lignin is 15%, 39%, 35%, and 12%, respectively, in Pineapple oranges. In Marsh grapefruit the distribution is 13%, 32%, 41%, and 14%, respectively (4). Juice vesicles and peel material not used as ingredients or processing aids in food products (5) are usually processed into cattle feed. Comminuted peel, juice sacs, segments, and seeds are mixed with lime, pressed to recover the "press liquor", dried, and pelleted.

Table I. Polysaccharide Composition in Pineapple Orange and March Grapefruit Residue[a]

| Fiber Fraction | g/100 g Fresh Component | |
|---|---|---|
| | Orange | Grapefruit |
| Hemicellulose | 6.6 | 5.6 |
| Cellulose | 17.6 | 13.7 |
| Pectin | 15.5 | 17.7 |
| Lignin | 5.2 | 6.1 |
| Total | 44.9 | 43.1 |

[a] Adapted from Braddock and Graumlich (4). Values reported are total of respective fraction in peel, juice sacs, segments and seeds.

Recent increases in energy costs have almost made drying of citrus waste cost ineffective. Utilization of fruit and vegetable processing waste material by microbial fermentation (6) for the production of other higher value products offers one solution to food and energy requirements. Fermentation by-products of microbial growth by submerged culture or solid state fermentation include vitamins, amino acids, hydrolytic enzymes, and single cell

protein. In addition to lowering disposal costs, enhancement of product value can be potentially realized.

Fermentation of citrus processing wastes without use of exogenous microorganisms or supplementation has been evaluated as a means to increase the nutritive quality of citrus waste for cattle feed. It is a low technology process with minimal process control. Ensilage of citrus peel and pulp waste was evaluated as a means to use citrus waste as ruminant feed (7). During ensilage, the authors reported a loss in dry matter weight of 40.6%. Soluble carbohydrates and gas accounted for 7.5% and 33.1%, respectively, of the loss in dry matter weight. In a subsequent study (8), the authors studied the loss of total sugar during ensilage and storage. The glucose content decreased from about 25.8% of the total sugar to less than 1% during ensilage. In the stored seepage, there was an initial decline followed by an increase in sugars to the initial level. The authors postulated that the increase in sugar content was due to hydrolysis of polysaccharide. Retention of the seepage within the ensilage during storage was suggested as a means to retain the nutritive value. The major fermentation products in ensilage of pre-dried shomouti peel were identified as ethanol (16%), lactic acid (3%), and acetic acid (3%) (9). The dominant microbial populations were lactobacilli and yeasts. It was postulated that the yeasts produced most of the ethanol and bacterial activity was inhibited by pH values less than 3.6. Selective inhibition of the yeasts could conceivably prevent seepage and nutritive losses.

Microorganisms that grow on citrus processing wastes and produce measurable quantities of by-products have been isolated from rotting lemons, decaying leaves, soil, infected vegetable tissue, orange peels, rice husks, partially rehydrated citrus pellets, or from personal libraries. Fermentation via exogenous microorganisms is most common with submerged, aerobic fermentations, although there are reports of solid state fermentations of citrus waste to recover by-products.

## Production of Single Cell Protein (SCP)

Submerged Culture. The production of single cell protein (SCP) has received the bulk of attention for recovery of value added products. SCP production is vastly influenced by the initial substrate concentration and composition of soluble sugars. The efficiency of acid vs. enzymatic hydrolysis of Mandarin orange peel to enhance production of reducing sugars as substrate for SCP was evaluated and it was observed that enzymatic hydrolysis resulted in more efficient release of reducing sugars (10). Enzymatic hydrolysis was also determined to result in more effective release of soluble solids into the press liquor of citrus waste than did conventional liming treatment (11). Moreover, the sugar content of the press liquor hydrolysates differed markedly. Virtually no arabinose or xylose is solubilized by lime treatment of peel, whereas 59.7 and 9.2 mg/g dry weight press liquor, respectively, were released following treatment with commercial pectinase and cellulase. About three times the amount of fructose and glucose were detected in peel extracts by enzyme treatment but the

difference in galactose and galacturonic acid content were not as great as from the lime treatment (11).

The utilization efficiency and growth yield from reducing sugars was evaluated for Saccharomyces, Candida, Debaryomyces, and Rhodatorula. Of these, Rhodatorula was better for SCP production but sugar utilization efficiency was better in the Debaryomyces (12). The growth parameters of Memmononella echinata and Fusarium rosuerm of lyophilized orange peel powder (LOPP) in a slurry system were compared with those of other organisms grown on different substrates. Clementi et al. (12) reported that the apparent specific growth rates appeared to depend on the initial substrate concentration for LOPP. The specific growth rate was obtained from a plot of protein dry weight vs. time. Values of specific growth rate for orange peel were similar to those of other substrates evaluated such as acid and alkali treated sawdust and avicel. Bioreactor design for laboratory scale production (10L) of SCP from orange peel by Fusarium avenaceum was optimized to achieve maximum protein production in less than eight hours (13).

Enhancement of substrate utilization and SCP production may occur from concerted utilization of fermentation by-products. If Aspergillus niger were introduced 24 hours prior to inoculation with Sporotrichum pulverulentum, then nearly 35% higher protein yields were obtained (3). If introduced simultaneously, the authors speculated that accumulation of glucose and cellobiose inhibited cellulase activity by S. pulverulentum and decreased protein yields. Although the A. niger has a weak cellulase system, it has high pectinase activity with some β-glucosidase activity. Utilization of glucose and cellobiose by A. niger minimizes the inhibition of the cellulase activity. Under optimized conditions and in mixed culture, the crude protein content of the produced biomass was increased from 7% to 36%.

Sufficient nutrient supplementation and fermentation control is also critical to SCP production. In submerged fermentation of Sporotrichum pulverulentum in single culture on dried Valencia orange peel, biomass containing nearly 32% protein was produced (14). The composition of the media and cultivation conditions were similar except that inorganic nitrogen as ammonium sulfate and phosphate as dibasic potassium phosphate, at 2 g/L each, were twice as high in the fermentation in single culture (14) than in mixed culture (3). Whereas, the source of inorganic nitrogen did not affect large differences in the crude protein production, pH values less than 5.0 had an adverse effect (14). Although the initial pH of the mixed culture fermentation (3) was adjusted to pH 4.6, the pH decreased to 3.2. The combination of mixed culture to minimize inhibition of the S. pulverulentum cellulase system, control of pH near 5.0, and sufficient mineral supplementation could result in higher SCP yields. However, single culture offers simpler process control and determination of the optimum stage of growth of the first culture before introduction of the second culture is not necessary. Furthermore, S. pulverulentum is thermotolerant and a doubling of protein production was observed with a 10°C increase in incubation temperature (14).

Fermentation of the press juice of Citrus unshuii Marcovitch by 125 strains of yeasts was conducted (15). Cell growth was near

35 g/L and a crude protein content was near 45% at three days fermentation for two species of Candida and one of Saccharomyces. The highest yield of cells and crude protein content was obtained at an optimized ratio of: peel dry matter (50):urea (5): dibasic potassium phosphate (1). Growth of a Fusarium isolate on dried citrus peel in slurry fermentation at 50 g/L yielded 38 g/L in 5 L fermentors (16). Further, the biomass was approximately 25 g/L protein. Characterization of the amino acid profile indicated high quality protein with methionine as the limiting amino acid. High biomass yields were reported when dried sweet orange residue, supplemented with $NaHO_3$, $KH_2PO_4$, $MgSO_4$, $FeCl_2$, and $(NH_4)_2SO_4$, was fermented for 12 hours with *Saccharomyces cerevisiae* (17). A biomass yield of 31.6% and a crude protein content of 57.6% was reported.

*Solid State Fermentation*. Solid state fermentation of citrus waste is less common and requires aeration for practical application. In fermentation of orange peel by *Agrocybe aegerita*, an increase in total nitrogen was observed only after 84 days; if orange peel were inoculated with *Armillariella mellea*, an increase was observed after 70 days (18). A more realistic fermentation time of two days was reported using orange peel inoculated with *Candida utilis* (19) and *Aspergillus niger* (20). Using comminuted orange peel supplemented with mineral salts and a tumbling device, an increase was observed in protein content of the dry matter from 7.3% to 18.5% (19). Stationary fermentation of orange peel in trays to increase surface area with *Aspergillus niger* resulted in a similar increase in the protein content of dry matter (20). Solid culture of an *Aspergillus niger* resulted in the production of 4.9 mg/mL of extracellular protein on orange peel, which was about three times that grown on wheat bran (1.7 mg/mL) (21).

## Recovery of Organic Acids and Vitamins

Bioconversion of high value products such as vitamins, organic, and amino acids has been most successfully exploited by the pharmaceutical industry. The use of citrus wastes as a substrate for fermentation of these types of products has recently received attention. Successful application depends on removal of nonspecific inhibitory agents from the peel, press residue, and distillation residue by ion exchange or by physical separation.

*Organic Acids*. The ability of 33 strains of yeast to grow and produce pyruvic acid using dried citrus peel as a substrate was evaluated (22). *Debaryomyces coudertii* IFO 1381 and *Candida utilis* IFO 0396 produced yields of pyruvic acid near 82 mg/100 mL after 24 hours of fermentation at 30°C. Fermentation conditions were optimized with respect to nitrogen source (0.5% ammonium sulfate, dibasic phosphate concentration (0.1%), yeast extract concentration (0.01%), calcium carbonate concentration (1.0%), and magnesium sulfate concentration (0.01%). Pretreatment of citrus peel powder with the sodium form of Amberlite IR-120B was observed to enhance pyruvic acid production. Under optimized conditions, a maximum of

970 mg pyruvic acid/100 mL could be produced with D. coudertii in 48 hours of fermentation (22).

The use of the residue following liming and pressing of peel has also been evaluated. Citrus molasses was not a suitable substrate for citric acid production by Aspergillus niger (NRRL 599) (23). The authors speculated that excessive levels of cations such as copper and iron inhibited citric acid synthesis since ion exchange treatment improved production. A procedure was described to prepare citrus molasses as a substrate for glutamic acid production by commercially used fermentation methods (24). After liming and pH adjustment to 6.0-7.0, press juice was briefly heated at 90°C and the sediment removed by centrifugation. The treated press juice was lower in calcium and other inhibitory agents and could be more easily concentrated to increase the sugar content (Table II).

Vitamins. Fermentation of citrus molasses by Eremothecuim ashbyii (NRRL 1363) produced nearly 720 μ grams/mL riboflavin with 7-9 days of fermentation under optimal conditions (23). Parameters critical to optimal riboflavin production were identified as removal of inhibitory agents from concentrated press liquor by filtration, pH control in a narrow range of 7-8, and adequate levels of reducing sugars.

Distillation residue from recovery of orange oil has been used as substrate for production of cobalamin (Vitamin $B_{12}$) by Propionibacterium freudenreichii and Propionibacterium shermanii fermentation (25). By filtration of the distillation residue, pH adjustment to 7.0 with NaOH, and use of a two-stage, anaerobic to aerobic fermentation, nearly 6.35 mg/L of cobalamin were produced in 192 hours of fermentation (Table II).

## Recovery of Endogenous Products

Limonene. Use of fungal cultures which produce hydrolytic enzymes were used to increase the yield of endogenous products in citrus. Enzymatic digestion of plant cell wall structures or other sub-cellular structures enhances solubilization of these by-products. In an attempt to increase the yield of limonene, citrus peel was soaked in fungal cultures of Neurospora crassa and Aspergillus terrius for 2.5 hours (26). The fungal culture filtrates were particularly rich in xylanase and amylase, but also contain significant amounts of polygalacturonase and cellulases. The yield of essential oil recovered by steam distillation was increased about 54% from 1.3 to 2.0 mL/100 g of orange peel in the fungal treated samples than in the control.

Pectin. Commercial extraction of pectin from citrus involves treatment of citrus waste at pH extremes with concentrated acids. In addition to the harsh extraction conditions, not all citrus waste is amenable to conventional pectin extraction. Mandarin orange peel becomes jelly-like on heating and separation of pectin from the residue is difficult (27). Milder extraction conditions and more effective extraction has been reported if mandarin citrus peel was suspended in water with Trichosporon pencillatum for 15 to

Table II. Production of Value Added Products by Microbial Fermentation of Citrus Processing Wastes[a]

| Microorganism | Substrate | Product | Quantity | Ref. |
|---|---|---|---|---|
| *Debaryomyces coudertii* | Dried peel[b] | Pyruvic acid | 970 mg/100 mL | 22 |
| *Propionibacteurium shermanii* ATCC 13673 | Filtered distillation residue[b] | Vitamin $B_{12}$ | 6.35 mg/L | 25 |
| *Eremothecium ashbyii* NRRL 1363 | Filtered distillation residue[b] | Riboflavin | 700 mg/L | 23 |
| *Aspergillus terreus* *Neurospora crassa* | Comminuted orange peel[c] | Limonene | 20 mg/kg | 26 |
| *Trichosporon penicillatum* SNO-3 | Comminuted orange peel[c] | Pectin | 20-25 g/kg | 27 |
| *Aspergillus niger* | Dried orange[b] | Soluble sugars | + | 21 |

[a] The quantity of product is reported if given by authors. Qualitative presentation of data is denoted by "+".
[b] Submerged culture fermentation.
[c] Solid substrate fermentation.

20 hours at 30°C (27). Yields of 20 to 25 g pectin/kg of peel were reported and represents about 100 to 110% of the yield obtained by the conventional method. The pectin extracted by this method had similar chemical and physical properties as commercial pectin and pectin extracted from Mandarin orange by acid. One notable difference was the neutral sugar content of pectin extracted by fermentation was twice that extracted by acid and four times the amount detected in commercial pectin. Although the molecular weight of the pectin extracted after fermentation was nearly the same as that determined for the commercial pectin, the molecular weight of acid extracted pectin from Mandarin orange was nearly half that extracted after fermentation.

Soluble Solids. The hydrolysis of fruit and vegetable processing waste is the result of a concerted action of multi-enzyme systems. The commercial enzymes typically are not standardized with respect to secondary enzymatic activities, which may vastly affect the rate and extent of hydrolysis. Since a spectrum of polysaccharide degrading enzymes is required for the bioconversion of fruit and vegetable processing waste, single or mixed cultures of micro-organisms grown on the substrate to be hydrolyzed may produce the most effective primary and secondary hydrolytic enzyme system.

An *Aspergillus niger* fungal culture was screened for the ability to produce enzymes which would macerate mandarin orange peel after growth on wheat bran or mandarin orange peel in submerged or solid state culture. Although no attempt was made to recover hydrolytic enzymes, activity was monitored by evaluation of the maceration activity and the increase in reducing sugars (21) (Table II). The production of reducing sugars and total sugars in either liquid or solid culture on orange peel was about half that grown on wheat bran. Correspondingly, the macerating value (ratio of final mass to initial mass) was less when Aspergillus was grown on orange peel. The macerating value of the enzymes produced on wheat bran were comparable to commercial fungal preparations.

## Recovery of Hydrolytic Enzymes

A procedure to produce pectinases from lemon peel by solid state fermentation with an Aspergillus species was described (28). Comparison for efficiency of clarification of the culture filtrate prepared by the authors with a commercial preparation showed that the two preparations were similar. Optimum results for polygalacturonase and pectinesterase activity were observed when the lemon peel was pretreated by careful drying at 100°C. Negative effects of temperature were observed when the peels were dried at a final temperature of 120°C. Washed peel had a negative impact on pectinesterase activity but no effect on polygalacturonase activity. The authors speculated that washing removed some co-factor essential for pectinesterase but not polygalacturonase synthesis. However, it has been shown that removal of water soluble carbohydrates from the peel by commercial leaching also removes approximately half of the endogenous pectinesterase (4). Unless specific steps were taken to distinguish fungal or plant pectinesterase, the decrease in pectinesterase activity observed by the authors could be due to

a leaching effect of endogenous pectinesterase. In addition, temperatures at 120°C may have partially denatured some pectic enzyme activity (Table III).

An Aspergillus species, previously isolated from wetted citrus pellets, was grown on untreated Valencia finisher pulp (29) (Table III). In inoculated pulp, protease activity, determined by a zone of diffusion on casein-agarose plate, was observed after 24 hours which was sustained for 72 hours. In inoculated samples, polygalacturonase activity was optimal at 24 hours if the pulp was supplemented with 1% exogenous citrus pectin. In the absence of exogenous pectin, optimal polygalacturonase activity was observed between 72 and 96 hours. It was noted that optimal polygalacturonase activity preceded the onset of liquefaction and syneresis by about 24 hours.

The ability of two strains of *Lachnospira multiparus* to grow on polygalacturonic acid, 36.8% esterified apple pectin, or 73.4% esterified citrus pectin and produce pectinesterase, polygalacturonase, and pectin lyase was evaluated (30) (Table III). The author noted that whereas low methoxyl pectin and polygalacturonic acid are the preferred substrates for polygalacturonase activity, no polygalacturonase production was detected when *L. multiparus* was grown on any of the three substrates. Pectin lyase and pectinesterase activities were both detected on all three carbon sources, although the lyase activity was low on the polygalacturonic acid. Since the nature of the pectin substrate had no effect upon production of pectinesterase, Silley speculated that pectinesterase is a constitutive enzyme and that the esterase and lyase could exist as a single inducible complex.

An *Aspergillus niger*, identified as *A. niger* 35-1 was selected for evaluation for production of hydrolytic enzymes with mandarin orange peel (31) (Table III). Enzyme activities for pectinesterase, amylase, xylanase, and carboxymethylcellulase were low for all strains evaluated, whereas polygalacturonase activity was relatively high in several strains, 35-1 was selected as one of the best polygalacturonase producers. The polygalacturonase was partially characterized. Optimal polygalacturonase activity was observed at pH values between 2.5 to 4.0 and was 95% inactivated at pH 6.5. The optimal growth temperature was determined to be 55°C with an estimated 50% activity loss at temperatures near 70°C.

Production of polygalacturonase was optimized with respect to concentration of mandarin orange peel and nitrogen source and concentration. Concentrations of peel higher than 5% were inhibitory and ammonium sulfate at 0.7% was optimum for polygalacturonase production. However, production of xylanase was optimal with sodium nitrate as the nitrogen source. The reduction in reducing and total sugars and the increase in mycelial growth coincided with an increase in polygalacturonase and xylanase activity. Interestingly, the authors noted that although the optimal concentrations of peel and ammonium sulfate coincide with an increase in polygalacturonase activity, there is not a corre-

Table III. Production of Polysaccharide Degrading Enzymes by Microbial Fermentation of Citrus Processing Wastes[a]

| Microorganism | Substrate | Product | Quantity | Ref. |
|---|---|---|---|---|
| Aspergillus sp. | Dried and undried peel[b] | PME<br>PG | +<br>+ | 28 |
| Aspergillus sp. | Valencia juice sacs[b] | Protease<br>PG | +<br>+ | 29 |
| Lachnospira multiparus D15d | HM citrus pectin[c] | PME[d]<br>PG[d]<br>PL[d] | 1.23 U/mL<br>0<br>18.49 U/mL | 30 |
| 2389 | | PME[d]<br>PG[d]<br>PL[d] | 2.16 U/mL<br>0<br>8.58 U/mL | 30 |
| Aspergillus niger 35-1 | Dried Mandarin orange peel[c] | PME[e]<br>PG[e]<br>CMC'ase[e]<br>Xylanase[e]<br>Amylase[e] | 0.26 U/mL<br>2.15 U/mL<br>0.06 U/mL<br>0.58 U/mL<br>0.13 U/mL | 31 |
| Aspergillus niger 35-1 | Dried Mandarin orange peel[c] | Endo-PGI[e]<br>Endo-PGII[e]<br>Exo-PG[e] | +<br>+<br>+ | 32 |
| Rhizopus oryzae ATCC 24563 | Valencia juice sacs[b] | PG[f]<br>CMC'ase[f] | 0.5 U/mL<br>0 | 32 |

[a] Qualitative presentation of data is denoted by "+".
[b] Solid substrate fermentation.
[c] Submerged culture fermentation.
[d] Extra- and intra-cellular activity. Units defined as: 1U PME = 1 μequiv. ester hydrolyzed/min/mL, pH 7.0, 30°C; 1U PG = 1 μmol reducing sugar/hr, pH 5.2, 25°C; 1U PL = 0.01 Δabs/min/mL, pH 8.5, 25°C.
[e] Units defined as: 1U PME = 1 μmol methanol/min/mL, pH 4.0, 40°C; 1U PG, CMCase, xylanase or amylase = 1 μmol reducing sugar/min/mL, pH 4.0, 40°C with 0.2% pectic acid, xylan or starch, respectively.
[f] Units defined as: 1U PG = 1 μmol GA/min/mL, pH 4.5, 37°C; 1U CMCase = 1 μmol glc/min/mL.

sponding increase in maceration of the tissue. Optimal maceration coincided more with the xylanase activity. It is conceivable that the polygalacturonase is produced in excess and that xylanase activity is limiting and is the enzyme primarily responsible for initiation of maceration.

In a subsequent paper (32), the polygalacturonase was further purified and determined to consist of an exo-PG, endo-PG I, and endo-PG II. The molecular weights and pI values of endo-PG I and endo-PG II were 46,000 and 35,000 and 5.6 and 6.5, respectively. Although the pH optimum for the endo-PG enzymes were near 4.5, the pH stability of endo-PG II was greater at pH values near 2.5 to 4.0. Endo-PG I was more stable in the pH range of 4.0-5.5.

Six ATCC cultures were screened for growth and production of cellulase and polygalacturonase activity on heat stabilized citrus finisher pulp (33) (Table III). No cellulase activity was detected in extracts prepared from the fermentation culture of any of the ATCC cultures. Aspergillus and Rhizopus appeared to be the better polygalacturonase producers. The absence of cellulase and detection of polygalacturonase production on lyophilized orange peel by Fusarium cultures has also been reported (13). The authors suggested that the cellulosic fraction of citrus waste will not be utilized until the pectin carbon source has been exhausted. The absence of detectable cellulase activity may also be related to the low pH during fermentation (34). Whereas production of pectinase activity was not related to initial microbial inoculum or cell growth, pectinase activity was preceded by the generation of reducing sugars. During the first 12 hours of fermentation, virtually no change was observed in pectinase activity. During the next 36 hours, *Rhizopus oryzae* (ATCC 24563) produced substantially higher quantities of pectinase with a maximum of 0.4 to 0.5 $\mu$mol GA/min/mL at 48 hours followed by a subsequent decline in activity at longer fermentation times. On a per gram basis, the Rhizopus produced approximately 2.5 U pectinase activity/g pulp on unsupplemented Valencia pulp.

Much of the fruit juice and beverage industries rely on the use of pectolytic enzymes to increase juice yields, to aide in clarification, and concentration. The unsubstituted regions of pectins are less esterified (~ 70%) and are more readily degraded than the highly branched, highly esterified segments, neutral sugar rich, segments along the rhamnogalacturon backbone of pectin (35). The degree of hydrolysis and type and amount of sugars released depends on the primary and secondary enzymatic activities. Incomplete hydrolysis may be related to the limiting amounts of secondary enzymatic activities, which are more active towards the highly branched regions of pectins, or pectinases which are most active on highly esterified pectin (36). Production and utilization of hydrolytic enzyme systems on the substrate to be degraded could conceivably result in more complete liquefaction of previously underutilized polysaccharide material and production of value added products.

In Quality Factors of Fruits and Vegetables; Jen, J.;
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Chapter 30

# Applications of Biotechnology to the Improvement of Quality of Fruits and Vegetables

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The integration of emerging biotechnologies with conventional breeding will greatly facilitate the modification of quality or "value-added" attributes of fruits and vegetables, (i.e. appearance, organoleptic, nutrition, physiological benefit and safety). Modern plant genetics will make possible the specific control of enzymes contributing to the development and deterioration of produce quality or the accumulation of secondary metabolites which provide added-value to a specific crop. However, to fully exploit this potential, an understanding of the biochemical and genetic control of these value-added attributes is necessary. Following is a brief discussion of the application of biotechnology to the improvement of the quality of fruits and vegetables.

Plant breeding has played a dominant role in providing the diversity and quality of fruits and vegetables enjoyed by the consumer and processor today, and will continue to make improvements related to production, enhancing such agronomic traits as pest resistance, yield, and stress tolerance. These production contributions by "supply-side" genetics affect the availability and cost of raw materials, whereas, "utilization" or "value-added" genetics determine the processibility, nutrition, overall quality, and functionality of the raw materials if subjected to processing. Traditionally, the food industry has used available raw materials as commodities and built in "added-value" through processing and packaging. The food industry is now requesting improved functionality of raw materials in the form of nutritional and physiological benefit, chemical and physical properties, and

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improved preservation; the consumer is demanding convenience, premium quality, year-round availability, and the absence of pesticides. Concomitantly, scientists and consumers are developing a better understanding and awareness of the nutritional, physiological, and even medicinal benefits of the different components of fruits and vegetables.

Produce quality is defined or referenced in several different ways depending upon what portion of the food production and distribution chain is being referred to, i.e. harvest, marketing, shipping, storage, or eating. These definitions can be summarized into five major quality factors: (1) appearance, (2) flavor, (3) nutrition and well being, (4) texture, and (5) safety, which are influenced by such preharvest and postharvest factors as: (1) genotype, (2) cultural and climatic conditions, (3) harvesting practices, including the physiological state of the crop, (4) postharvest treatment, and (5) the various interactions among these factors. Although substantial progress has been made in the quality improvement of fruits and vegetables which reach the consumer, emerging biotechnologies will greatly broaden the scope of products targeted by plant breeding. Modern cellular and molecular genetic approaches will provide new strategies to improve those value-added characteristics mentioned. However, the realization of this potential is greatly dependent on progress in the understanding of functional traits at the biochemical and genetic levels. Thus, the following perspective is based on a limited number of examples of realized applications, work in progress, and speculation.

## EMERGING BIOTECHNOLOGIES AND CROP IMPROVEMENT

This section will provide a very brief review of the biotechnological state of the art as of this writing. Any present treatment is rendered obsolete by rapid advances. The interested reader is urged to consult one or more excellent recent volumes on this subject (1-2).

Biotechnology consists of a body of interrelated methods wherein sub-organismal units, e.g. organs, tissues, cells, organelles, chromosomes, genes, etc, are manipulated. The field of plant cell and tissue culture has its origin in the early 20th century (3). The finding by Skoog and Miller (4) that developmental changes in cultured tissues could be influenced by phytohormones initiated an era of rapid advances: in vitro meristem propagation for virus elimination, micropropagation, protoplast isolation, culture, and regeneration, microspore culture, and transformation with T-DNA vectors via infection with the crown gall pathogen (*Agrobacterium tumefaciens*). Most of the methods of plant cell and tissue culture were derived empirically. In fact, we still understand very little of the mechanisms which underlie developmental transformation. Experiments in the early to mid 1970's quickly established that plant species and even genotypes within species varied tremendously in the relative ease with which they could be cultured and regenerated. For example, the genotypes A188 and Black Mexican Sweet of maize would regenerate while other genotypes were recalcitrant (5-6). Until recently, regeneration from cell and tissue culture of certain important crops such as cotton (7) and soybean (8) was only demonstrated in closely related

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wild species. Persistence in exploring new empirical approaches has been largely successful in overcoming recalcitrance. However, while it is clear that predisposition for cell proliferation in vitro and regeneration has a genetic component, no absolute barriers exist in the development of successful protocols.

Numerous successful applications of cell culture technologies to crop improvement have now been reported. Most notable among these are protoplast-mediated alloplasmic conversion, doubled haploids, somaclonal variation, and in vitro mutant selection. The doubled haploid approach (9) is universally lauded by plant breeders, because the possibility of proceeding directly from a hybrid to an inbred harboring fixed recombinant gene assortments eliminates the need for inbreeding. Unfortunately, gaps in our knowledge and capabilities in gametophyte development are numerous. Culture and regeneration of microspores or megaspores is successful in only a handful of plant species (e.g. Nicotiana, Brassica, Datura, Oryza, Capsicum) and the list is not growing very quickly (9). In certain rare cases, natural or genetically mediated processes during gametogenesis give rise to progeny derived from the egg only, thus imparting the same end result as anther culture (10). Also, in barley, it is possible to recover haploid plants by hybridizing with the wild relative Hordeum bulbosum (11).

Alloplasmic conversion is a strategy which emerged from capabilities developed with plant protoplasts. Originally touted as a method for the recovery of exotic interspecific hybrids (12), protoplast fusion has proven to be an excellent way to approach the wholesale manipulation of mitochondrial genes (13). The predominant trait of note is cytoplasmic male sterility (cms), which is of interest to the seed industry as a tool to facilitate hybrid seed production. Little or no impact on possible value-added traits impacting on food quality, however, is anticipated from alloplasmic conversion.

Somaclonal variation in regenerated plants has been observed and reported since cell culture technology was very young (14). Early reports of such variation were dismissed as exceptional or artifactural because the notion conflicted with the widely accepted view of genetic fidelity in both eukaryotic and prokaryotic cell cultures. Larkin and Scowcroft (15) were noteworthy in first suggesting that genetic instability is an intrinsic feature of plant cell cultures. Mounting evidence has much supported this view.

The original Larkin and Scowcroft paper also espoused the exploitation of 'somaclonal variation' in crop improvement. It has been argued that using tissue culture to induce mutations is operationally and perhaps even mechanistically equivalent to mutagenesis via chemicals or ionizing radiation. A small number of papers have recently appeared, however, which demonstrate that the frequency and spectrum of mutants generated via mutagens vs. cell culture are indeed different (16).

The application of somaclonal variation to crop improvement is attractive because it is cheap, simple and requires few assumptions as compared to more directed approaches (17). The glaring disadvantage of the phenomenon is that it is not qualitatively controllable. If one possesses a line which is genetically superior in all respect with one or few deficiencies, somaclonal variation can theoretically be induced to mitigate the deficiencies while

leaving the desirable fraction of the genotype undisturbed (17). There are, unfortunately, few concrete examples where this has been accomplished convincingly.

Cell selection is a process wherein medium composition or physical environment are engineered to differentiate the growth rate of mutant vs. non-mutant cultured cells. This is attractive because the probability of success is much enhanced as compared with screening large populations of random somaclones. Moreover, searching for mutants is much more efficient among populations of cultured cells than for whole plants. Numerous examples of the successful application of this approach exist in the literature and within commerce (18). The strategy is most successfully applied when information exists regarding the biochemical pathways which culminate in compounds of interest. For example, tryptophan auxotrophs can be selected by exposing cells to 5-methyltryptophan (19). Exposing normal cells to one amino acid involved in a branch synthetic pathway is inhibitory because the control enzyme is inhibited resulting in deficiencies. Cells containing mutants which render the enzyme insensitive to feedback inhibition can grow under these conditions. Such cells should also accumulate free amino acids by virtue of acquired feedback insensitivity.

Direct pertinence of molecular approaches to crop improvement was first demonstrated by Fraley et al. (20), who successfully used T-DNA of Agrobacterium tumefaciens to introduce foreign DNA into a plant in a permanent and heritable fashion. Subsequently, methods have been devised for the direct introduction of foreign DNA sequences (21-24) into the genomes of higher plants. At this juncture, it would appear reasonable to conclude that no theoretical barriers exist to the stable integration and expression of foreign DNA sequences or transformation into higher plants.

Until recently, major issues have imparted limitations which much constrain the widespread utility of higher plant transformation. First, how can genes which condition a desirable genetic outcome be captured? Secondly, conventional thought holds that most economically important characters are conditioned by many genes which interact in a spacial and developmental matrix. Moreover, many traits whose inheritance is simple are either codominant or recessive.

New techniques have now been forged which mitigate these limitations. Progress in the isolation and capture of desirable genes of economic importance has been much facilitated by transposon mutagenesis (25). Until recently isolated genes of known function were relatively rare because properties of polypeptide, mRNA, or nuclear sequence abundance were necessary. High abundance of genes or gene products makes it possible to isolate desired DNA sequences directly from libraries or to work backward enzymatically from mRNA to isolate clones from a random genomic library. If the polypeptide which conditions a desired trait has been isolated and sequenced, it is also possible to deduce the nucleotide sequence of an oligomer which could encode a part of the protein. The oligomer can then be used to locate the genomic sequence based on binding affinity.

Most aspects of phenotype are, however, encoded by a gene or genes whose products are not relatively abundant, and it is not generally possible to ascertain the contribution of isolated polypeptides and mRNAs based on sequence alone. An exception to

this is for genes which are highly conserved over long periods of evolutionary history. Thus, if one obtains a gene from a prokaryote which encodes an enzyme in a fundamental biochemical pathway, a possibility exists that sufficient homology remains within the corresponding gene of higher eukaryotes to permit isolation by binding affinity (26).

New methods of gene isolation, which take advantage of natural polymorphisms and genetic transformation capabilities, have now been developed which transcend limitations imposed by the aforementioned techniques. If a plant cell heterozygous Aa (respectively active and inactive alleles) at a given locus is transformed, the sequence or multiple copies thereof integrate into the host nuclear (and organelle) genome presumably at random. If the sequence integrates in or near the active allele, it will be turned off, thus changing the phenotype of cells or plants from dominant A to recessive a. The transforming sequence can then be used to locate this gene from a mixture of random DNA fragments. Since plant genomes are very large, and the likelihood of desired events low, transformation cassettes containing known transposable elements, such as AC (maize) and Tam-1 (*Anthirrhinum*) have been employed to improve such probabilities (27). Examples of traits which have thusfar been recovered by this approach include nuclear male sterility, altered seed fatty acid composition, and corolla pigmentation. Recently, it has been demonstrated that the T-DNA of *A. tumefaciens* can affect the insertional inactivation of genes by random integration.

Implicit in any molecular transformation approach to crop improvement is the need to modulate the expression of the foreign gene. It has been well documented that DNA sequences upstream from the 5' end of coding genes, or promoter, are necessary for normal transcription. While some promoters confer constitutive transcription of the corresponding coding sequence, most appear to respond to a stimulus such that they are only expressed at the proper place and time. Examples of such promoters include developmental, tissue-specific, and signal mediated (28). It has been demonstrated that polypeptides are produced following environmental or development stimuli which bind specifically to 5' sequences and permit transcription to initiate and proceed. At least some promoters have been shown to function similarly in divergent plant species (29). The fact that most economic traits are inherited in a recessive or complex fashion is a much more daunting problem than finding simply inherited genes. Recessive traits appear to be at least partially approachable by transformation of targeted gene loci with sequences which encode an mRNA complementary to that of the native coding sequence, or "antisense RNA" (30). Antisense RNAs theoretically inactivate sense mRNAs by forming duplex structures which interfere with normal translation. Many processes can be postulated, however, which might antagonize the desired result. More evidence on the efficacy of the antisense approach to recessively inherited traits will be necessary before any judgement regarding effectiveness is possible.

Recent findings about traits exhibiting complex inheritance are very encouraging from the standpoint of accessibility to molecular approaches. A general assumption in the field of quantitive genetics is that traits are conditioned by a large number of genes with equal, additive effects. Cosegregation experiments involving

restriction fragment length polymorphisms (RFLPs) suggest that complex traits such as yield and solids content are controlled by genes with different quantitative effects (31). In fact, most of the phenotypic variation may be accounted for by fewer than five distinct genes. If this is the case, and if one was able to isolate desirable alleles of genes exerting major effects on quantitative traits, then transformation would appear quite possible for the manipulation of such traits (32).

As mentioned above, RFLPs have been very useful for dissecting the genetic components of very complex characters. RFLPs are differences in base sequence resulting in the addition or deletion of restriction enzyme recognition and cutting sites, or other changes which affect sequence length between two restriction sites. Mutations of this type apparently occur relatively frequently in that they are abundant even among some closely related genotypes (31). Since they occur randomly within the genome and are physiologically silent, many can be detected simultaneously within a single organism, thus facilitating studies of linkage and attempts to derive genetic "fingerprints" for proprietary protection.

Commercializing these biotechnological strategies will require a careful integration with plant breeding, postharvest physiology, food science, and packaging. Transformation, alloplasmic conversion, somaclonal variation, and mutant selection are all powerful techniques to broaden and/or manipulate germplasm. RFLPs and dihaploids are methods to assist the breeder in reducing the time and effort necessary to achieve desired recombinants. A thorough understanding of the product targets is essential to realization of the full economic potential of biotechnological strategies.

## VALUE-ADDED GENETICS

Although the improvement of functional properties can be greatly enhanced by complementation of conventional breeding with the new technologies, progress is still limited by the lack of knowledge regarding the biochemical control of specific attributes and the fact that many such traits are multigenic, which greatly complicates improvement strategies. Even with these complications, however, there will be increased emphasis on the development of raw materials genetically designed for the processor and consumer.

A few examples of fruits and vegetables can be cited, where focus has been on the improvement of composition or functional properties. A fairly thorough understanding of the biochemical and genetic control of carbohydrate metabolism in sweet corn has allowed considerable progress in the development of genotypes with an array of sweetness, texture, color, and rates of sugar to starch conversion (33). In spite of poor seedling vigor, differentiated sweet corn varieties, based on the sh2 gene (endosperm gene shrunken-2), are being grown commercially. Over 90 percent of the sweet corn produced in Florida is sh2 hybrids.

Increasing the solids content of tomato is a demonstration of the modification of multigenic traits by modern biotechnology. Although various institutions have utilized several approaches, only somaclonal variation has proven successful, with DNA Plant

Technology Corporation (DNAP) receiving a PVPA certificate (Certificate of Plant Variety Protection No. 8400146) for DNAP-9. DNAP-9, a derivative of UC82B, a standard open pollinated processing variety, was obtained through the regeneration of plants from tissue culture. Data indicated that the solids increase of approximately 20% was the only principal difference from the parental material. Although DNAP-9 did demonstrate the utility of a somaclonal variation approach, it has not proven to be competitive with recently developed hybrids with higher yields and comparable solids content.

The most effective means of leveraging biotechnology for the quality improvement of fruits and vegetables is to complement the traditional sciences. To provide unique high quality fruit and vegetable products will require synergies provided by optimizing the raw material, and the cultural and environmental conditions for production, processing, packaging, and distribution conditions. The Vidalia onion is an example of the combination of genotype and soil properties that provides a unique product. Varieties of some crops such as apples respond differently to controlled and modified atmosphere. The maturity of fruits and vegetables is also a factor in determining their response or susceptibility to preservation environments. This suggests that for increased shelf life, new varieties may be selected for biochemical modifications in their response or resistance to specific environments. An example is VegiSnax[R] vegetable sticks, which DNAP and DuPont, Agricultural Products Division will market. This product is based on optimizing the quality of the raw celery and carrots through variety improvement, then packaging in a modified atmosphere to provide a high quality product with a shelf life of 30 days under refrigerated distribution.

## NEW GENOTYPES WITH UNIQUE PROPERTIES WHICH ENHANCE THE QUALITY OF FRESH PRODUCE

Because of increasing public concern regarding pesticide application and pesticide residues, insect and disease resistance, traditionally considered as part of the "supply side" genetics, may now be accounted for on the "value-added" genetics column. Because of its economic importance, tomato is the major vegetable crop which biotechnology and agricultural products firms have targeted to further improve resistance to insects and pathogens. This has resulted in recent field studies with genetically engineered tomato plants exhibiting resistance to TMV and lepidopteran insects.

Postharvest losses due to the presence of plant pathogens are significant. Recent reports (34) regarding the isolation of a chitinase gene, suggests the possibility of transforming plants with genes encoding for fungal cell wall degrading enzymes.

Health and Well Being. Fruits and vegetables have always had a strong relationship to human health and nutrition. Such benefits as dietary fiber and several essential nutrients are contributed with minimal caloric contribution and no cholesterol.

Recently, because of the established physiological benefit of dietary and soluble fiber (i.e. the reduction of cholesterol), fruits and vegetables have taken on additional significance as one

In Quality Factors of Fruits and Vegetables; Jen, J.;
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of the major sources of dietary fiber. There has also been some interest in the potential of horticultural crops as possible sources of Omega-3 fatty acids associated with reduced incidence of heart disease, (35). Vegetables, although low in fat, are a source of alpha-linolenic acid, the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Linolenic acid predominates in the chloroplasts of green plants while seeds are generally richer in linoleic acid, precursor to archidonic acid. Currently there are no studies directed toward the evaluation of breeding green vegetables to enhance Omega-3 fatty acid content.

There is also interest in crops for anticarcinogenic compounds. Consumption of cruciferous vegetables has been associated with a reduction in the incidence of some specific site cancers, i.e. colon and stomach (36); and their increased consumption has been endorsed by The American Cancer Society (37). Epidemiological studies have suggested that higher intake of carotene may reduce the risk of cancer (38). For example, carrots (Daucus carota) are thought to be the most important source of pro-vitamin A carotenes, and at the same time are a source of high quality fiber. Strawberries contain ellagic acid, a substance speculated to protect normal cells from becoming cancerous.

Traditionally, antiviral, antithrombotic, and diuretic, benefits have been associated with specific metabolites in a range of spices. Allium species, for example, have a long history and folklore pertaining to their ability to inhibit specific diseases. Other herbs have been associated with a diversity of remedies, generally attributed to secondary metabolites. Quantitative changes in secondary products produced by plant cells are quite responsive to modification by plant cell culture. This often results in the regeneration of plants exhibiting abnormally high concentrations (39). This suggests that components exhibiting such benefits could be induced to accumulate at higher concentrations in new varieties.

Breeding programs are slow, expensive, and very time consuming, even with the complementation of modern biotechnology. However, rapid analytical screening procedures, nondestructive analysis, i.e. NMR and half seed analysis, integrated with a breeding program make genetic modification increasingly effective. As the physiological and anticarcinogenic benefits of these plant components become clarified, and considering the general absence of undesirable compounds such as cholesterol and saturated fatty acids, the selection and development of varieties with such enhanced properties may become appropriate. Key metabolites which exhibit physiological and medicinal attributes could be enhanced in new varieties. The American Heart Association has indicated that it will endorse certain types of foods, a position they have avoided in the past, perhaps providing incentive to develop varieties with such enhanced properties, or unique combination of traits previously unattainable.

Texture, Flavor, and Color of Fruits and Vegetables. Considerable variability is found in the texture, flavor, and color of the edible portion of horticultural crops. Obviously much of this variability is due to preharvest cultural and environmental conditions, postharvest treatment, and the stage of maturity at consumption. However, there is also considerable genetic variability which accounts for the range of visual and organoleptic properties.

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Strawberries range from almost white to deep red centers; from a pale red exterior to almost black due to the high concentration of anthocyanins; the texture of ripe strawberries can range from mushy to crisp. The texture and flavor of apple varieties differ due to genotype and the varied responses to postharvest storage environments. Cauliflower and broccoli differ in concentrations of glucosinolates, which are responsible for off and pungent flavors. In most cases conventional breeding has provided adequate diversity in these quality attributes for the consumer. However, further genetic modification of key components could facilitate the distribution of quality produce as one envisions greater distribution ranges, and longer term storage or remote production areas to provide year-round availability. The improvement of flavor, through the enhancement of key flavor components, levels of reducing sugars and key amino acids or the removal of undesirable components can be facilitated by biotechnology. Secondary metabolites such as flavor compounds are quite responsive to cell culture manipulation, with somaclonal variants generated with decreased or increased levels of targeted components (39). By precisely regulating starch biosynthetic enzymes, antisense technology may offer the opportunity to elevate sucrose levels of sweet corn without the concurrent reduction of phytoglycogen associated with shrunken-2 mutants (40). Phytoglycogen is responsible for providing much of the mouth feel of normal sugary sweet corn varieties.

Key enzymes involved in the depolymerzation of cell wall components or the development of flavor could be modulated up or down. The use of developmentally regulated promoters would allow the precise regulation of such biochemistry. For example, transformation with an active cloned gene for up-modulation of lipoxygenase activity could alter the flavor characteristics of fruit tissue. A reduction in postharvest activity of lipoxygenase would be useful in minimally processed vegetables such as peas (41). Color and flavor deficiencies greatly reduce the quality of tomatoes. Through the enhancement of the levels of ß-carotene, lycopene, key flavor components and a more strict control of the processes of ripening and senescence, the quality of non-seasonal tomatoes can be greatly improved. The isolation of genes responsible for pigment accumulation in plant materials and subsequent transformation with the active genes for up-modulation could enhance specific colors. The potential to modify such quality attributes in plant tissue will be greatly accentuated by biotechnology; the cost/benefit ratio will be the determining factor as to whether such utility will be realized.

## MAINTENANCE OF HARVEST QUALITY

Quantitative and qualitative losses in produce are due to microbial degradation; deterioration due to the activity of endogenous enzymes associated with maturation, ripening, and senescence; enzymatic activity resulting from physical injury and the subsequent decompartmentalization of substrate and enzymes; and the interaction of these various activities. The extent of such biological activity determines the appearance, texture, and flavor of the plant tissue when it reaches the consumer. To what degree these deteriorative

processes can be controlled through the genetic modification of the raw material or the use of microbial pesticides developed through modern biotechnology remains to be seen.

There are sufficient examples of genetic variability among genotypes in their rates of biological maturation and deterioration to suggest that varieties which progress perhaps more slowly through maturation, ripening, and senescence or which exhibit a reduction in the rate of key biochemical events could be developed. The genetic diversity in the conversion rate of sucrose to starch among sweet corn genotypes is well documented (33). Genotypes incorporating the bt (brittle) or sh2 gene not only provide elevated levels of sucrose but also exhibit a reduction in the conversion rate of sucrose to starch.

Several spontaneous mutants of tomatoes have been identified specifically as "ripening" mutants. The ripening inhibitor (rin) and non-ripening (nor) mutations result in non-climacteric fruits (42). These fruits fail to produce system 2 ethylene and thus do not ripen normally. Other mutant alleles such as "never ripe" (NR), "green ripe" (GR), and "alcobaca" cause all ripening to proceed much more slowly and to occur over an extended period of time. These slow-ripening mutants with a depressed and extended climacteric accumulate pigment slowly or not at all, and often ununiformally. They also tend to exhibit reduced polygalacturonase activity, which very slowly causes a depolymerization of the cell walls and subsequent softening of the tissue.

Cultivars of apples Vamos-Vigyazo et al., 1976) and apricots (43), and avocados (44) demonstrated different rates of enzymatic browning. Such browning tendencies were dependent upon substrate levels as well as the concentration of polyphenol oxidase in the fruit tissue (45).

Quality attributes such as appearance, texture, and flavor are susceptible to change after harvesting, but very little has been accomplished in establishing the effect of genotype. During storage, plant tissue pigments can undergo considerable change depending upon the storage environment and the endogenous biochemistry of the plant tissue. The more common catalytic agents of carotenoid degradation appear to be peroxidases and lipoxygenases. Anthocyanin synthesis is stimulated by light and is often effected by temperature. One of the more obvious changes which occurs in senescing tissue is the loss of characteristic green color. Peel and often pulp degreening is associated with the ripening of most fruit and "yellowing" is a common characteristic of many detached leaf, stem, or flower tissue consumed as vegetables. The highly respiring florets of broccoli are very susceptible to postharvest loss of chlorophyll and the generation of off and pungent flavors. Although one would expect such enzyme mediated processes to be effected by genotype, the differences among existing genotypes is apparently so subtle that they are many times accountable to preharvest growing conditions. Variability among broccoli genotypes with regards to the rate of color change has not been observed.

Uniformity of ripening, and rate of ripening will provide targets for the application of emerging technology. Polygalacturonase (PG) is the major enzyme responsible for the depolymerization of cell walls and the subsequent softening of the

tissue during the ripening of fruits such as tomato (46). With the anticipation that reduced PG activity would result in improved shelf life and processing quality, scientists at the University of Nottingham and ICI have reported the inhibition of the expression of endogenous developmentally regulated gene for PG in transgenic tomato expressing antisense mRNA (47). However, there was no observed reduction in softening as measured by compressibility. In the case of avocado, a fruit which does not ripen while attached to the tree, cellulase probably plays a major role in the solubilization of the cell wall polymers (48). Further understanding of the interaction of ethylene and cellulase accumulation may offer other opportunities for the control of cell wall deterioration in specific tissue. The control of ripening through the use of tissue specific promoters for the control of ethylene mediated ripening and senescence processes could yield substantial rewards in the uniformity of ripening and postharvest stability. Ethylene synthesis is generally limited by the supply of the immediate precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). The control of ethylene induced ripening would necessitate the control of ACC synthase or the ethylene forming enzyme (EFE). The other possibility would be to genetically modify plant tissue to be devoid of ethylene receptors. Blecker et al. (49) recently reported a dominant mutation in Arabidopsis which appears to affect the ethylene receptor.

## DIAGNOSTICS

As a related area, biotechnology will greatly impact the protection of the food supply. Immuno-diagnostic techniques will provide simple, rapid, and inexpensive means for monitoring a wide range of variables including: plant pathogens, food pathogens, aflotoxins, pesticides, etc. As the consumer continues to demand freshness and convenience, and the processor attempts to meet such demands, quality control will take on increasing significance.

## SUMMARY

The maintenance of harvest quality through control of the physiology of the harvested tissue, pathogens, and interaction of the commoditywith the environment provides one of the greatest opportunities for modern plant genetics to have an impact. The enhancement of quality components of fruits and vegetables (appearance, texture, flavor, nutrition and physiology, and safety) is made more feasible by biotechnology. Diagnosis or monitoring of food pathogens and pesticides will be one of the first applications of biotechnology to fruits and vegetables. Advances will depend on the integration of biotechnology with traditional sciences, and an understanding of the biochemical basis of the targeted attributes.

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