REFRIGERATED FRUIT JUICES: QUALITY AND SAFETY ISSUES

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Fruit juices are an important source of bioactive compounds, but techniques used for their processing and subsequent storage may cause alterations in their contents so they do not provide the benefits expected by the consumer.

In recent years consumers have increasingly sought so-called ''fresh'' products (like fresh products), stored in refrigeration. This has led the food

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industry to develop alternative processing technologies to produce foods with a minimum of nutritional, physicochemical, or organoleptic changes induced by the technologies themselves. Attention has also focused on evaluating the microbiological or toxicological risks that may be involved in applying these processes, and their effect on food safety, in order to obtain safe products that do not present health risks. This concept of minimal processing is currently becoming a reality with conventional technologies (mild pasteurization) and nonthermal technologies, some recently introduced (pasteurization by high hydrostatic pressure) and some perhaps with a more important role in the future (pulsed electric fields). Nevertheless, processing is not the only factor that affects the quality of these products. It is also necessary to consider the conditions for refrigerated storage and to control time and temperature.

I. INTRODUCTION

The consumption of refrigerated juice in the United States is currently of the order of 4.4 billion liters (Jago, 2004). Orange juice is in highest demand, followed by apple, pineapple, and grapefruit. The preferred tropical juices are pineapple, passion fruit, and mango, followed by guava and soursop, which are generally used for mixtures and not in single-flavor beverages. The countries most receptive to consumption of tropical fruit juices are France and Spain, although demand is also high in the UK. The presence of Asian and Latin-American ethnic groups stimulates demand for these flavors. In the European Union (EU) the production of concentrated orange juice is low in comparison with Brazil and the United States. Spain and Italy are the main producers and have a reputation for high quality. Sales of juices and concentrates among the 15 members of the EU were 9.7 billion liters in 2004, and the trend has been increasing during the last decade. Germany is the main market for fruit juices and concentrates, with a consumption of 40.3 liters per person per year. It is followed by Finland, Austria, Spain, and Denmark.

According to the Mexican–European Union Business Centre, consumer preferences currently show a tendency toward processed products that satisfy safety and hygiene regulations, and that are low in fats and contain no artificial preservatives (Legiscomex.com, 2006).

Governments throughout the world advocate the inclusion of fruit juices in a healthy diet. A juice that is 100% derived from its parent fruit or fruits is almost universally regarded as a healthy and nutritious part of a human diet. The main emphasis in health-promoting dietary recommendations is increased consumption of fruit and vegetables. According to the CODEX General Standard for Fruit Juices and Nectars, an authentic fruit juice product

must maintain the "essential physical, chemical, organoleptical, and nutritional characteristics of the fruit(s) from which it comes." Juices are more convenient to consume and generally have a longer shelf life than fresh fruit (IFFP, 2005).

Although fruits and vegetables are generally consumed fresh, many of them have to be processed and/or preserved, for financial or logistic reasons, to improve their digestibility, because of culinary needs, or to make them easier to consume for certain consumer groups (children, the elderly, the sick, or people with little time to prepare food, and so on). The consumer demands safe foods obtained by processing, with a preparation that takes up as little time as possible. These new habits have led to an increase in the consumption of prepared fruit and vegetable juices.

A recent WHO/FAO joint report recommends consumption of about 400 g of fruit and vegetables a day as an invaluable aid to prevent chronic diseases, including cardiovascular diseases, cancer, type II diabetes, and obesity. According to WHO data, low fruit and vegetable intake causes some 2.7 million deaths each year and is one of the 10 risk factors contributing to mortality (WHO, 2003).

In a study on food sources of nutrients in the diet of Spanish children, fruit juices and citrus fruit were shown to be the principal sources of vitamin C, accounting for 43% (Royo-Bordonada *et al.*, 2003).

Fruit juices contain a complex mixture of nutrients that are beneficial to the maintenance of good health, and they have intrinsic disease risk reduction properties. In addition to the major nutrients (e.g., vitamins, minerals) inherent in the fruit itself, juices also contain phytochemicals (often referred to as phytonutrients) derived from the fruit. The biological activity of phytochemicals has been studied in numerous *in vitro* and *in vivo* tests and in tests involving humans (Cassano *et al.*, 2003; Duthie, 1999; Giovannucci *et al.*, 1995; Granado *et al.*, 1997; Hertog *et al.*, 1994; Kurowska *et al.*, 2000; Omaye and Zhang, 1998; Simon *et al.*, 2001; Topuz *et al.*, 2005; Tribble, 1998). Antioxidant activity is a common characteristic for all these bioactive compounds because of its ability to capture oxygen radicals (hydroxyl, peroxyl, superoxide, and simple oxygen), nitrogen radicals, and organic radicals (lipid hydroperoxides, and so on). Free radicals appear in tissues in situations of oxidative stress, polluted atmospheres, and so on. The accumulation of these species causes the appearance of oxidative damage in DNA, and also in proteins and lipids in cell membranes. All this damage leads to a consequent aging of tissues and the appearance of degenerative diseases (Halliwell, 1996). Lampe (1999) describes various mechanisms by which fruits and their constituents can have a protective effect.

Epidemiological studies show that consumption of fruit and vegetables has a great protective effect against the risk of certain diseases connected with age,

such as cancer, cardiovascular diseases, cataracts, macular degeneration, and diabetes, because these foods are rich in antioxidant vitamins such as vitamins C and E, phenolic compounds, and carotenes (Bazzano *et al.*, 2002; Block *et al.*, 2001; Burns *et al.*, 2003; Epler *et al.*, 1993; Forastier *et al.*, 2000; Gardner *et al.*, 2000; John *et al.*, 2002; Lichtenthäler and Marx, 2005; Schieber *et al.*, 2001; Simopoulos, 2001; Slattery *et al.*, 2000). Some studies of supplementation with these antioxidant compounds, especially β-carotene, have produced contradictory results. Many epidemiological studies (ATBC, CARET, Women's Health Study) with β-carotene supplements, alone or associated with vitamin E, show that they do not avoid cardiovascular diseases or some types of cancer, and they are not very recommendable for smokers (ATBC, 1994; Hennekens *et al.*, 1996; Taylor, 1996). Nevertheless, epidemiological studies with fruits and vegetables or derivatives do show beneficial results for the health (Aviram *et al.*, 2000; Kris-Etherton *et al.*, 2002; Temple and Gladwin, 2003).

Adapting to new trends and consumer demands is one of the primary objectives of orange juice producers. For some years, therefore, they have been producing juices with mild pasteurization, marketed in refrigerated conditions and with limited shelf life. Although conventional thermal processing ensures the safety and extends the shelf life of foods, it often leads to detrimental changes in the sensory qualities of the product (Bull *et al.*, 2004). New products are being introduced, with juice mixtures that provide increased quality (nutritive value, color, and so on), this being the factor that most contributes to consumer acceptance and an increase in the value added to the product.

As a result of the problem that has arisen because of the development of pathogens in some (unpasteurized) fresh orange juices (Ghenghesk *et al.*, 2005; Parish, 1998a; Parish *et al.*, 1997), the image and safety of these juices have been damaged, and the FDA is recommending manufacturers of these juices to increase safety measures by introducing the Hazard Analysis and Critical Control Points (HACCP) system in their processes and by applying a pasteurization or treatment that will ensure five decimal reductions of *Escherichia coli* (Food and Drug Administration, 1998).

Product quality is determined by a number of parameters. Without going into detail about quality concepts, the most important attributes for a food product, in our view, are: organoleptic properties (taste, flavor, texture, appearance, and color); microbiological (absence of pathogens and microbial toxins) and toxicological safety; nutritive value; shelf life; convenience; and, last but not least, healthiness. Processing is generally done to achieve some desired results such as killing or inactivating microorganisms or inactivating enzymes and antinutritional factors. Such treatments also frequently result in undesired reactions, mostly of a chemical nature, especially during heat treatment (Van Boekel and Jongen, 1997).

Many studies have been carried out on the quality and stability of pasteurized orange juices (Graumlich *et al.*, 1986; Kaanane *et al.*, 1988; Martín *et al.*, 1995). But in some cases the juices are obtained from concentrates (Fellers and Carter, 1993), while in other cases (Graumlich *et al.*, 1986; Martín *et al.*, 1995) the pasteurization conditions applied are fairly intense (from 90°C, 15 s to 110°C, 15 s), anticipating storage at ambient temperature or else a very long shelf life in refrigeration. In these latter cases the impact of pasteurization on quality is clearly appreciable. In other cases, high storage temperatures have been studied in order to observe clearly the effects of temperature on certain factors (browning, development of hydroxymethylfurfural, loss of vitamin C, and so on), and to deduce the kinetic models that define these changes (Manso *et al.*, 2001a). And, finally, in other cases there are studies on the effects of certain processes, processing conditions, canning, or storage, and on one or more specific quality parameters (Ayhan *et al.*, 2001; Choi *et al.*, 2002; Decio and Gherardi, 1992; Fan *et al.*, 2002; Johnston and Bowling, 2002; Manso *et al.*, 2001b; Nienaber and Shellhammer, 2001a; Parish, 1998b; Sánchez-Moreno *et al.*, 2003; Trammell *et al.*, 1986).

Refrigerated juices that are not obtained from concentrates and have been subjected to mild pasteurization (75°C for 30 s) partly satisfy the requirements of the higher quality demanded by consumers. The shelf life of these juices ranges between 28 and 45 days in refrigeration and their quality approaches that of freshly squeezed juices.

Newly developed food technologies usually focus on preservation while keeping food quality attributes. Therefore, the frequently used concept of "minimal processing" is not absolutely apt because really the principle of "as little as possible, but as much as necessary" is meant. Nonthermal methods allow processing of foods below the temperatures used during thermal pasteurization, so flavors, essential nutrients, and vitamins undergo minimal or no changes. Obstacles to commercialization include the lack of systematic inactivation kinetic data, the interpretation of nonlinear death kinetics, and the need to establish equivalent control measures for nonthermal treatments in comparison with traditional heat processes (Stewart *et al.*, 2002).

Foods can be nonthermally processed by irradiation, high hydrostatic pressure, antimicrobials, ultrasound, filtration, and electrical methods such as pulsed electric fields (PEFs), light pulses, and oscillating magnetic fields. As a result of technological developments, high-pressure and high-intensity PEF processing have received increased attention during the last decade (Butz and Tauscher, 2002).

The main requirement that these new technologies must meet is to ensure product microbial safety while preserving sensory and nutritional characteristics so as to obtain products as similar as possible to fresh foods.

Food preservation using high pressure is a promising technique in the food industry as it offers numerous opportunities for developing new foods with extended shelf life, high nutritional value, and excellent organoleptic characteristics (Cheftel, 1995). High pressure is an alternative to thermal processing. Industrial applications are already known in Japan, the United States, France, and Spain. The European Commission includes products obtained by this technology in the Novel Foods group, subject to Novel Foods Legislation. After successful application and verification of this technology, the Food Standards Agency, UK, released a statement saying that the technology is not regarded as novel provided the foods are of fruit or vegetable origin, with a pH below 4.2, and germination of clostridia is prevented during shelf life (Houska *et al.*, 2006).

The first attempts to treat foods with electroimpulses were reported at the end of the 1920s in the United States. During recent years, research in this technology has been reinforced again. Various laboratory and pilot-scale treatment chambers have been designed and used for high-intensity PEF treatment of foods. Two industrial-scale systems are available, including treatment chambers and power supply equipment (Butz and Tauscher, 2002). Energy loss due to heating of foods is minimized, reducing detrimental changes in the sensory and physical properties of the foods. Microbial inactivation by PEFs has been explained by several theories. The possibility most studied is electrical breakdown and electroporation (Barbosa-Cánovas *et al.*, 1999; Grahl and Maerkl, 1996; Jeyamkondan *et al.*, 1999).

To study optimum treatment conditions for these new refrigerated juices, and also to guarantee the absence of microorganisms (whether pathogenic or otherwise), it is essential to know the characteristics (physicochemical and quality) of these juices and their possible storage variations in order to establish their shelf life. For all these reasons, in this chapter we propose to give details of studies that have been performed in recent years on refrigerated juices, first to ensure their safety, and second (but also importantly, as the many studies show) to ensure juices that are organoleptically as similar as possible to fresh juice and with the maximum content of bioactive compounds.

II. REVIEW

A. PHYSICOCHEMICALS

Physical measurements are important because of their potential impact on sensory evaluation parameters such as mouthfeel. Viscosity, density, and amount of cloud are related to the quantity and consistency of the juice pulp.

Pasteurization appears to have a major impact on the stability of juice cloud in orange juice concentrates because of the deactivation of pectinesterase enzymes (Chandler and Robertson, 1983).

Rodrigo *et al.* (2003a) studied the physicochemical and quality characteristics of various refrigerated mixed orange and carrot juices, and their changes with storage time and temperature. Density, dry extract, °Brix values, acidity, turbidity, formol index, pectin methylesterase (PME), hydroxymethylfurfural, essential oils, ascorbic acid, and color varied with storage time and temperature. Some of the parameters could be used as indicators of quality loss or spoilage of the juices. Except for calcium, diacetyl index, and pulp, there were statistically significant differences ($p < 0.05$) between the juices, attributable to differences in the raw material and the processes used. However, the contents of ash, calcium, phosphorus, magnesium, potassium, total nitrogen, nitrates and nitrites, pH, conductivity, proline, diacetyl index, and particle size did not vary during storage, irrespective of temperature.

Esteve *et al.* (2005) studied the physicochemical and quality characteristics of various minimally pasteurized refrigerated Spanish orange juices, and their changes with storage time and temperature. Except for pH, all the characteristics studied (essential oils, acidity, color, conductivity, density, diacetyl index, and viscosity) varied with storage time at 4 and 10°C, but density variation was not statistically significant at 4°C.

In a study by Farnworth *et al.* (2001), Mexican orange juice bottled without pasteurization and frozen (–18°C); orange juice that was pasteurized, bottled, and frozen; and orange juice pasteurized and stored at 1°C in plastic bins were sampled monthly for 9 months. The viscosity and cloud of the orange juice stored in refrigeration for 9 months decreased significantly, whereas the density did not vary. The density of the unpasteurized orange juice was less than the density of the pasteurized juices (subsequently stored at –18 and 1°C). The cloud measurement indicated that pasteurization had a dramatic effect on this physical property of the juice. Orange juices that were not pasteurized settled out over time, but no settling or clearing was observed for pasteurized orange juices. However, no significant changes were observed in the value of the density. The concentration of sugars (glucose, sucrose, and fructose) in the orange juice did not vary during storage, but the °Brix increased with time. No significant differences in °Brix were observed with type of treatment, although the pasteurized juices had a greater sugar content and higher °Brix value.

Sadler *et al.* (1992) reported that sucrose concentrations in Valencia orange juice decreased during storage at 4°C, apparently due to microbial contamination. The smallest decrease in sucrose was observed in unpasteurized orange juice. The malic acid content of the unpasteurized orange juice was significantly lower than of the pasteurized juices, and its concentration

increased during storage. However, the authors did not find changes in the concentration of citric acid during storage or as a result of processing.

Yeom *et al.* (2000a) compared PEF-treated orange juice (35 kV/cm, 59 μs) and heat-treated orange juice and observed that the PEF-treated juice had a smaller particle size than that of the juice pasteurized by heat. There were no differences between the PEF-treated juice and fresh juice. They did not observe differences between the °Brix and pH of the juices after treatment or during storage.

Del Caro *et al.* (2004) did not see significant changes in the pH, acidity, °Brix, and dry matter of freshly squeezed juice of Shamouti oranges, Red Blush grapefruit, and Salustiana oranges during 15 days' storage at 4°C.

Polydera *et al.* (2003) treated orange juice with HHP (500 MPa at 35°C for 5 minutes). They found that the consistency index did not change significantly during storage of thermally treated orange juice, leading to an almost constant apparent viscosity. In the case of high pressurized orange juice, the consistency index increased with storage time. Higher apparent viscosity values were determined for high-pressurized orange juice compared with thermally treated orange juice immediately after processing and on each storage day. The same authors (Polydera *et al.*, 2005a) found similar results when they treated the juice with HHP (600 MPa, 40°C, 4 minutes) and thermal pasteurization (80°C, 60 s). However, a small decrease in the consistency index, which also means a decrease in the corresponding apparent viscosity values, was observed during storage in different conditions. This decrease was more pronounced in the case of high pressurized orange juice, while the consistency index of thermally treated juice did not change significantly with storage time.

Bull *et al.* (2004) compared the quality and shelf life of high-pressure-processed (600 MPa, 20°C, 60 s) Valencia and Navel orange juices with fresh juice and thermally pasteurized juice (65°C, 1 minute), and their subsequent storage at 4 and 10°C for 12 weeks. For both juice types, the pH, °Brix, viscosity, titratable acid content, and alcohol insoluble solids of the pressure or thermally treated juices were not significantly different from fresh, untreated juices. The parameters did not change significantly over storage time. Clarification (cloud loss) occurred in all treatments, but no difference was found between treatments. The degree of clarification increased significantly over time across all treatments. Similar results were obtained by other authors (Goodner *et al.*, 1999; Parish, 1998a), who required treatments of at least 700 or 500 MPa/60°C to obtain cloud stable juices.

Fiore *et al.* (2005) compared different juices purchased in the market and found that pH was slightly lower in sterilized (long shelf life) orange juices (2.66–3.20) than in pasteurized and refrigerated orange juices (3.12–3.34).

Garde-Cerdan *et al.* (2007) studied the effect of thermal and PEF treatments on various physicochemical properties of Parellada grape juice.

No significant changes were noticed in the physicochemical properties measured such as reducing sugar content, total acidity, and pH.

B. NONENZYMATIC BROWNING

The control of furanic aldehydes is important in the evaluation of nonenzymatic browning, adulterations, heating, incorrect storage, and sensory characteristics of food. Rodrigo *et al.* (2003a) and Esteve *et al.* (2005) observed an increase in 5-hydroxymethyl-furaldehyde (5-HMF) during storage (up to 6 weeks) of mixed orange and carrot juice and orange juice, respectively. The increase was greater at 10°C than at 4°C.

Fan (2005a) investigated the formation of furan from sugars, ascorbic acid, and organic acids affected by ionizing radiation and thermal treatments. The results showed that both thermal treatments and irradiation induced formation of furan from ascorbic acid, fructose, sucrose, or glucose. Little furan was produced from malic acid or citric acid. The pH and concentration of sugars and ascorbic acid solutions had strong influences on furan formation due to either irradiation or thermal treatment. The rate of irradiation-induced furan formation increased with decreasing pH from 8 to 3. Approximately 1600 times less furan was formed at pH 8 than at pH 3. At the same pHs, the amounts of furan formed from irradiation of ascorbic acid, fructose, and sucrose were always higher than that from glucose. As the pH decreased from 7 to 3, an increase in thermally induced furan was observed for sucrose and ascorbic acid solutions; for glucose solution, however, less furan was formed at pH 3 than at pH 7. The levels of sugars commonly found in fruits and fruit juices would, on irradiation, be high enough potentially to produce low parts per billion (ppb) levels of furan. The concentration of ascorbic acid at which a maximum of furan was produced on irradiation was about 0.5 mg/ml, a level commonly found in some foods. Five furan derivatives were tentatively identified in thermally treated ascorbic acid solution, while one furan derivative was tentatively found in both irradiated and thermally treated samples. The same author (Fan, 2005b) studied the formation of furan in freshly prepared apple and orange juices affected by ionizing radiation and thermal treatments, using a newly developed solid-phase microextraction method coupled with gas chromatography–mass spectrometry (GC–MS). The results showed that furan levels increased linearly as the radiation dose increased from 0 to 5 kGy. Irradiation induced more furan in apple juice than in orange juice. During postirradiation storage at 4°C, furan levels increased in both apple and orange juices, particularly in the first 3 days. On the other hand, irradiation degraded deuterated furan (4-furan) spiked in water and fruit juices. The rate of degradation as a function of radiation dose was highest in water and lowest in orange juice. Submerging

the juice samples in boiling water for 5 minutes induced higher amounts of furan in orange juice than in apple juice, but autoclaving (121°C, 25 minutes) resulted in more furan formation in apple juice than in orange juice. The results reported suggest that both ionizing radiation and thermal treatments induce furan formation in fruit juices.

Yeom *et al.* (2000a) observed a linear increase in the browning index in PEF-treated orange juice (35 kV/cm, 59 μs) and pasteurized juice (94.6°C, 30 s) after a storage period of 28 days at 4°C. The browning index was lower in the PEF-treated juice than in the heat-treated juice during storage at 4°C, although no differences were observed when the juices were stored at 22°C.

Roig *et al.* (1999) studied the occurrence of nonenzymatic browning during storage of freshly produced commercial citrus juice, aseptically filled in Tetra-Brik cartons. The rate of browning of the samples was directly related to temperature (room temperature and 5°C). Although formation of 5-HMF has been detected in degraded juice samples, its presence could not be used as an index of browning. 5-HMF has been found to be unreactive in the browning process in citrus juices and its contribution to browning in products of this type is insignificant if not negligible. Increasing the L-ascorbic acid (added as an antioxidant) concentration extends the nutritional value of the products but also increases the severity of browning.

Bull *et al.* (2004) did not find significant differences in the browning index of high-pressure-processed (600 MPa, 20°C, 60 s) Valencia and Navel orange juices, fresh juice, and thermally pasteurized juice (65°C, 1 minute). The significant increase in the browning index seen over time was observed across all treatments.

C. FATTY ACIDS AND FREE AMINO ACIDS

Although the formol index is not specific, it is used to estimate the total content of amino acids in a juice. Rodrigo *et al.* (2003a) and Esteve *et al.* (2005) determined the formol index of refrigerated (mild pasteurization, 77°C for 20 s) mixed orange and carrot juice and orange juice, respectively, and their evolution during storage for 6 weeks at 4 and 10°C. In both cases, the formol index decreased with storage time and temperature. This decrease might be due to consumption of amino acids by microorganisms responsible for the start of fermentation of the juices. Kaanane *et al.* (1988) observed that the formol index of pasteurized orange juices did not vary during storage. However, when Trifirò *et al.* (1995) studied the effect of storage time and temperature on the quality of fresh orange juices, they observed an increase in the formol index which they attributed to a proteolysis effect, and they found that it was related to the origin of the juice and storage temperature. Villamiel *et al.* (1998) studied the influence of heat treatment (conventional and microwaves) on orange juice and reported that there were no differences

in the content of amino acids between the untreated juices and the juice treated by microwaves, but they observed a decrease in some amino acids in the juice treated conventionally.

Garde-Cerdan *et al.* (2007) studied common thermal and PEF treatments to assess their effect on fatty acid and free amino acid contents of Parellada grape juice. These compounds are of great importance in winemaking as nutritive compounds for yeast growth. Neither thermal nor PEF treatments modified the total content of fatty acids and free amino acids in Parellada grape juice. However, the concentration of lauric acid diminished after PEF processing, and the concentration of some amino acids varied after both treatments. Lipids and nitrogen compounds play an important role in the fermentative steps of winemaking. Fatty acids and sterols have a great influence on the growth of fermentative yeast and thus on the development of alcoholic fermentation. Zulueta *et al.* (2007) evaluated the effect of HIPEF treatment on various physicochemical properties and fatty acid profile changes of the orange juice–milk beverage. After HIPEF treatment, nonsignificant changes in the contents of saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids were observed, only a small reduction in fat content (p, 0.05) was found.

D. AROMA AND FLAVOR

The flavor of orange juice is easily altered during processing and storage. Irreversible changes are produced in the flavor of the juice as a result of chemical reactions that are initiated or occur during thermal processing (Braddock, 1999). The changes in flavor are also associated with a number of deteriorative reactions that take place during storage, giving rise to the development of off-flavor. Nonenzymatic browning such as ascorbic acid degradation causes deterioration of flavor as well as loss of nutrients and darkening (Kaanane *et al.*, 1988).

Jordan *et al.* (2003) performed a comparative study between the aromatic profile of fresh orange juice versus deaerated and pasteurized juices. At the qualitative level, all the volatile components in the fresh orange juice were also found in the counterparts after deaeration and pasteurization processes. According to statistical analyses, significant losses in the concentration of volatile components occurred during the deaeration process, while there were no statistically significant differences between the concentrations of volatile components in the deaerated and pasteurized juices. The results show that during industrial processing of orange juice the biggest losses in the concentration of volatile components occur during deaeration. The pasteurization process does not significantly change the analytical composition of deaerated orange juice for any of the 42 quantitated volatile compounds (alcohols, aldehydes, esters, ketones, and terpenic hydrocarbons).

In a study performed by Butz and Tauscher (2002), in high-pressure-processed orange juice the changes in aroma and flavor and general quality

after 21 days' storage were imperceptible. However, Baxter *et al.* (2005) found that the odor and flavor of the HPP juice was acceptable to consumers after storage for 12 weeks at temperatures up to 10°C.

Farnworth *et al.* (2001) found that in Mexican orange juice the concentrations of acetaldehyde and ethyl acetate were higher in unpasteurized juice. α-Pinene, β-myrcene, limonene, α-terpineol, 1-hexanol, 3-hexen-1-ol, and sabinene concentrations were higher in the unpasteurized juice than in the pasteurized juice.

As storage time increased, PEF-treated orange juice showed a significantly higher content of flavor compounds than heat-pasteurized orange juice during storage at 4°C (Yeom *et al.*, 2000a).

Polydera *et al.* (2003, 2005a) found that high-pressure processing resulted in better retention of the flavor of untreated juice and superior sensory characteristics compared with thermal pasteurization.

E. VITAMIN C

Vitamin retention studies to assess the effects of food processing on the nutritive value of foods are of great importance to food technologists and consumers. Vitamin C is thermolabile and therefore in fruit and vegetables it provides an indication of the loss of other vitamins and acts as a valid criterion for other organoleptic or nutritional components such as natural pigments and aromatic substances. Its concentration decreases during storage, depending on storage conditions such as temperature, oxygen content, and light (Alwazeer *et al.*, 2003; Blasco *et al.*, 2004; Esteve *et al.*, 1996; Polydera *et al.*, 2003; Zerdin *et al.*, 2003).

Kabasakalis *et al.* (2000) studied the ascorbic acid content of commercial juices and its loss with storage time and temperature. The juices that they analyzed were divided into three groups: long-life commercial fruit juices without preservatives (100% orange; 100% grapefruit; 100% cocktail of orange, peach, grapefruit, pineapple, apple, mango, kiwi; 17% lemon; 50% cocktail of apple, orange, apricot, peach, grapefruit, pineapple); short-life commercial fruit juices (refrigerated) without preservatives (100% orange, 9% lemon); and fresh fruit juice (orange). A loss of ascorbic acid was observed in short-life 100% orange juice as the expiration date approached (42.7-mg ascorbic acid/100 ml at 26 days before expiration and 38.9-mg ascorbic acid/100 ml at 8 days before expiration). Loss of ascorbic acid in various commercial fruit juices stored in closed containers for a period of 4 months at room temperature ranged between 29% and 41%. When the containers were opened for consumption and then stored in the refrigerator for 31 days, commercial 100% orange juice lost 60–67% of its ascorbic acid, whereas under the same conditions ascorbic losses in fresh orange juice were much lower (7–13%).

Farnworth *et al.* (2001) also obtained similar results. They studied Mexican orange juice bottled without pasteurization and frozen, orange juice that was pasteurized, bottled, and frozen, and orange juice pasteurized and stored at 1°C in plastic bins, sampled monthly for 9 months. The concentration of ascorbic acid was affected by the method of production. The amount of ascorbic acid diminished as storage time increased.

Vitamin C was evaluated by Gil-Izquierdo *et al.* (2002) in orange juices manufactured by different techniques (squeezing, mild pasteurization, standard pasteurization, concentration, and freezing). They found that mild and standard pasteurization slightly increased the total vitamin C content and the contribution from the orange solids parts, whereas concentration and freezing did not produce significant changes.

Rodrigo *et al.* (2003a) determined the concentration of ascorbic acid in various refrigerated mixed orange and carrot juices, and their changes with storage time (for 5 weeks) and temperature (4 and 10°C). They calculated the mean life of the juices on the basis of the vitamin C concentration, obtaining a period of 32 and 43 days at 10 and 4°C, respectively. This would ensure that if the storage temperature increased the juice would conserve its nutritive characteristics during its shelf life.

Del Caro *et al.* (2004) stored squeezed juices of various species and cultivars (Red Blush grapefruit, Salustiana, and Shamouti oranges) for 15 days at 4°C and only in the Salustiana orange juice did they observe a significant decrease (13%) in the vitamin C concentration. Esteve *et al.* (1996) found a decrease of 5% in the concentration in freshly squeezed orange juice after 7 days' storage at 4°C. Trifirò *et al.* (1995) also reported a maximum decrease of 8% in the ascorbic acid concentration in fresh blood orange juice stored at 3°C, although the juice was pasteurized and stored for 30 days.

Vikram *et al.* (2005) studied the status of vitamin C during thermal treatment of orange juice heated by different methods (conventional heating, electromagnetic processing including infrared, ohmic heating, and microwave heating) and at different treatment temperatures (50, 60, 75, and 90°C) and times (0–15 minutes). The degradation kinetics of vitamin C in terms of reaction rate constant, destruction kinetics, enthalpy, and entropy for the different heating methods were discussed. The destruction of vitamin C was influenced by the heating method and the processing temperature. The degradation was highest during microwave heating, owing to the uncontrolled temperature generated during processing. Of the four methods studied, ohmic heating gave the best result, facilitating better vitamin retention at all temperatures. The activation energies for both vitamin and color were within the range of the literature values, 7.54–125.6 kJ/mol. The activation enthalpies agreed with the literature values of vitamin destruction in other food products. The z-values were also within the literature values of 20–30°C for vitamin destruction, except for microwave heating.

Choi *et al.* (2002) studied the retention of ascorbic acid with storage in blood oranges and observed a linear reduction in concentration with time. Fan *et al.* (2002) also observed a linear degradation of ascorbic acid in orange juice with time, whether irradiated or not.

Alwazeer *et al.* (2003) studied the effect of both redox potential (Eh) and pasteurization of orange juice on its stability during storage at 15°C for 7 weeks. Gassing the juice with N_2 or N_2–H_2 increased color retention and ascorbic acid stability. The study showed that the juice must be reduced just after heat treatment in order to stabilize color and ascorbic acid during storage. The effects of storage temperature and time on the stability of PEF and thermally pasteurized orange juice were studied by Yeom *et al.* (2000a). PEF-treated orange juice retained a higher ascorbic acid content than that of heat-pasteurized orange juice during storage at 4°C. PEF-treated orange juice showed no difference in ascorbic acid concentration compared with heat-pasteurized orange juice during storage at 22°C.

Polydera *et al.* (2003, 2005a,b) studied the effect of HPP treatment (500 MPa, 35°C, 5 minutes or 600 MPa, 40°C, 4 minutes) and thermal pasteurization (80°C, 30–60 s) on orange juice and its subsequent storage (0–30°C, 1–3 months). In all cases, the ascorbic acid degradation rates were lower for high pressurized juice, leading to an extension of its shelf life compared with conventionally pasteurized juice. The shelf life of the HAP-treated juice (based on ascorbic acid retention) was greater than that of pasteurized juice. However, Bull *et al.* (2004) did not find significant differences between HAP-treated juice (600 MPa, 20°C, 60 s), pasteurized juice (65°C, 1 minute), and fresh juice. Nevertheless, they found a decrease in ascorbic acid concentration in all the juices with storage time, irrespective of the treatment applied and storage temperature (4 and 10°C).

Fiore *et al.* (2005) did not find differences in vitamin C content (38.9–89.0 mg/100 ml) between sterilized (long shelf life) orange juices and pasteurized (refrigerated) orange juices.

Esteve *et al.* (2005) studied nutritional characteristics of orange juices that can be found on the market and their evolution with time (1–6 weeks), and storage temperature in refrigeration (4 and 10°C). The ascorbic acid content of the juices decreased during storage, faster at 10 than at 4°C. The shelf life of the juices, based on 50% of the initial ascorbic acid concentration, was 42 days at 4°C and 35 days at 10°C.

Torregrosa *et al.* (2006) compared the shelf life of a PEF-treated mixture of orange and carrot juice with a heat-treated juice (98°C, 21 s), kept in refrigerated storage at 2 and 10°C. The concentration of ascorbic acid remaining in the pasteurized orange–carrot juice was 83%, whereas in the PEF-treated juice it was 90%. The ascorbic acid degradation rate in the juice stored at 2°C was less than in the juice stored at 10°C, and in the pasteurized juice it was greater.

F. CAROTENOIDS/VITAMIN A

Although they can easily be degraded, carotenoids can be retained during industrial processing if good technological practices are observed. Processing at lower temperatures and for the shortest possible treatment times is recommended. Retention of provitamin A is favored during storage at low temperatures, protected from the light, with the exclusion of oxygen and the presence of antioxidants. Rodriguez-Amaya (1993) reviewed the susceptibility or resistance to degradation of carotenoids during storage, performing a detailed analysis of the effects of factors such as carotenoid structure, nature of the matrix, available oxygen, moisture content/water activity, light, temperature, antioxidants, pro-oxidants, fatty acids, sulfites, and sodium chloride in models and foods. In 1997, the same author made an exhaustive review of the influence of manipulation of foods on carotenoids, analyzing the retention of provitamin A carotenoids in prepared, processed, and stored foods (Rodriguez-Amaya, 1997).

Lee and Coates (2003) studied changes in carotenoid pigments as a result of thermal pasteurization of Valencia orange juices. Total carotenoid pigment content loss was significant after thermal pasteurization at 90°C for 30 s. Thermal effects on carotenoid pigment contents, especially violaxanthin (−46.4%) and antheraxanthin (−24.8%), were clearly observed. With the loss of violaxanthin and antheraxanthin, lutein became the major carotenoid, followed by zeaxanthin, in pasteurized Valencia orange juice.

Effects of high-pressure treatment on orange juice carotenoids (β-carotene, α-carotene, zeaxanthin, lutein, and β-cryptoxanthin) associated with nutritional (vitamin A) values were investigated by De Ancos *et al.* (2002). Various high-pressure treatments (50–350 MPa) combined with different temperatures (30 and 60°C) and treatment times (2.5, 5, and 15 minutes) were assayed. The juice was subsequently stored at 4°C. The authors found that high-pressure treatments at 350 MPa produced significant increases of 20–43% in the carotenoid content of fresh orange juice (from 3.99 to 4.78–5.70 mg/liter). In the treatment at 350 MPa/30°C/5 minutes, they observed an increase in the vitamin A value from 164 to 238 RE/liter (45%). During storage of the orange juice subjected to high pressures, it was better preserved and even increased its total content of carotenoids and vitamin A activity. The authors indicated, therefore, that high-pressure treatment might be an efficient processing method for preserving orange juice as freshly squeezed for up to 30 days from the point of view of sensory (carotenoids) and nutritional (vitamin A) quality. However, when Bull *et al.* (2004) studied high-pressure-processed (600 MPa, 20°C, 60 s) Valencia and Navel orange juices, thermally pasteurized juice (65°C, 1 minute), and fresh juice, they did not find changes in the β-carotene concentration. They also observed no significant variations during storage at 4 and 10°C (12 weeks).

Torregrosa *et al.* (2005) studied the effect of pulse treatment on carotenoids in an orange–carrot mixture (80:20, v/v), using different field intensities (25, 30, 35, and 40 kV/cm) and treatment times (30–340 ms). In parallel, a convectional heat treatment (98°C, 21 s) was applied to the juice, and the results were compared. Of all the carotenoids studied, only five decreased significantly: 9-*cis*-violaxanthin + neoxanthin, antheraxanthin, α-cryptoxanthin, 9-*cis*-α-carotene, and 9-*cis*-β-carotene; the rest increased significantly, with the exception of lutein, mutatoxanthin, β-carotene, and ξ-carotene. The largest increase was in the concentration of 13-*cis*-β-carotene, followed by zeaxanthin and *cis*-β-cryptoxanthin. The decrease in violaxanthin + neoxanthin and antheraxanthin after pasteurization coincided with an increase in mutatoxanthin. Vitamin A increased in the orange–carrot juice in comparison with the untreated juice. When the juice was treated with pulses the authors observed that the concentrations of the 9-*cis*-violaxanthin + neoxanthin mixture, antheraxanthin, *cis*-β-cryptoxanthin, and 9-*cis*-α-carotene increased with treatment time, and that the rate of formation of those carotenoids increased with treatment intensity. They concluded that PEF processing generally caused a significant increase in concentrations of the various carotenoids identified in the orange–carrot mixture as treatment time increased, whereas when conventional pasteurization was used to process the juice, the concentrations of most of the carotenoids decreased or else showed a nonsignificant increase. With PEF treatment of the orange–carrot mixture at 25 and 30 kV/cm, it was possible to obtain a vitamin A concentration higher than that found in the pasteurized juice.

Cortés *et al.* (2006a) studied the effect of pasteurization and PEF treatment on carotenoids in orange juice. In their study, they found that PEF processing generally caused an increase in the concentrations of the carotenoids identified as treatment time increased. The decrease in the concentrations of carotenoids with provitamin A activity was very small, although it always decreased in comparison with untreated fresh juice. The concentration of total carotenoids decreased by 12.6% in the pasteurized orange juice in comparison with untreated fresh orange juice, as opposed to decreases of 9.6%, 6.3%, or 7.8% when fields of 25, 30, or 40 kV/cm were applied.

The same authors (Cortés *et al.*, 2006b) subsequently compared the evolution and modification of various carotenoids and vitamin A in untreated orange juice, pasteurized orange juice (90°C, 20 s), and orange juice processed with high-intensity pulsed electric fields (HIPEF) (30 kV/cm, 100 μs) during 7 weeks of storage at 2 and 10°C. The concentration of total carotenoids in the untreated juice decreased by 12.6% when the juice was pasteurized, whereas the decrease was only 6.7% when the juice was treated with HIPEF. Vitamin A was greatest in the untreated orange juice, followed by HIPEF-treated

orange juice (decrease of 7.52%), and, finally, pasteurized orange juice (decrease of 15.62%). The decrease in the concentrations of total carotenoids and vitamin A during storage in refrigeration was greater in the untreated orange juice and the pasteurized juice than in the HIPEF-treated juice. During storage at 10°C, auroxanthin formed in the untreated juice and the HIPEF-treated juice. This carotenoid is a degradation product of violaxanthin. The concentration of antheraxanthin decreased during storage and it was converted into mutatoxanthin, except in the untreated and pasteurized orange juices stored at 2°C. From the results obtained the authors concluded that nonthermal treatments had less effect than conventional thermal treatments on concentrations of total carotenoids and vitamin A in refrigerated orange juice. With HIPEF treatment there was no significant decrease in the concentration of any carotenoid in comparison with the untreated juice. During storage in refrigeration, total carotenoids and vitamin A were maintained for longer in the juice treated with HIPEF than in the juice conserved using conventional pasteurization treatments.

G. ANTHOCYANINS/FLAVONOIDS

Anthocyanins are included in the list of natural compounds known to work as powerful antioxidants. Blood orange anthocyanins are not very stable: during thermal treatment and storage they can degrade and form colorless or undesirable brown-colored compounds; the juice loses its bright red color and gains a brown color (Maccarone *et al.*, 1985).

Gil-Izquierdo *et al.* (2002) determined the effect of individual orange juice-processing techniques at industrial scale (pasteurization, concentration, and freezing) on phenolic compounds, and the effect of pasteurization on the pulp added to the final juice. In pulp, pasteurization led to degradation of several phenolic compounds, that is, caffeic acid derivatives, vicenin 2, and narirutin, with losses of 34.5%, 30.7%, and 28%, respectively. Flavonones were the major phenolic compounds in orange juice (narirutin, hesperidin, and didymin). Therefore, these flavonones were stable in the whole juice at pasteurization temperatures. Mild and standard pasteurization techniques did not show changes in the total content of phenolics in either the soluble fraction or the cloud fraction. No important changes were observed during the juice concentration process. In the case of the freezing technique, there was a dramatic decrease in phenolic compounds in comparison with the contents before this process (loss of 35%).

Del Caro *et al.* (2004) stored squeezed juices of various species and cultivars (Red Blush grapefruit, Salustiana, and Shamouti oranges) for 15 days at 4°C. They found a decrease in the amount of single flavonoids (narirutin,

hesperidin, and didymin), and therefore in the total flavonoid content in the orange juices. Grapefruit juice showed significant differences only for narirutin, hesperidin, and total content, whereas they did not observe variations in didymin, naringin, neohesperidin, and poncirin.

Fiore *et al.* (2005) studied the anthocyanin contents in pasteurized pure orange juice with 40 days of shelf life, and a sterilized beverage containing from 25% to 35% of concentrated orange juice, with a long shelf life (1 year). In the refrigerated juices, the major compounds were cyanidin glucoside and cyanidin 3-(6″-malonylglucoside). The concentration of anthocyanins found in the refrigerated juices was similar to those found in fresh orange juices, indicating that the pasteurization treatment and storage conditions applied to this type of commercial sample do not damage anthocyanins. In the sterilized juice almost no anthocyanins were detected, which was partly due to the lower percentage of fruit juice present in the beverage and also to the severe degradation of the anthocyanins. Kirca and Cemeroglu (2003) showed that losses of anthocyanins were 14.4%, 21.5%, and 60.9% after heating for 120 minutes at 70, 80, and 90°C, respectively. They also confirmed that anthocyanin levels decreased very rapidly for samples stored at 37 and 20°C, whereas samples stored at 5°C showed a remarkably slower degradation. Choi *et al.* (2002) pasteurized orange juice at 90°C for 30 s and observed a decrease of 25% in the total anthocyanin contents after 7 weeks' storage at 4.5°C.

Vanamala *et al.* (2006) studied the variation in the bioactive flavonoid contents in 12 made-from-concentrate (MFC) orange juices, 14 pasteurized not-from-concentrate (NFC) orange juices, and 5 NFC grapefruit juices. The results obtained showed that the total flavonoid content of the MFC orange juices (53 mg/100 ml) was significantly higher than that of the NFC orange juices (36 mg/100 ml). Hesperidin was found to be the major flavonoid, followed by narirutin and didymin in orange juice. Naringin, narirutin, and poncirin were the major flavonoids in all brands of grapefruit juices. The concentration of didymin was considerably higher in the NFC orange juices than in the MFC orange juices.

H. ANTIOXIDANT ACTIVITY

Fruits and vegetables contain many antioxidant compounds, especially ascorbic acid, phenolic compounds, thiols, carotenoids, and tocopherols.

Polydera *et al.* (2005b) studied the total antioxidant activity of high-pressure-processed fresh Navel orange juice (600 MPa, 40°C, 4 minutes) compared with thermally pasteurized fresh Navel orange juice (80°C, 60 s) as a function of storage in different isothermal conditions (0–30°C). They also evaluated the contribution of ascorbic acid (among other antioxidant

compounds of orange juice) to the total antioxidant activity. The reaction rate constant of *n*th order kinetics of the decolorization of ABTS radical cation solution, after addition of orange juice, was used as a measure of the total antioxidant activity. A mathematical description of this reaction rate constant as a function of storage temperature and time was established. Total antioxidant activity of both juices decreased during storage, mainly owing to ascorbic acid loss. High-pressure treatment led to a better retention of the antioxidant activity of orange juice compared with conventional pasteurization.

Piga *et al.* (2002) evaluated the evolution of the ascorbic acid concentration and overall antioxidant properties of the water-soluble fraction of minimally processed mandarin juice during storage at 4°C for 4, 8, and 12 days. The evolution of the antioxidant properties as affected by processing and storage conditions was not entirely related to ascorbic acid changes. The mandarin juices showed good retention of the original antioxidant activity at the end of storage.

Gil-Izquierdo *et al.* (2002) found that mild pasteurization, standard pasteurization, concentration, and freezing of orange juices did not affect the total antioxidant capacity of the juice, but they did affect it in pulp, where it was reduced by 47%. In their study, they also found that ascorbic acid provided at least 77% of the antioxidant capacity, whereas the contribution of phenolic compounds was comparatively irrelevant.

De Ancos *et al.* (2002) studied the effect of high-pressure treatments (50–350 MPa) combined with different temperatures (30 and 60°C) and times (2.5, 5, and 15 minutes) on the antioxidant capacity of orange juice, measured as free radical-scavenging capacity. During storage of the high-pressure-treated juice at 4°C, a decrease in the free radical-scavenging capacity of the untreated and high-pressure-treated orange juices was observed. There were significant differences between the untreated sample (37.5% inhibition) and the orange juices treated at 350 MPa/30°C for different treatment times (2.5, 5, and 15 minutes), with approximately 20% inhibition. They did not find a correlation between the carotenoid concentrations and free radical-scavenging capacity.

Lo Scalzo *et al.* (2004) studied the effect of thermal treatment on the antioxidant activity and antiradical activity of blood orange juice. The samples were processed in different ways: blanching at 80°C for 6 minutes before squeezing; pasteurizing the juice at 80°C for 1 minute; and blanching the fruit, then squeezing it, and pasteurizing the juice. The results obtained showed that the inhibition of enzymatically mediated linolenic acid peroxidation was increased by thermal treatments, while the scavenging effects toward $OH^{\bullet}$, generated by Fenton reaction, and $DPPH^{\bullet}$, decreased. The authors indicated

that the first point was sustained by the amounts of some phenolic substances with antioxidant action (anthocyanins and hydroxycinnamates). It was evident that the thermal treatments induced a decrease in the free radical-scavenging activity and were also responsible for the degradation of ascorbic acid in the blood orange juice.

During storage of orange juice (Salustiana and Shamouti oranges) and Red Blush grapefruit juice at 4°C for 15 days, Del Caro *et al.* (2004) found that the antioxidant capacity increased significantly in the Red Blush grapefruit juice, decreased in Salustiana orange juice, and did not change in Shamouti orange juice. They found that the antioxidant capacity of the juices was significantly correlated with the ascorbic acid content rather than with the presence of flavonone glycosides.

Fiore *et al.* (2005) studied two orange-based products: pasteurized pure juice with 40 days of shelf life (refrigerated), and a sterilized beverage containing a minimum 12% of fruit juice concentrate. All the assays gave clearly different values for the two groups of juice, with the refrigerated juice having greater antioxidant power than the sterilized juice. The total concentration of anthocyanin was positively correlated with ABTS (2,2′-azino-*bis* (3-ethyl benzthiazoline-6-sulfonic acid)) values and, of course, with the cyanidin glucoside content.

I. COLOR

The bright color of citrus juices is one of the important quality factors in citrus products. Detrimental changes in color, primarily caused by nonenzymatic browning, reduce consumer acceptance of citrus juices (Klim and Nagy, 1988). Storage can also cause an alteration in the color of juice because the action of heat, air, and light cause carotenoids to suffer oxidation, *cis*/*trans* changes, and changes in epoxide rings, with alteration of color.

Rodrigo *et al.* (2003a) studied the color of various refrigerated mixtures of orange and carrot juice (mild pasteurization, 77°C, 20 s) and their stability over time (1–6 weeks). The juices were studied at an optimal storage temperature of 4°C and a suboptimal storage temperature of 10°C. The color variations at 4°C were minimal (nonsignificant differences). At 10°C, no variations were seen in a^* (red to green color), but there was a reduction in the yellow component (b^*, yellow to blue color), a decrease in hue which was translated into a color that was more red and less yellow.

Lee and Coates (2003) studied the changes in color due to thermal pasteurization (90°C, 30 s) of Valencia orange juices. There was a perceptible color change after pasteurization of the juice, which led to the juice color becoming lighter and more saturated. Decreases in CIE a^* value and increases in CIE L^* (brightness), b^*, h^* (hue angle), and C^* [chroma, $(a^{*2} + b^{*2})^{1/2}$] were the

major color changes after pasteurization. Overall increases in reflected light might also influence the perception of color to a great extent in pasteurized orange juice. The total color difference ($\Delta E^* = \Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}$) compared with the fresh juice was 2.92 ± 0.97 ($p < 0.05$).

Fiore *et al.* (2005) studied sterilized (long shelf life) orange juices, and pasteurized and refrigerated orange juices purchased in the market. Measurement of the color density and polymeric color gave an immediate distinction between the two groups of samples. In the refrigerated orange juices, the color density was higher and the percentage of polymeric color was considerably lower than in the long shelf life orange juices. This clearly indicates that the color of sterilized orange juice is mainly due to the presence of chemical colorant added.

Vikram *et al.* (2005) studied changes in visual color (as an index of carotenoids) during thermal treatment of orange juice heated by conventional, infrared, ohmic, and microwave heating at various treatment temperatures (50, 60, 75, and 90°C) and times (0–15 minutes). The degradation of visual color was expressed by the combined ($a \times b$) values. The results obtained confirmed the influence of temperature on the degradation of color. The highest activation energy value corresponded to ohmic heating, followed by infrared, and it was lowest for microwave. Higher activation energy implies that a smaller temperature change is needed to degrade color more rapidly. The thermal resistance (Z) and activation energy values indicated that, with microwave heating, color degradation requires a higher temperature.

Esteve *et al.* (2005) studied various refrigerated orange juices purchased in the market and evaluated color variation during storage at 4 and 10°C for 6 weeks. During storage at 4°C, there were slight decreases in L^* and variations in a^* and b^* which were not significant in any of the three indices. With storage at 10°C there was a significant increase in L^* compared with the initial value, and there was also a significant increase in a and significant reductions in b^* in the juices. The color evaluations were always higher in the samples stored at 4°C than in those stored at 10°C.

Ibarz *et al.* (2005) presented UV–visible irradiation as a possible process for destruction of the polymeric compounds (melanoidins) present in fruit derivative juices. In order to study the process, apple, peach, and lemon juices with different soluble solid contents and different browning degrees were irradiated using a lamp that emitted in the UV–visible irradiative spectrum. The data obtained showed an increase in the brightness of the juices with irradiation time. It was possible to describe the increase by means of a first order kinetics. The authors found that the colorimetric parameters a^* and b^* both decreased with irradiation time, indicating that the effect that produced the irradiation was contrary to the browning process. An increase in soluble solids and in higher colored polymer contents led to a smaller percentage decrease in the colorimetric parameters.

When they studied the effect of PEF and heat treatment on the color of orange juice, and its subsequent storage at 4 and 10°C, Yeom *et al.* (2000a) observed that the PEF-treated orange juice had higher brightness (L), and higher hue angle values than the heat-pasteurized orange juice during storage at 4°C, whereas they saw no differences when the juice was stored at 22°C.

Cortés *et al.* (2006a) found that HIPEF-treated and pasteurized orange juices showed a greater tendency to yellow color and a lesser tendency to red compared with untreated orange juice, although this tendency was greater in the pasteurized juice.

Polydera *et al.* (2003) found that color measurements of orange juice stored in laminated flexible pouches indicated that, although the color changed slightly with storage time (1–2 months), the change did not correlate with the type of processing (500 MPa at 35°C and thermal pasteurization at 80°C for 60 s) and storage temperature (0–15°C). The same authors (Polydera *et al.*, 2005a) subsequently studied a high-pressure treatment of 600 MPa at 40°C for 4 minutes and postprocessing storage of fresh orange juice at 0–30°C compared with conventional thermal pasteurization (80°C, 60 s). HPP treatment led to lower rates of color change compared with thermal pasteurization at all the storage temperatures studied, except at 30°C (which is above the range of normal storage temperatures). An increase in storage temperature resulted in higher rates of browning of the orange juice. Similar results to those found in these studies were obtained by Bull *et al.* (2004) when they studied high-pressure processed (600 MPa, 20°C, 60 s) Valencia and Navel orange juices and compared them with thermally pasteurized juice (65°C, 1 minute) and fresh juice, and stored them at 4 and 10°C for 12 weeks. In comparison with untreated orange juice, HPP or thermal treatment had no effect on the color of the juices. The results showed that there was an increase in the total color difference with time, regardless of the treatment.

J. PECTINESTERASES

Collet *et al.* (2005) stated that the study of pectinesterase inactivation behavior is important because pectinesterase is responsible for juice cloud stability loss, is composed of several isoenzymes, and occurs naturally in orange. Freshly squeezed juice of Pera orange (*Citrus sinensis*) was pasteurized at temperatures of 82.5, 85.0, and 87.5°C. At least five runs with different holding times were performed for each temperature. As the isothermal curves obtained showed deviations from the expected first-order kinetics, the data was statistically treated by applying a nonlinear regression, and the estimated best fit was a three-parameter-multicomponent-first-order model. At 82.5°C, the isothermal curves showed a nonzero asymptote of inactivation, indicating that at this temperature the most heat-resistant

isoenzyme could not be totally inactivated. The 87.5°C isotherm showed the highest inactivation among the temperatures studied. These observations agree with the batch inactivation data found in the literature, but the holding time required for a satisfactory inactivation was significantly shorter than the time found in the literature, suggesting that the proposed model can be used to design continuous processes with more accuracy.

Ingallinera *et al.* (2005) compared total pectinesterase activity of Sicilian blood oranges (Sanguinello, Moro, and Tarocco) with the blonde Navel cultivar, checking enzyme stability with various pasteurization time and temperature (70–85°C) conditions in order to optimize the heat treatment and increase the shelf life of the pasteurized juice. To do this they stored the juices at 4, 15, and 25°C for times ranging between 10 minutes and 50 days. Decimal reduction time and temperature (D and z) and the kinetic constant (k) were established to optimize and increase the shelf life of the pasteurized juice. Finally, a heat treatment (85°C × 3 minutes) of both microbiological and enzymatic efficacy was developed that does not compromise anthocyanin stability.

Heat pasteurization of orange juice is designed to inactivate PME, which is more heat resistant than vegetative microorganisms (Chen and Wu, 1998). PME exhibits greater heat and pressure resistance than common orange juice spoilage microorganisms and can thus be used as a processing index for both HHP and thermal processes (Goodner *et al.*, 1998; Parish, 1998c; Versteeg *et al.*, 1980).

Quoc *et al.* (2006) studied the development of a process that permitted quick inactivation of PME, which is present in cloudy or unclarified apple juice. This enzyme is responsible for opalescence instability. In order to achieve this objective, acidification of the apple juice to pH 2.0 was performed by electrodialysis, followed by mild heat treatment at temperatures of 40, 45, and 50°C for 0–60 minutes. Opalescence of the adjusted juice was more stable than for an untreated cloudy apple juice when stored at 4°C for 3 months.

Bayindirli *et al.* (2006) studied the effectiveness of treatment on pectinesterase activity in orange juice, comparing the application of high hydrostatic pressure with a mild heat treatment. The residual pectinesterase activity in the orange juice after treatment at 450 MPa and 50°C for 30 minutes was determined as approximately 7 ± 1.6%. This compares with 12 ± 0.2% after a treatment of 40°C and 450 MPa for 60 minutes. The inactivation was irreversible and the enzyme was not reactivated when stored at 4 and 25°C for 1 week.

Guiavarc'h *et al.* (2005) studied combined thermal and high-pressure inactivation of PME in white grapefruit. The results showed that combined mild heat and high-pressure processing cannot be used for full inactivation of PME in grapefruit juice. However, by eliminating up to 80% of the PME

activity (labile fraction), this treatment can probably contribute to a significant delay of the cloud loss defect observed in grapefruit juices while allowing pasteurization and good quality retention. Combining high-pressure and mild-temperature processing with other nonthermal approaches (e.g., use of PME-inhibitor) could be of interest for the creation of juices with extended shelf life.

Lacroix *et al.* (2005) studied the effect of dynamic high-pressure (DHP) homogenization, alone or in combination with prewarming, on PME activity and opalescence stability of orange juice. DHP without heating reduced PME activity by 20%. PME inactivation was further increased by adjusting the pH downward prior to treatment. The orange juice was stored for 16 days at 30°C in order to accelerate loss of opalescence compared with storage at 4°C. These results suggest that the opalescence stability of orange juice treated by DHP does not depend entirely on PME activity but also depends on particle size reduction and structural changes to pectin resulting from treatment. The freshness attributes of orange juice treated by warming were improved by DHP treatment. This treatment at pH 3.8 resulted in opalescence being maintained for 8 days, compared with 1 day for the control, 2 days for prewarmed but not pressure-treated, and about 10 days for pasteurized juice.

In the study carried out by Bull *et al.* (2004), PME was not completely deactivated in the Valencia juice (pH 4.3) by HPP (600 MPa, 20°C, 60 s) or thermal treatment. In the Navel orange juice (pH 7.3), PME was reduced with thermal treatment (85°C for 25 s) and with HPP (45%).

Polydera *et al.* (2004) studied the inactivation kinetics of endogenous PME in freshly squeezed orange juice under high hydrostatic pressure (100–800 MPa) combined with moderate temperature (30–60°C). PME inactivation followed first order kinetics with a residual PME activity (5–20%) at all pressure–temperature combinations used. Pressure and temperature were found to act synergistically, except in the high temperature–low pressure region, where an antagonistic effect was found.

Yeom *et al.* (2000b) studied the effect of PEF treatment (35 kV/cm, 59 μs) and heat (94.6°C for 30 s) on the relative PME activity of orange juice during storage at 4 and 22°C. PEF treatment decreased 88% of PME activity, and the inactivated PME was not restored at 4 and 22°C for 112 days. Heat pasteurization inactivated 98% of PME activity.

K. POLYPHENOL OXIDASE

The purpose of the study by Quoc *et al.* (2006) was to develop a process to enable quick inactivation of the polyphenol oxidase enzyme, which is present in cloudy or unclarified apple juice. This enzyme is responsible for enzymatic

browning. In order to achieve this objective, the apple juice was acidified to pH 2.0 by electrodialysis (bipolar–anionic membranes), followed by mild heat treatment at temperatures of 40, 45, and 50°C for a duration of 0–60 minutes. It was shown that the application of mild heat treatment at 45°C for 5 minutes to the acidified juice was sufficient for quick inactivation of the enzyme. The authors also found that the organoleptic properties of the juice were preserved after treatment, and the adjusted juice (pH readjusted to its initial value) had a better color than untreated apple juice when the juice was stored at 4°C for 3 months.

Bayindirli *et al.* (2006) studied the effectiveness of treating polyphenol oxidase activity in apple juice by applying high hydrostatic pressure with mild heat treatment (350 MPa at 40°C).

L. YEAST

Many organisms, particularly acid-loving or acid-tolerant bacteria and fungi (yeasts and molds), can use fruit as substrate and cause spoilage, producing off-flavors and odors and product discoloration. If the contaminating microorganisms are pathogens, they could also cause human illness.

Toxigenic fungi, on the other hand, under favorable conditions could produce mycotoxin in fruit products such as juice (Varma and Verma, 1987). Before pasteurization, fruit juices contain a microbial load representative of the organisms normally found on fruits during harvesting plus contaminants added postharvest (during transport, storage, and processing). Many reports of bacterial growth in fruit juices exist in the literature, but most of the ones describing human illness due to contaminated juice deal with unpasteurized juice (Besser *et al.*, 1993; Krause *et al.*, 2001). Some investigations regarding fungal contamination of pasteurized fruit juice are also available (Kurtzman *et al.*, 2001; Mendoza *et al.*, 1982).

Most of these reports have shown yeasts to be the predominant fungi involved in juice spoilage (Parish and Higgins, 1989). Yeast spoilage of fruit juice can result in formation of haze, production of CO_2 and off-odors, and changes in color.

Tchango *et al.* (1997) studied the resistance of *Candida pelliculosa* and *Kloeckera apis*, two spoilage yeasts, isolated from pasteurized tropical fruit juices and nectars produced in Cameroon, in pineapple juice, guava nectar, and passion fruit nectar, as it relates to the pasteurization process of this beverages. The results showed that 22% of the pasteurized fruit juice samples tested contained live fungi, due either to inadequate pasteurization or to postpasteurization contamination during cooling, bulk storage, and bottling. Juice processors and packers should therefore take care to eliminate yeasts from juices and pack these products under strict aseptic conditions in

order to avoid losses due to yeast spoilage, which results in products of poor or unacceptable quality.

Silva *et al.* (1999) investigated the influence of temperature (85–97°C), total soluble solids (5–60 °Brix or wt.%), and pH (2.5–6.0) on *D*-values (decimal reduction time) of *Alicyclobacillus acidoterrestris* (type strain, NCIMB 13137) spores, and they fitted a model using response surface methodology. Within the factor ranges studied, temperature was the parameter that most affected the *D*-value. Soluble solids came next, and pH value was last. In general, *D*-values measured in real fruit systems, such as orange, apple, and grape juices, black currant concentrates, cupuaçu (exotic fruit) extract, and orange juice drink, were higher than those predicted by the malt extract broth model.

Parish (1998c) obtained decimal reduction times (*D*-values) for *Saccharomyces cerevisiae* ascospores inoculated into pasteurized orange juice ranging from 4 to 76 s at pressures between 500 and 350 MPa. At the same pressures, *D*-values of *S. cerevisiae* vegetative cells ranged from 1 to 38 s, while for the native microflora in nonpasteurized Hamlin orange juice they ranged between 3 and 74 s. The corresponding *z*-values were 123, 106, and 103 MPa for ascospores, vegetative cells, and native microflora, respectively.

Alwazeer *et al.* (2003) studied the effect of both redox potential (Eh) and pasteurization of orange juice on growth recovery of *S. cerevisiae* during storage at 15°C for 7 weeks. Oxidizing conditions were the most effective for thermal destruction of *S. cerevisiae*, while reducing conditions decreased recovery of heated cells of *S. cerevisiae*.

Tahiri *et al.* (2006) evaluated the potential of DHP technology to inactivate *S. cerevisiae*. The inactivation efficacy of DHP depended on the pressure applied and the number of passes.

Garde-Cerdan *et al.* (2006) studied the effect of thermal and PEF treatments on several physicochemical properties and a population of inoculated *S. cerevisiae*. Both technologies reduced the population of the spoilage microorganism inoculated in grape juice. No viable cells were observed after thermal processing of grape juice, whereas PEF treatment achieved four logarithmic reductions of the microbial viability.

Tournas *et al.* (2006) studied 65 pasteurized fruit juice samples (apple, carrot, grapefruit, grape, and orange juices, apple cider, and soy milk) purchased from local supermarkets in the Washington, DC area and found that 22% of the pasteurized fruit juice samples tested contained live fungi due either to inadequate pasteurization or to postpasteurization contamination during cooling, bulk storage, and bottling. Some of the yeasts isolated from these products were capable of growing under refrigeration, completely spoiling the product before its expiration.

Yeast species commonly isolated from fruit juices were *Rhodotorula rubra, Candida lambica, Candida sake*, and *K. apis*, with *C. lambica* being the organism most frequently encountered in these products. Small numbers of *Penicillium* and *Fusarium* spp. were isolated from 20%, whereas *Geotrichum* spp. were present in 40% of the grapefruit juice samples tested. All other products contained no molds. The fact that these organisms were present in very low quantities indicated that they were random contaminants not able to grow in the refrigerated juice.

Various authors have studied the inactivation of pathogenic and nonpathogenic microorganisms, mesophile flora, molds, and yeast flora (Abram *et al.*, 2003; McDonald *et al.*, 2000; Rodrigo *et al.*, 2001, 2003b; Spilimbergo *et al.*, 2003). A number of authors have studied the evolution of quality and safety factors in orange juice after nonthermal treatment, in some cases making a comparison with the evolution after heat treatment (Ayhan *et al.*, 2001; Jia *et al.*, 1999; Linton *et al.*, 1999; Yeom *et al.*, 2000a,b; Zook *et al.*, 1999).

M. *LACTOBACILLUS BREVIS*

Elez-Martínez *et al.* (2005) studied the inactivation of spoilage microorganisms such as *Lactobacillus brevis* by HIPEF and pasteurization. The effects of HIPEF parameters (electric field strength, treatment time, pulse polarity, frequency, and pulse width) and heat pasteurization (90°C/1 minute) were evaluated on samples of orange juice inoculated with *L. brevis* (108 CFU/ml). HIPEF processing of orange juice was more effective in inactivating *L. brevis* than thermal processing. The extent of microbial inactivation depended on the processing parameters ($p < 0.01$). *L. brevis* destruction was greater when the electric field strength and treatment time increased, and also when the pulse frequency and pulse width decreased. *L. brevis* was inactivated to a maximum of 5.8-log reductions when inoculated orange juice was processed at 35 kV/cm for 1000 μs using a 4-μs pulse width in bipolar mode and 200 Hz at less than 32°C. Mechanical breakdown of cell walls was observed in *L. brevis* when orange juice was processed by HIPEF.

N. *LACTOBACILLUS PLANTARUM*

Alwazeer *et al.* (2003) studied the effect of both redox potential (Eh) and pasteurization of orange juice on growth recovery of microorganisms during storage at 15°C for 7 weeks. Three Eh conditions, +360 (ungassed), +240 (gassed with N_2), and −180 mV (gassed with N_2–H_2) were applied to orange

juice. Both thermal destruction and recovery of sublethally heat-injured cells of *Lactobacillus plantarum* were investigated. Oxidizing conditions were the most effective for thermal destruction of *L. plantarum*.

Tahiri *et al.* (2006) evaluated the effect of DHP technology to inactivate pathogenic and spoilage microflora in orange juice. The inactivation efficacy of DHP depended on the pressure applied and the number of passes.

O. *E. COLI*

Exposure of *E. coli* to microwave treatments results in a reduction of the microbial population in apple juice. Cañumir *et al.* (2002) determined the effect of pasteurization at different power levels (270–900 W) on the microbial quality of apple juice, using a domestic 2450 MHz microwave. The data obtained were compared with conventional pasteurization (83°C for 30 s). Apple juice pasteurization at 720–900 W for 60–90 s resulted in a 2- to 4-log population reduction. Using a linear model, the *D*-values ranged from 0.42 ± 0.03 minutes at 900 W to 3.88 ± 0.26 minutes at 270 W. The value for z was 652.5 ± 2.16 W (58.5 ± 0.4°C). These observations indicate that inactivation of *E. coli* is due to heat.

Heinz *et al.* (2003) focused on improving the energy efficiency of PEFs treatment for pasteurization of apple juice inoculated with *E. coli* by investigating the relation between the reduction achieved in the survivor count and electric field strength and treatment temperature. To evaluate the thermal load of the product the pasteurization unit and cook value, key benchmarks for the thermal load, were used to compare PEF and conventional heat treatment.

Tahiri *et al.* (2006) evaluated the potential of DHP technology to inactivate *E. coli* O157:H7 ATCC 35150 in orange juice. Complete inactivation and 5-log reduction of *E. coli* O157:H7 were achieved in orange juice at 200 MPa and 25°C after 5 and 3 passes, respectively.

Bayindirli *et al.* (2006) found that high hydrostatic pressure with mild heat treatment (350 MPa at 40°C) caused inactivation of *E. coli* O157:H7 933 in apple, orange, apricot, and sour cherry juices.

P. *STAPHYLOCOCCUS AUREUS*

Bayindirli *et al.* (2006) studied the effect of high hydrostatic pressure with mild heat treatment on *Staphylococcus aureus* 485 in apple, orange, apricot, and sour cherry juices. The results showed that commercially practicable pressure processes can be used to inactivate even the most pressure-resistant microorganisms. The use of HPP (350 MPa) at 40°C could be considered

for treating the fruit juices studied to improve microbial kill, with respect to the pressure-resistant strains of the pathogens studied.

Q. *SALMONELLA ENTERITIDIS*

Korolczuk *et al.* (2006) used a pilot-scale continuous PEF treatment of liquid products to study the effects of energy input (0–300 kJ/kg), electric field strength (25–70 kV/cm), square wave pulse width (0.05–3 μs), and initial product temperature (4–20°C) on *Salmonella enteritidis*. For energy input (Q), 0–100 kJ/kg, the decimal reduction number can be considered as linearly related to Q, with the decimal reduction energy (Q_D) varying between 44 ± 3.2 kJ/kg for 0.05 μs, 37 ± 2.5 kJ/kg for 0.1 μs, and 32 ± 1.4 kJ/kg for 0.25–3 μs pulse width. For Q = 0–300 kJ/kg, the relation between Q and $\log(N_0/N)$ was of power law type, with the threshold energy level $Q_0 = 9 \pm 2.6$ kJ/kg and the power coefficient 3.17 ± 0.21.

Bayindirli *et al.* (2006) studied the effect of high hydrostatic pressure on *S. enteritidis* FDA in apple, orange, apricot, and sour cherry juices. They found that commercially practicable pressure processes (350 MPa at 40°C) can be used to inactivate *S. enteritidis*.

R. *NEOSARTORYA FISCHERI*

Ascospores of heat-resistant molds can survive the heat pasteurization treatments normally applied to fruits and derivatives, and may spoil these products by germination and subsequent growth under reduced oxygen conditions. *Neosartorya fischeri, Byssochlamys fulva, Byssochlamys nivea, Talaromyces flavus*, and *Eupenicillium* are some of these fungi. These heat-resistant molds are also known to produce various mycotoxins during their growth in fruit products (Rajashekhara *et al.*, 2000). *N. fischeri* (anamorph *Aspergillus fischeri*) is one of the most frequently reported heat-resistant molds causing spoilage in fruit products (Nielsen, 1991).

Salomao *et al.* (2007) studied the heat resistance of *N. fischeri* in three different juices (apple, pineapple, and papaya). The optimum heat activation temperature and time for ascospores of *N. fischeri* (growth for 30 days at 30°C) was 85°C for 10 minutes. The z-values for apple, papaya, and pineapple juices were 5, 5.5, and 5.9°C, respectively. The sterilization F-values (4-log reduction) for apple, pineapple, and papaya juices were 56.3, 38.0, and 7.2 s, respectively. Considering the thermal treatments commercially applied to pineapple (96°C/30 s) and apple juices (95°C/30 s), the authors concluded that such treatments would not guarantee that less than 1 ascospore in each set of 103 packs survives. Only the treatment applied to papaya juice (100°C/30 s) would be sufficient because the F-value was less than 30 s.

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