REVIEWS

Phenolics of Apple, Pear, and White Grape Juices and Their Changes with Processing and Storage—A Review[†]

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The phenolic composition of apple, pear, and white grape juices is reviewed. Attention is given to the importance of phenolics in fruit juices, the factors that affect the phenolic composition of fruit juices, and the improvements in the techniques that have been employed in the analysis of phenolics.

INTRODUCTION

Phenolic compounds are secondary plant metabolites that have important roles in providing flavor and color characteristics of fruit juices and wines. Phenolic compounds have also been characterized as "potential causes of instability" as they are involved in formation of undesirable sediments (Heatherbell, 1984) and yellow and brown pigments (Montgomery, 1983). The international fruit juice market has been growing rapidly over the past few years (Hartog, 1988). This is partly due to the rise in the standard of living, the increased demand for natural products, and the introduction of many new products formulated with fruit juices. An understanding of the phenolic composition and the factors that affect the phenolics is critical in the design of juice products. Data on the phenolic composition can also be useful in determining the authenticity of juice products.

CLASSIFICATION OF PHENOLICS IN APPLE, PEAR, AND WHITE GRAPE

The phenolic constituents of importance in apple, pear, and grape juices can be divided into two groups: (a) phenolic acids and related compounds and (b) flavonoids.

The term phenolic acid includes the cinnamic acids (C_6–C_3) and the benzoic acids (C_7). Figure 1 shows the structures of commonly occurring cinnamic and benzoic acids. Caffeic and coumaric acid are cinnamics widely distributed and common to apple, pear, and grape. Cinnamic acids have the trans configuration, but exposure to ultraviolet light can cause isomerization to cis (Engelsma, 1974). They occur naturally in combination with other compounds, usually in the form of esters. The ester of caffeic with quinic acid, chlorogenic acid, is a classic example. On the contrary, benzoics usually occur as free acids.

Flavonoids are built upon a diphenylpropane skeleton (C_6–C_3–C_6) in which the three-carbon bridge between the phenyl groups is usually cyclized with oxygen. The different classes within the group differ in the number of substituent hydroxyl groups, degree of unsaturation, and degree of oxidation of the three-carbon segment. Figure 2 shows the flavonoid structures that are present in apple, pear, and white grape. Flavan-3-ols (catechins) are some of the most widely occurring flavonoids. They have two asymmetric carbons (2, 3) and therefore four possible isomers. The (+)- and (−)-catechins have the number 2 and 3 hydrogens in trans configuration, while they are cis in the epicatechins. Flavan-3,4-diols are also referred to as leucoanthocyanidins. Polymeric structures based on the flavan-3,4-diols and flavan-3-ols make up the procyanidins (condensed tannins). Widely present procyanidin dimers are shown in Figure 3. These dimers, on heating with acid, release one molecule of catechin and one of anthocyanidin (cyanidin). Both C–C and C–O bonds are

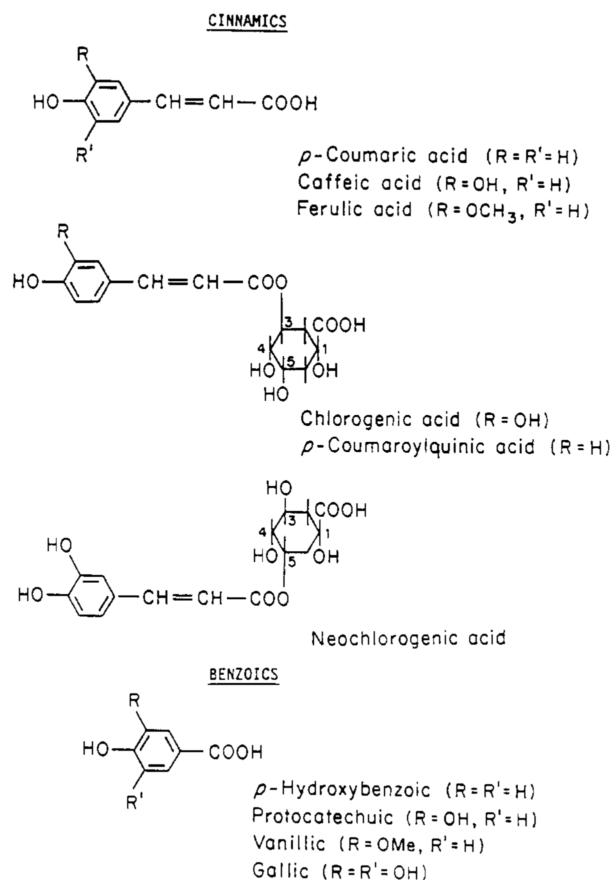

Figure 1. Structures of common phenolic acids.

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[†] Technical Paper No. 8787 from the Oregon State University Agricultural Experiment Station.

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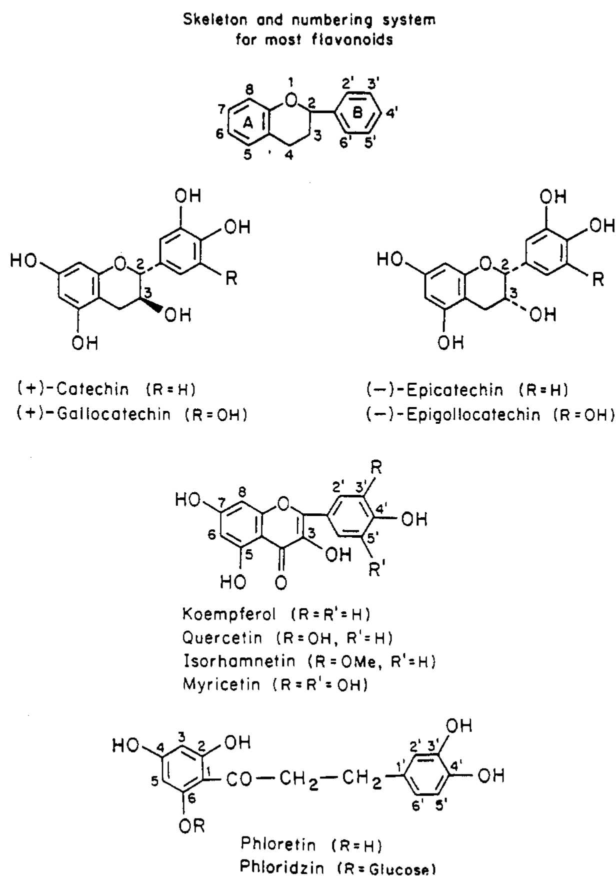

Figure 2. Important flavonoid structures present in apple, pear, and white grape.

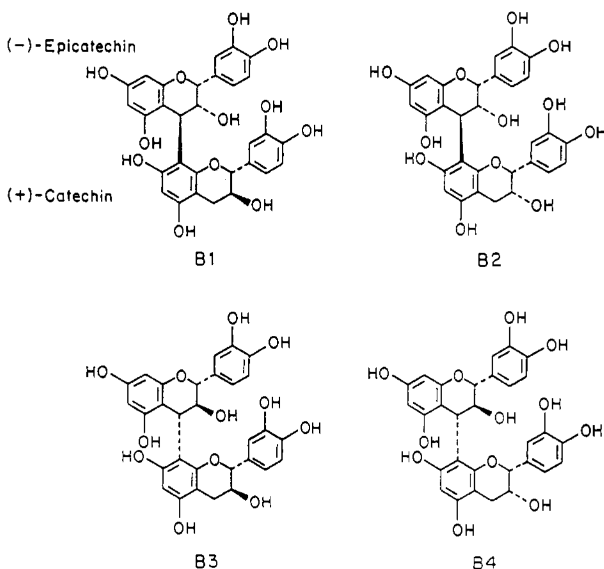

Figure 3. Common procyanidins of group B.

possible in these structures, but usually there is a C–C bond from the C-4 of one unit to the C-6 or C-8 of the other. Flavonols, another widespread flavonoid group, occur mostly as glycosides. Since the formation of flavonol glycosides depends on light (Herrmann, 1976), it is not surprising that they are found mainly in the skins of the fruit (Walker, 1964). The most common aglycon is quercetin, while the most common glycosides are the rutinoside, galactoside, glucoside, arabinoside, xyloside, and rhamnoside. Phloridzin, a dihydrochalcone glucoside, is characteristic to the genus *Malus*, and it has been reported to have regulatory effects on apple seedlings (Jones, 1976). Anthocyanins, a major flavonoid class, impart the characteristic color of red juices. However, they are not present in white grapes, and their presence in the skins of apples and pears does not influence typical juice processing operations. Therefore, anthocyanins will not be discussed in this paper.

Arbutin, which fits neither the group of phenolic acids nor the group of flavonoids, is a hydroquinone glucoside characteristic of pear, *Pyrus communis* L. (Williams, 1957; Duggan, 1967).

Importance of Phenolics in Fruit Juices. Bitterness and astringency have long been associated with phenolic fractions (Rossi and Singleton, 1966; Singleton and Esau, 1969). Bitterness, detected mostly at the back and the sides of the tongue, is generally considered to be caused by an interaction between polar molecules and the lipid portion of the taste papillae membrane (Koyama and Kurihara, 1972) and thus is critically dependent on the relative lipid solubility of the bitter material. On the other hand, astringency affects the whole tongue uniformly and results from nonspecific and somewhat irreversible hydrogen bonding between *o*-diphenol and protein in the mouth (Bate-Smith, 1973). This causes the distinctive drying and puckering sensation which is difficult to remove and makes aftertaste assessment a problem. These mechanisms suggest that procyanidins are the most significant phenolic fraction for these sensory characteristics. Studies reported in the literature show that the contribution of phenolic acids to astringency is small; phloridzin tastes bitter, but at its low levels in most apple juices can make but a small contribution to the overall bitterness (Lea and Timberlake, 1974). Although the pure procyanidins display both bitterness and astringency, the balance between these sensations depends on the molecular weight. Taste panel work has shown that tetrameric procyanidins are the most bitter, while the more polymeric ones are more astringent on an equivalent weight basis (Lea and Arnold, 1978).

Browning of fruits and fruit juices has also been related for a long time to the phenolic constituents (Hulme, 1953). The formation of yellow and brown pigments in fruit juice is controlled by the levels of phenolic compounds, the presence of oxygen, and the amount of polyphenol oxidase (PPO) activity (Coseteng and Lee, 1987). An excellent review on the properties of the enzyme has been presented by Mayer and Harel (1979). In summary, PPO is a membrane-bound enzyme that contains copper and catalyzes the hydroxylation of monophenols leading to formation of *o*-diphenol compounds (cresolase activity) and the oxidation of *o*-dihydroxy compounds to quinones (catecholase activity). It is these quinones that undergo polymerization to impart the characteristic yellow and brown pigments. Oxidative activity on *p*-diphenols (laccase activity) is usually present in fruits infected with molds. The phenolic yields of juices are critically dependent on the activity of the enzyme during processing and will be discussed under the section of factors affecting the phenolic composition.

The procyanidins play a particularly important role in the formation of hazes and sediments by both oxidative and nonoxidative mechanisms (Lea, 1984). Unoxidized procyanidins can hydrogen bond with proteins and subsequently form insoluble complexes. Polymerization of procyanidins, which can occur by partial hydrolysis in acid solutions and repolymerization, may lead to the formation of large and unstable polymers. Consequently, procyanidins may eventually throw down hazes and deposits with or without the involvement of proteins (Johnson et al., 1968; Heatherbell, 1984). Such hazes are likely to be dissolved in temperatures around 60 °C (Lea, 1984).

Procyanidin oxidation in the juice generates highly reactive intermediates that can complex irreversibly with

Table I. Phenolic Composition of Apple Fruit and Apple Juice

phenolic compound	content[a]	variety/comments	references
cinnamics			
chlorogenic, caffeic coumarylquinic, coumaric, chlorogenic and coumarylquinic isomers, ferulic	qualitative		Hulme, 1953; Sondheimer, 1958; Williams, 1958; Scarpati and Esposito, 1963; Walker, 1963; Durkee and Poapst, 1965; Mosel and Herrmann, 1974; Whiting and Coggins, 1975
chlorogenic	93–232, 0–208	laboratory and commercial juice samples, resp	Brause and Raterman, 1982
	25.6–136	fruit; Empire, Cortland, McIntosh, G. Delicious, Rome, Rhode Island Greening, Classic Delicious	Coseteng and Lee, 1987
	1.5–228	Golden Delicious, Jonathan, McIntosh, G. Smith	Lee and Wrolstad, 1988
	8.8–113.7	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
	40–60, 30–60	flesh, skin resp; range average during maturation–storage; includes G. Delicious, Empire, Rhode Island Greening	Burda et al., 1990
chlorogenic isomers	0.5–14.5	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
coumarylquinic	1–34	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
caffeic	1.9–9.6	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
coumaric	1.5–6.2	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
catechins/procyanidins			
catechin epicatechin; catechin/epicatechin-based polymers up to heptamers	qualitative		Siegelman, 1955a,b; Schmidt and Neukom, 1969; Mosel and Herrmann, 1974; Lea and Timberlake, 1974; Lea, 1978, 1984
catechin	0–18.4	fruit; Empire, Cortland, McIntosh, G. Delicious, Rome, Rhode Island Greening, Classic Delicious	Coseteng and Lee, 1987
	1.7–16.9	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
epicatechin	30–23.4	fruit; Empire, Cortland, McIntosh, G. Delicious, Rome, Rhode Island Greening, Classic Delicious	Coseteng and Lee, 1987
	3.5–44.4	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
	10–140, 130–670	flesh, skin resp; range average during maturation–storage; includes G. Delicious, Empire, Rhode Island Greening	Burda et al., 1990
B2	40–150, 120–600	flesh, skin resp; range average during maturation–storage; includes G. Delicious, Empire, Rhode Island Greening	Burda et al., 1990
total procyanidins[b]	8.6–86.5	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
flavonols			
gal, glu, xyl, arab, rha, rut glycosides of quercetin	qualitative		Siegelman, 1955a; Walker, 1964; Duggan, 1967; Dick et al., 1987; Oleszek et al., 1988
quercetin rutinoside	trace–28.8	fruit; Empire, Cortland, McIntosh, G. Delicious, Rome, Rhode Island Greening, Classic Delicious	Coseteng and Lee, 1987
total quercetin glycosides (gal, glu, xyl, arab, rha)	6.3–51.8	juice; includes diffusion extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
	0, 720–1070	flesh, skin resp; range average during maturation–storage; includes G. Delicious, Empire, Rhode Island Greening	Burda et al., 1990
dihydrochalcones			
phloridzin, phloretin xylglu	qualitative		Duggan, 1967; Whiting and Coggins, 1975; Wilson, 1981; Oleszek, 1988
phloridzin	4.4–18.8	fruit; Empire, Cortland, McIntosh, G. Delicious, Rome, Rhode Island Greening, Classic Delicious	Coseteng and Lee, 1987
	3.3–56	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
	10, 100–150	flesh, skin resp; range average during maturation–storage; includes G. Delicious, Empire, Rhode Island Greening	Burda et al., 1990
phloretin xylglu	2.2–18.6	juice; includes diffusion-extracted samples; G. Smith, R. Delicious, McIntosh, Spartan	Spanos et al., 1990
phloretin xylgal	10–30, 60–230	flesh, skin resp; range average during maturation–storage; includes G. Delicious, Empire, Rhode Island Greening	Burda et al., 1990

[a] Micrograms per milliliter of juice or micrograms per gram of fruit. [b] Dimers (B1, B2, B3, B4), trimers, tetramers, and other unknown procyanidins.

each other and with proteins to form insoluble complexes which are not dissolved on warming (Lea, 1984). Therefore, oxidation of juice, which contains high levels of procyanidins, tends to promote formation of haze. An early oxidation of juice in the presence of pulp may prevent the formation of hazes in the finished juice products (Pilnik and de Vos, 1970). In this case, oxidation of procyanidins leads to the tanning of the pomace and significant removal of these constituents. Excessive pulp oxidation, however, can progressively reduce the "normal" character (color and flavor) of apple juice, and it has been considered detrimental for the production of ciders (Lea and Timberlake, 1978).

PHENOLIC COMPOSITION OF APPLE JUICE

The phenolic composition of apple fruit and fruit juice is summarized in Table I. Table I includes data from various varieties, maturity stages, and processing treatments and as a result reflects the effect of these parameters on the phenolic composition.

Several investigators (Hulme, 1953; Walker, 1963; Durkee and Poapst, 1965) have reported that chlorogenic acid (3-*O*-caffeoyl-D-quinic) is the major cinnamic derivative found in apples. Other positional isomers of chlorogenic acid are also present in apple fruits. Sondheimer (1958) reported trace amounts of isochlorogenic, "band 510" (band name relates to elution fraction), and neochlorogenic acid in McIntosh apples. Scarpati and Esposito (1963) identified "band 510" as 4-*O*-caffeoyl-D-quinic acid and neochlorogenic as 5-*O*-caffeoyl-D-quinic acid. Isochlorogenic acid was later identified as a mixture of several dicaffeoylquinic acids (Mosel and Herrmann, 1974). The presence of 3-*O*-*p*-coumarylquinic acid and 4-*O*-*p*-coumarylquinic acid has also been reported (Williams, 1958; Whiting and Coggins, 1975). Traces of ferulic acid have also been found in hydrolyzed core tissue extracts from McIntosh apples (Durkee and Poapst, 1965).

The presence of (+)-catechin and (−)-epicatechin in apple juice has been well documented (Siegelman, 1955a,b; Mosel and Herrmann, 1974). Procyanidins are also known

to occur in apple juice. It is important to stress that these oligomeric and polymeric compounds are preformed in the fruit and are not the result of processing or storage of apple juice. Schmidt and Neukom (1969) characterized the structure of the major procyanidin in Waldhoefler apples as the dimer B2 on the basis of mass, IR, and NMR spectra. Later studies (Lea and Timberlake, 1974; Lea, 1978, 1984) have revealed a range of procyanidins from dimeric up to heptameric, where seven epicatechin or catechin units are joined together. Mixed procyanidins of epicatechin and catechin and procyanidins of alterntive stereochemistry also occur, so the system is exceedingly complex. The exact conformation, however, of polymeric procyanidins is difficult to elucidate as the mass spectral data do not reveal differences between closely related isomers (Weinges and Freudenberger, 1965) and the NMR data cannot be interpreted in an unambiguous manner (Geisman and Dittmar, 1965). Confirmation of procyanidins up to trimers has been achieved on the basis of procyanidin hydrolysis with acidic toluenethiol (Lea et al., 1979). The method relies on acidic cleavage of procyanidin at the C–C bond to give a carbocation from "the top half" and a free catechin from the "bottom half", the stereochemistry of both halves being preserved. In the presence of toluene-α-thiol, the carbocation is then captured to form a stereospecific thiol derivative. For steric reasons, the carbocation derived from (+)-catechin gives rise to two thiol derivatives, but the corresponding carbocation from (−)-epicatechin produces only one such derivative (Thompson et al., 1972). All of the products of these reactions are separable by paper chromatography, and so from an examination of the breakdown pattern the structure of the original molecule may be deduced.

Preparative and analytical separation of procyanidins has been achieved by a variety of techniques including paper chromatography, TLC on silica or cellulose, open column chromatography on Sephadex LH-20, and countercurrent distribution between ethyl acetate and water (Lea and Timberlake, 1974; Lea, 1978). These techniques are now being replaced by HPLC. Lea (1979) examined HPLC methods for procyanidin separation based on adsorption, reversed phase, and gel permeation, and he concluded that of the three modes reversed phase offered the greatest potential. Gel permeation provided inadequate resolution, and adsorption chromatography led to infinitely long retention times. The disadvantages of reversed phase HPLC, however, are the coelution of procyanidins with cinnamics and the lack of relation between molecular weight and retention time. TLC, especially the new high-performance silica plates, provides a very good method for assessing the degree of polymerization of procyanidin oligomers; dimeric up to hexameric procyanidins have been separated in the order of decreasing molecular weight on silica plates (Lea et al., 1979).

Quercetin is the major flavonol aglycon of apples (Duggan, 1967), while kaempferol derivatives have also been found in much smaller concentrations (Van Buren, 1970; Herrmann, 1976). Flavonol derivatives are mainly concentrated in the peel tissue, with values ranging between 500 and 1800 μg/g (Workman, 1963; Walker, 1964). Quercetin 3-*O*-galactoside (hyperin), 3-*O*-glucoside (isoquercetrin), 3-*O*-xyloside (reynoutrin), 3-*O*-arabinoside (avicularin), and 3-*O*-rhamnoside (quercetrin) have been reported by many investigators (Walker, 1964; Siegelman, 1955a; Dick et al., 1987; Oleszek et al., 1988). The presence of quercetin 3-*O*-rutinoside (rutin) has also been reported (Siegelman, 1955a). Quercetin glycosides have been implicated in the inhibition of β-galactosidase and the suppression of softening in Golden Delicious apples (Dick et al., 1985; Lidster et al., 1985; Dick and Smith, 1990). The earlier studies on these quercetin glycosides were limited to qualitative data as the similarities in molecular weight and polarity did not allow for adequate resolution by conventional methods such as TLC and column chromatography. The recent works by Oleszek et al. (1988) and Spanos et al. (1990) demonstrate the utility of an HPLC system equipped with diode array detector for characterization of phenolics; spectral data enabled these researchers to distinguish the quercetin arabinoside from the closely eluting phloretin xyloglucoside.

Derivatives of the dihydrochalcone phloretin are characteristic of apple fruit and apple juice (Duggan, 1967). The major derivative is phloridzin (Whiting and Coggins, 1975; Wilson, 1981), but lower levels of phloretin xyloglucoside are also present (Wilson, 1981; Oleszek et al., 1988; Spanos et al., 1990). These dihydrochalcone glycosides are present in both flesh and skin, but the highest levels occur in the seeds (Durkee and Poapst, 1965).

Factors Affecting the Phenolic Composition of Apple Juice. *Factors Related to Fruit.* Studies on the changes in cinnamics and catechins during the development of apples report that large quantities of catechin and cinnamic acid derivatives are formed early in the fruit but during the rapid growth of the fruit and concentrations of these compounds drop drastically. This decline stops as the fruit matures (Mosel and Herrmann, 1974). Similar patterns have been reported (Walker, 1963) on the levels of chlorogenic acid and *p*-coumarylquinic acid in Cox's Orange and Sturmer apples.

Data on the phenolic profile of different varieties indicate a very similar qualitative pattern. Quantitative differences among varieties exist. For instance, Lea and Arnold (1978) found that the variety Tremlett's, which is quite bitter, had a higher ratio of oligomeric procyanidins to polymeric than the variety Vilberrie, which is more astringent and less bitter. Coseteng and Lee (1987) studied the changes of PPO and phenolic concentration in relation to degree of browning in Empire, Cortland, McIntosh, Golden Delicious, Rome, Rhode Island Greening, and Classic Delicious apples. The results of the study show that PPO activity and phenolic concentrations decreased during maturation and remained nearly constant during postharvest storage. In some varieties, PPO activity was directly related to the degree of browning, while in others the degree of browning was related more to phenolic concentrations without, however, any particular phenolic compound to be accountable for the differences in browning. A similar pattern in phenolic changes was reported by Burda et al. (1990). The authors also reported a direct correlation between concentration of phenolics in the flesh and that in the skins.

Cultural conditions can also affect the phenolic composition of fruits. Lea and Beech (1978) measured a 17% decrease in phenolics of ciders from apples harvested from trees that had been fertilized as compared to those from nonfertilized trees.

Factors Related to Juice Processing. The cinnamic acids and the flavans are good substrates for apple PPO (Siegelman, 1955a,b; Stelzig et al., 1972), while the flavonols appear to be less suited as PPO substrates (Baruah and Swain, 1959). Unit operations such as crushing, prepress enzymatic treatments, and pressing provide opportunities for PPO activity. The literature reports that the most significant oxidation occurs at the pulp before and during pressing, with cinnamics and catechins being affected the most (Van Buren et al., 1976; Lea and Timberlake, 1978). Oxidation after the extraction can also be very important if a long time elapses until PPO inactivation. Spanos et al. (1990) reported that a high-temperature short-time (HTST) treatment immediately after pressing protected

phenolic compounds from oxidation during subsequent processing operations. Enzyme inactivation requires heating to ca. 90 °C for 30 s (Montgomery and Petropakis, 1980; Beveridge and Harrison, 1986). Pressing in the presence of agents suitable for PPO inhibition, such as sulfur dioxide or ascorbic acid, drastically increases the phenolic yields (Van Buren et al., 1976; Lea and Timberlake, 1978). The kinetics of apple PPO have been the subject of many recent studies (Klapp et al., 1990a,b; Oszmianski and Lee, 1990). The latter study demonstrated an inhibitory effect of honey on PPO and on browning reactions in apple slices, grape juice, and phenolic model systems. The compound responsible for this inhibitory effect appeared to be a small peptide with an approximate molecular weight of 600.

A variety of enzymatic clarification and fining agents are used in apple juice processing. Commercial pectolytic enzyme preparations can cause hydrolysis of cinnamic acids (Spanos et al., 1990). Fining agents recommended for fruit juices include gelatin, bentonite, silica sol, activated carbon, and polyvinylpolypyrrolidone (PVPP) (Heatherbell, 1984). Reports on the effects of these agents on the phenolics emphasize those on gelatin, mainly because of its widespread application. Lea and Timberlake (1978) reported that gelatin fining results in reduction of total phenolics, but it was the polymeric phenolics that were the most affected as 20% of the polymeric procyanidins were removed by gelatin fining. Spanos et al. (1990), however, reported that fining with bentonite, gelatin, and silica sol had a minor effect on the HPLC profile of Granny Smith apple juice. Activated carbon is considered nonspecific and has been used as a decolorizing agent (Beveridge, 1986). PVPP is known to bind phenolics by hydrogen bonding (Loomis, 1974), but it does not appear to be extensively used by the fruit juice industry. Bentonite and silica sol are mainly used to remove unstable proteins (Hsu and Heatherbell, 1987).

The application of hot water (above 57 °C) in the extraction of juice from fruits has recently been adopted as an alternative to conventional pressing to increase juice yield. The diffusion process, however, tends to favor extraction of phenolic compounds, and there can be a tendency for high rates of browning and obvious aroma and taste differences from the normal apple juice character (Schobinger et al., 1978). Temperature of extraction is considered very important, as the true diffusion requires a significant change to the fruit membrane permeability (Luthi and Glunk, 1974). The extraction of phloridzin, which is concentrated in the seeds and has low solubility in cold water, was found to be markedly increased in diffusion-extracted juice (Lea and Timberlake, 1978; Spanos et al., 1990). Similar results were found for the quercetin glycosides, which are concentrated in the skins and poorly soluble in cold water (Spanos et al., 1990).

The quantitative data on the phenolic composition of diffusion-extracted juices relative to juices extracted with conventional pressing show a strong increase in total phenolics. Up to a 5-fold increase in total phenolics by HPLC was measured in diffusion-extracted juices relative to that in pressed juices (Spanos et al., 1990). Schobinger et al. (1978) reported the total phenolics by colorimetric assay of pressed juice to be 255 μg/mL, while the total phenolics of diffusion-extracted juice were 659 μg/mL. The change in procyanidins was found to be even more drastic as these compounds were 30 μg/mL in pressed juice and 372 μg/mL in diffusion-extracted juice. With a similar assay Kardos (1979) reported a 3-fold increase in the phenolic content in diffusion-extracted juice relative to that of pressed juice.

Storage of apple juice and juice concentrates results in degradation of phenolics. A 9-month storage at 25 °C of apple juice concentrates showed an approximately 36% degradation of cinnamics, 60% degradation of quercetin and phloretin glycosides, and total loss of procyanidins (Spanos et al., 1990). Van Buren et al. (1976) found that flavonol glycosides decreased considerably during storage of juice concentrates at 35 °C for 90 days. Polymerization of flavans was also concluded by the decreased mobility of these compounds on TLC plates. The colorimetric quantitation (based on Folin–Ciocalteu reagent) of total phenolics, however, showed an increase with storage. Similar increase in total phenolics, quantitated with Folin–Ciocalteu colorimetric assay, during storage has been reported by Babsky et al. (1986) and Spanos et al. (1990), which indicates that such data should be interpreted cautiously. Formation of nonenzymatic browning intermediates, such as enediols and reductones, during storage undoubtedly accounts for these "apparent" increases in total phenolics.

PHENOLIC COMPOSITION OF PEAR JUICE

A summary of the phenolic composition of pear fruit (various varieties, maturity stages) and fruit juice (various treatment) is presented in Table II. The cinnamic composition of pears is similar to that of apples, as the major cinnamics of pears are chlorogenic and *p*-coumarylquinic acid (Cartwright et al., 1955; Hulme, 1958; Sioud and Luh, 1966; Challice and Williams, 1972). The positional isomers of these cinnamics reported in apples are also found in pears (Mosel and Herrmann, 1974).

Several investigators have reported the presence of (+)-catechin and (−)-epicatechin in pears (Sioud and Luh, 1966; Ranadive and Haard, 1971; Mosel and Herrmann, 1974). Procyanidins are also present, but the data on specific conformations are very limited. Sioud and Luh (1966) detected cyanidin as a hydrolysis product of procyanidins of Bartlett pears. These procyanidins were identified as B1 and B2 by comparing their R_f values on two-dimensional paper chromatography with those of procyanidins found in cacao beans.

Glycosides of the aglycons quercetin, isoquercetin, and kaemferol make up the flavonol composition of pears (Duggan, 1967, 1969a,b). Sioud and Luh (1966) observed the flavonol glycosides of Bartlett pears as two spots on paper chromatograms of ethyl acetate extracts. They identified one of the spots as isoquercetin (quercetin 3-*O*-glucoside); the other was considered as another glycoside of quercetin. Nortjé and Koeppen (1965) isolated five flavonols from Bon Chretien pears: isoquercetin, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-rhamnoglucoside, isorhamnetin 3-*O*-rhamnogalactoside, and a fifth compound believed to be an acid-conjugated form of isorhamnetin 3-*O*-glucoside. Duggan (1969b) confirmed the presence of these flavonol glycosides in Packingham, Bartlett, Bosc, and d'Anjou pears using mass spectrometric and NMR as identification techniques and reported the presence of quercetin 7-*O*-xyloside as a new compound not previously identified. A distribution of flavonols in the fruit presented by Herrmann (1976) shows that 28 μg/g of quercetin and 12 μg/g of kaempferol were found in skins and peels of Williams Christ pears. The levels of these flavonols in the remaining tissues amounted to less than 0.1 μg/g.

The major difference between the phenolic profile of pear and apple fruit is the presence of arbutin and the lack of phloretin derivatives in pears (Williams, 1957; Duggan, 1967).

An excellent chemotaxic survey of the phenolic compounds in the genus *Pyrus* has been presented by Challice and Williams (1968).

Table II. Phenolic Composition of Pear Fruit and Pear Juice

phenolic compound	content[a]	variety/comments	references
cinnamics			
chlorogenic, coumarylquinic, caffeic, coumaric, ferulic, isomers of chlorogenic and coumarylquinic	qualitative		Cartwright et al., 1955; Hulme, 1958; Sioud and Luh, 1966; Challice and Williams, 1972; Mosel and Herrmann, 1974
chlorogenic	30–70	d'Anjou, Bosc; flesh; harvest to 170 days at −1 °C	Meadows, 1983
	125, 238.7	SO_2 processed juice; Bartlett, d'Anjou resp	Beveridge, 1986
	0.6–131.3	±SO_2 processed juice; Comice, d'Anjou, Bartlett	Spanos and Wrolstad, 1990a
chlorogenic isomers	1, 2	SO_2 processed juice; Comice, d'Anjou resp	Spanos and Wrolstad, 1990a
coumarylquinic	1, 2	SO_2 processed juice; Comice, d'Anjou, resp	Spanos and Wrolstad, 1990a
caffeic	30, 17.7	SO_2 processed juice; Bartlett, d'Anjou resp	Beveridge, 1986
	1.1–9.1	±SO_2 processed juice; Comice, d'Anjou, Bartlett	Spanos and Wrolstad, 1990a
coumaric	1–2.1	SO_2 processed juice; Comice, d'Anjou, resp	Spanos and Wrolstad, 1990a
catechins/procyanidins			
catechin epicatechin; catechin/epicatechin-based polymers	qualitative		Sioud and Luh, 1966; Ranadive and Haard, 1971; Mosel and Herrmann, 1974
catechin	13–30	d'Anjou, Bosc; flesh; harvest to 170 days at −1 °C	Meadows, 1983
	3.24	SO_2 processed juice; Bartlett	Beveridge, 1986
	1.5–3.1	SO_2 processed juice; Comice, d'Anjou, resp	Spanos and Wrolstad, 1990a
epicatechin	25.3, 8.5	SO_2 processed juice; Bartlett, d'Anjou, resp	Beveridge, 1986
	19, 4.3	SO_2 processed juice; Comice, d'Anjou, resp	Spanos and Wrolstad, 1990a
total procyanidins[b]	74.2–11.3	SO_2 processed juice; Comice, d'Anjou, resp	Spanos and Wrolstad, 1990a
flavonols			
quercetin, isorhamnetin, kampferol glucosides; glu, gal, xyl, rha, rhaglu, rhagal	qualitative		Nortje and Koeppen, 1965; Sioud and Luh, 1966; Duggan, 1967, 1969a,b
quercetin	0.1, 28	Williams Christ; flesh, skins resp	Herrmann, 1976
kaempferol	0.1, 12	Williams Christ; flesh, skins resp	Herrmann, 1976
quercetin and isorhamnetin glycosides[c]	1.2–10.4	±SO_2 processed juice; Comice, d'Anjou	Spanos and Wrolstad, 1990a
arbutin	qualitative		Williams, 1957; Duggan, 1967
	10–19	d'Anjou, Bosc; flesh; harvest to 170 days at −1 °C	Meadows, 1983
	6.7–16.8	±SO_2 processed juice; Comice, d'Anjou, Bartlett	Spanos and Wrolstad, 1990a

[a] Micrograms per milliliter of juice or micrograms per gram of fruit. [b] Dimers (B1, B2, B3, B4), trimers, tetramers, and other unknown procyanidins. [c] Glycosides (glu, gal, rha, rhaglu, rhagal, xyl) of quercetin and isorhamnetin.

Factors Affecting the Phenolic Composition of Pear Juice. *Factors Related to Fruit.* The changes of cinnamics and catechins during the development of pear fruit are very similar with those occurring during the development of apple fruit (Mosel and Herrmann, 1974). Meadows (1983) studied the changes in phenolics during long-term cold storage of d'Anjou and Bosc pears. The data show that the levels of phenolic compounds in flesh at harvest were for d'Anjou fruit 50 μg/g (fresh weight) chlorogenic acid, 13 μg/g catechin, 10 μg/g arbutin, and no epicatechin or *p*-coumarylquinic acid; and for Bosc pears 50 μg/g chlorogenic acid, 14 μg/g catechin, 19 μg/g arbutin, and no epicatechin or *p*-coumarylquinic acid. During the 170 days of storage at −1 °C, chlorogenic acid fluctuated but rose and leveled to 70 μg/g in d'Anjou but fell to 30 μg/g in Bosc. Catechin rose to 30 μg/g in d'Anjou and to 20 μg/g in Bosc, while arbutin remained nearly constant. At 120 days epicatechin/*p*-coumarylquinic acid (compounds were not resolved) rose to 18 μg/g in d'Anjou but not in Bosc.

Duggan (1969b) compared the flavonol glycosides of d'Anjou, Bartlett, Bosc, and Packingham, pears and found these varieties very similar, with d'Anjou differing most from the group. It contained much more quercetin 7-*O*-xyloside and much less isorhamnetin 3-*O*-galactoside.

Several studies have focused on the isolation and properties of PPO of different pear varieties (Rivas and Whitaker, 1973; Halim and Montgomery, 1978; Smith and Montgomery, 1985). Since color deterioration during processing of pear juice is caused by endogenous PPO activity and presents a major problem, it is worthwhile to review some of the properties of the enzyme. Rivas and Whitaker (1973) purified two isoenzymes from Bartlett pears. The two isoenzymes differ in the effect of ionic strength on activity, but they are similar with respect to substrate specificity, pH activity relations, inhibition by *p*-coumaric and benzoic acids, and heat stability. Both enzymes are *o*-diphenol oxidases with no monophenolase or laccase activity. Chlorogenic acid and catechin were good substrates of the enzymes. Dependence of activity on oxygen and chlorogenic acid concentrations indicated a sequential mechanism for binding of these substrates to the enzyme. Maximum activity on chlorogenic acid was at pH 4.0. *p*-Coumaric acid was a linear noncompetitive inhibitor with respect to chlorogenic acid. Halim and Montgomery (1978) separated by gel electrophoresis and detected by catechol activity eight isoenzymes from d'Anjou pears. None of these enzymes exhibited laccase activity. Some of these multiple forms, however, were artifacts from the interaction between enzyme and phenolics (Smith and Montgomery, 1985). Extraction of d'Anjou PPO in the presence of adsorbents such as PVPP, Amberlite XAD-4, and ion-exchange resins resulted in a PPO extract with optimum pH 5.1. At 4 °C the extract lost 11% of the PPO activity during an 11-day storage period. Gel electrophoresis of the extract revealed only three isoenzymes (Smith and Montgomery, 1985). Heat inactivation of the crude d'Anjou PPO extracts follows first-order kinetics. Approximately 50% of PPO activity was inactivated after heating for 11.7, 6.25, 2.24, and 1.1 min at temperatures of 70, 75, 80, and 85 °C, respectively (Halim and Montgomery, 1978).

Factors Related to Juice Processing. Most of the studies concerning pear juice deal with the problem of the rapid browning during processing (Dimick et al., 1951; Montgomery, 1983; Petropakis and Montgomery, 1984). Color deterioration occurring during storage has been mainly ascribed to the Maillard reaction (Cornwell and Wrolstad, 1981).

Heat treatment, ascorbic acid, and SO_2 have been applied to prevent browning during processing. Thermal treatment, the only permanent treatment, and ascorbic acid have resulted in production of pear concentrates of lighter color (Petropakis and Montgomery, 1984). Ap-

Table III. Phenolic Composition of White Grape Juice

phenolic compound	content[a]	variety/comments	references
cinnamics			
trans- and *cis*-caftaric, *trans*- and *cis*-coutaric, caffeic, coumaric, ferulic, 2-*S*-glutathionylcaftaric	qualitative		Ribereau-Gaynon, 1965; Singleton and Noble, 1976; Ong and Nagel, 1978a,b; Singleton et al., 1978; Baronowski and Nagel, 1981; Okamura and Watanabe, 1981; Cheynier et al., 1986; Singleton, 1987
trans-caftaric	181	mean value for white grape cultivars grown in NY	Lee and Jaworski, 1987
	0.5–2.5	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad 1990b
cis-caftaric	6	mean value for white grape cultivars grown in NY	Lee and Jaworski, 1987
	0–0.3	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad 1990b
2-*S*-glutathionylcaftaric	6.0–7.8	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad 1990
coutaric	10	mean value for white grape cultivars grown in NY	Lee and Jaworski, 1987
	0.2–0.3	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b
caffeic	1.1–7.6	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b
coumaric	0.8–3.1	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b
ferulic	0.2–0.5	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b
benzoics			
gallic	qualitative		Singleton and Trousdale, 1983; Oszmianski et al., 1986
	0.5–1.6	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad 1990b
catechins/procyanidins			
catechin, epicatechin; polymeric, up to hexameric, procyanidins based on these units; dimeric procyanidin–gallate structures	qualitative		Lea et al., 1979; Oszmianski et al., 1986; Oszmianski and Sapis, 1989
catechin	19	mean value for white grape cultivars grown in NY	Lee and Jaworski, 1987
	1.7–6.5	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b
epicatechin	23	mean value for white grape cultivars grown in NY	Lee and Jaworski, 1987
	0.3–1.9	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b
dimers (B1, B2, B3, B4)	21	mean value for white grape cultivars grown in NY	Lee and Jaworski, 1987
	2.6–20.3	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b
trimers, tetramers and other unidentified procyanidins	1–11.4	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b
catechin–gallate	26		Lee and Jaworski, 1987
catechin–catechin–gallate isomers	44		Lee and Jaworski, 1987
flavonols			
quercetin and kaempferol glycosides; 2,3-dihydrokaempferol rha and 2,3-dihydroquercetin rha	qualitative	localized in the solid parts of the clusters	Ribereau-Gaynon, 1964; Singleton and Trousdale, 1983; Cheynier and Rigaud, 1986
quercetin glycosides	7.2–9	Thompson Seedless; ±SO_2 processed juice	Spanos and Wrolstad, 1990b

[a] Micrograms per milliliter of juice or micrograms per gram of fruit.

proximately 300 ppm of ascorbic acid was required to prevent browning in pear pulp and juice up to and during the time of heating (Montgomery and Petropakis, 1980). Ascorbic acid, however, is not always desirable as it can participate in nonenzymatic browning in later storage (Tatum et al., 1969). Heating the fruit mash is an alternative. However, heating pears solubilizes middle lamella pectins (Reeve and Leinback, 1953) resulting in cell separation and "saucing" of the fruit. Serious problems in extraction and clarification of juice from such mashes have been encountered (Beveridge, 1986).

Spanos and Wrolstad (1990a) studied the influence of variety, maturity, processing, and storage on the phenolic composition of pear juice. Variety and maturity introduced major quantitative differences in the phenolic profile. Considerable loss of cinnamics and total loss of procyandins occurred in the juices processed without SO_2. Cinnamic hydrolysis was observed during enzymatic clarification. There was no measurable reduction of phenolics with fining (bentonite, gelatin, silica sol). Arbutin and flavonol glycosides were less affected by SO_2 and processing. Some compositional data reported by Beveridge (1986) showed only minor quantitative differences between SO_2 and ascorbic acid processed juice from Bartlett and d'Anjou fruit.

Inhibition of browning in pear juice concentrate by cysteine has been demonstrated by Montgomery (1983). Control of browning with addition of cysteine seems to result from the formation of colorless products by the reaction of *o*-quinones with the sulfhydryl compounds rather than direct inhibition of PPO; for effective inhibition of browning, a thiol/phenolic ratio greater than unity is required (Joslyn and Ponting, 1951; Walker and Reddish, 1964). Cysteine, due to the absence of the characteristic sulfite odor, is attractive for use as an antioxidant, but its use is not approved at present in the United States.

Clear amber-colored pear juice was obtained by ultrafiltration on hollow fibers (Kirk et al., 1983). The method offers an alternative means for removing proteins, polysaccharides, and polymerized phenolics, but it does not affect phenolics of low molecular weight (Heatherbell et al., 1977).

PHENOLIC COMPOSITION OF WHITE GRAPE JUICE

The phenolic composition of white grape juice is summarized in Table III. The phenolic composition of grapes has been exhaustively studied and reviewed (Singleton and Esau, 1969). Varieties important in wine making have received most of the attention.

For a long time, conflicting results appeared in the literature about the cinnamic composition of grapes. It was assumed that the esters of caffeic acid with quinic acid, which are the predominant cinnamic derivatives in most fruits, are present in grapes. Ribéreau-Gaynon (1965) showed with paper chromatography that the esters actually contained tartaric rather than quinic acid. No mention of the tartaric esters of *p*-coumaric or ferulic acids was made at that time. Singleton and Noble (1976) considered that the nature of the cinnamic derivatives in grapes was not yet fully understood. In a more recent study aiming to resolve the confusion of cinnamic esterification in grapes, Singleton et al. (1978) identified crystalline products isolated from Müller-Thurgau white wine as *cis*- and *trans*-*p*-coumaryltartaric acid (coutaric acid) and *trans*-caffeoyltartaric acid (caftaric acid). They also reported that the corresponding quinates were not present. In later

studies several investigators confirmed that cinnamics in grapes exist as tartrate esters and the absence of often reported chlorogenic and neochlorogenic was substantiated (Ong and Nagel, 1978a,b; Baranowski and Nagel, 1981; Okamura and Watanabe, 1981). Ong and Nagel (1978b) reported the occurrence of glucose–tartrate–cinnamic acid esters, but in a later study by Baranowski and Nagel (1981) it was acknowledged that these glucose esters were indeed cis isomers of caftaric and coutaric acid. Although feruloyl tartrate has been detected in red grapes (Ribéreau-Gaynon, 1965), there was some question as to its presence in white grapes. Baranowski and Nagel (1981) showed that low levels of this cinnamic were present in white Riesling wine.

Gallic acid was one of the phenolic compounds separated by HPLC in Thompson Seedless juice and wine (Singleton and Trousdale, 1983). It is also found in grape seed extracts (Oszmianski et al., 1986). Mahler et al. (1988) utilized HPLC and electrochemical detection to identify nonflavonoid phenols in wines. The authors claimed that more compounds were identified with electrochemical detection than by diode array techniques.

Lea et al. (1979) characterized the procyanidins of white grapes and wines (Müller-Thurgau). Four main dimers were isolated and identified as procyanidins B1–B4. A further procyanidin fraction was isolated and shown to consist of a mixture of two stereoisomeric trimers, one composed of three epicatechin units and the other of two epicatechins and a terminal catechin. Identification of specific structures was achieved with acidic toluenethiol degradation of procyanidins. Higher degree procyanidin polymers (up to six) were also separated on high-performance TLC plates. Catechin, epicatechin, and procyanidins B1–B4 have also been extracted from grape seeds (Oszmianski et al., 1986).

Jaworski and Lee (1987) fractionated grape phenolics into acidic and neutral on C_{18} Sep-Pak cartridges. The juice sample was adjusted to pH 7, and it was passed through a C_{18} Sep-Pak preconditioned with water. The acidic phenolics, being negatively charged at pH 7, are not retained on the cartridge. Neutral phenolics were eluted with methanol and analyzed by HPLC to reveal the presence of catechin, epicatechin, and procyanidins B2 and B3. The authors claimed that this fractionation resulted in higher procyanidin recoveries than those achieved by ethyl acetate extraction at pH 7 described by Salagoïty-Auguste and Bertrand (1984).

Most of the gallocatechins and galloyl esters of catechins found in wines are extracted from seeds (Lea et al., 1979). These highly substituted flavanols have been reported in many studies (Singleton et al., 1966; Su and Singleton, 1969; Oszmianski et al., 1986; Lee and Jaworski, 1987) which show that their seed concentrations diminish considerably or disappear as the grapes ripen.

Ribéreau-Gaynon (1964) identified the 3-glucosides of kaempferol, quercetin, and myricetin and quercetin 3-glucuronide in red grapes and observed that white varieties lack the myricetin derivatives. Flavonols are localized in the solid parts of the cluster and therefore are not usually found in juices or wines that have been prepared with traditional wine-making procedures. Most of the studies on grape flavonols focus on their levels in wines fermented in the presence of the pulp (Cheynier and Rigaud, 1986). Singleton and Trousdale (1983) reported, for the first time, trace amounts of astilbin (rhamnoside of 2,3-dihydrokaempferol) and engeletin (rhamnoside of 2,3-dihydroquercetin) in Thompson Seedless grape juice.

Factor Affecting the Phenolic Composition of White Grape Juice. *Factors Related to Fruit.* Lee and Jaworski (1987) found large differences in the phenolic makeup of white grape cultivars grown in New York. Varietal differences in the chromatographic pattern of phenolics have also been shown by Singleton and Trousdale (1983).

Studies on activity of PPO during the maturation of several white grape varieties grown in the northeastern United States revealed varieties with low, medium, and high levels of PPO activity. The activity for any variety fluctuated throughout the ripening period, and no correlation between PPO activity and phenolic content was found (Wissemann and Lee, 1980). Over a 3-year period investigation of the development of PPO activity throughout growth and maturation of five grape varieties revealed differences for certain years in the intensity and speed of development of the enzymatic activity (Sapis et al., 1983).

Factors Related to Juice Processing. In previous sections the effect of the different processing operations on the phenolic composition of apple and pear juice was explained. It is expected that operations of similar nature would have comparable effect on the phenolic composition of grape juice. The literature in the area of grape processing emphasizes wine technology, and some interesting aspects of that technology are pointed out. Depectinization of grape juice often is an operation common to both wine and juice processing. Complete hydrolysis of cinnamic esters (caftaric and coutaric acid) in wines clarified with pectinases has been reported by many investigators (Baranowski and Nagel, 1981; Cheynier et al., 1986). Extensive hydrolysis of caftaric, coutaric, and quercetin derivatives was measured during enzymatic clarification of Thompson Seedless grape juice (Spanos and Wrolstad, 1990b).

The oxidation of cinnamic acids in grapes has drawn the attention of researchers in the field (Singleton et al., 1984; 1985; Cheynier et al., 1986, 1989; Cheynier and Van Hulst, 1988). It is now established that oxidation of caftaric and coutaric acid by PPO leads to the formation of 2-*S*-glutathionylcaftaric acid. The reaction involves as a first step the enzymatic oxidation of caftaric acid to caftaric acid *o*-quinone and as the second step the spontaneous addition of the sulfhydryl to make the thioether. According to Singleton (1987) glutathionylcaftaric acid is not a substrate for PPO, in spite of its *o*-dihydroxyphenol structure. This conversion of caftaric acid is therefore believed to be a way of limiting must browning by trapping caftaric acid quinones in the form of a stable glutathione substituted product (Cheynier et al., 1986). Thus, the varietal differences in grape susceptibility to browning, which are not determined by the grape PPO activity or by the cinnamic acid content (Sapis et al., 1983), might depend on the glutathione to caftaric ratio (Cheynier and Van Hulst, 1988).

In grapes such as Thompson Seedless, which have an adequate content of glutathione, quantitative conversion of caftaric to the glutathione derivative is easily achieved (Cheynier et al., 1986, 1989). Thorough protection from enzymatic oxidation during pulping of grapes is required to avoid the formation of glutathionylcaftaric in grape juices (Singleton, 1987). Although enzymatic clarification of Thompson Seedless grape juice resulted in hydrolysis of caftaric and couteric, it showed no hydrolytic effect on 2-*S*-glutathionylcaftaric acid (Spanos and Wrolstad, 1990b).

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Received for review April 20, 1992. Accepted June 9, 1992.