# Phenolic Composition of Industrially Manufactured Purées and Concentrates from Peach and Apple Fruits

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Purées and concentrates are intermediate products in the elaboration of commercial fruit juices. In this paper, the phenolic composition was established and quantified in a large number of samples of peach and apple purées and concentrates. Different benzoic acids and aldehydes, cinnamic acids and their derivatives, flavan-3-ols, procyanidins, flavonols, and dihydrochalcones have been identified. The concentration of cinnamic acids and derivatives is higher in the concentrates than in the purées for both fruits. However, flavan-3-ols and procyanidins are only present in the purées. Peach-based products are completely devoid of flavonol and dihydrochalcone derivatives due to the removal of the skin of the fruit in the manufacturing process. On the other hand, different quercetin and phoretin glycosides were detected in apple purées and concentrates. The results show that phenolic compounds can prove to be helpful in the characterization of fruit purées and concentrates as well as in the detection of adulterations in the manufacturing of commercial fruit juices from these intermediates.

**Keywords:** *Phenolics, peach, apple, fruit purées, fruit concentrates*

## INTRODUCTION

Most industrial fruit juice manufacturing processes consist of two separate stages normally carried out at different plants. The first stage is comprised of processing the fresh fruit into a stabilized, semiliquid intermediate product, e.g., purées and concentrates (Figure 1), that can be stored for extended periods. The second stage is comprised of reconstitution of these intermediates by adding water and other additives to yield commercial juice with a limited shelf life.

Characterization of fruit juices and jellies, jams, nectars, etc., as well as the detection of adulterated juices, has mostly been based on identification and quantification of a variety of components: amino acids, sugars, hydroxy acids, carotenoids, lipids, and proteins (Mears and Shenton, 1973; Wills *et al.*, 1987; Schnüll, 1990; Dizy *et al.*, 1992; Krueger *et al.*, 1992; Van Gorsel *et al.*, 1992; Lo Voi *et al.*, 1995). Phenolic compounds, secondary metabolites common throughout the vegetable kingdom, can also be used in the characterization of fruit products (Fernández de Simón *et al.*, 1992; García-Viguera *et al.*, 1992; Van Gorsel *et al.*, 1992; Hernández *et al.*, 1997), though use of such compounds is less widespread because of the difficulties entailed in identifying the phenolic composition with precision.

Certain phenolic compounds are characteristic of certain fruits; for example, cinnamic esters of tartaric acid are present in grapes (Fernández de Simón *et al.*, 1992) but not in other fruits, and hence, they can be used in the characterization of juices containing grape juice. Grape varieties can also be differentiated on the basis of their phenolic compositions (Estrella *et al.*, 1984; Gónzalez-San José and Díez, 1993). Apple juices are characterized by the main dihydrochalcone present, phlorizin (phloretin-2′-glucose) (Macheix *et al.*, 1990; Fernández de Simón *et al.*, 1992; Hernández *et al.*, 1997). Some work has also been carried out on differentiating apple varieties on the basis of the polyphenolic composition (McRae *et al.*, 1990; Pérez-Ilzarbe *et al.*, 1991). Similarly, flavanone glycosides such as hesperidin and naringin are characteristic of citric fruits and have even been employed as the basis for some official methods of detecting the adulteration of citric fruit juices (Rouseff, 1988; Mouly *et al.*, 1994).

Phenolic compounds are not uniformly distributed in fruits either at the subcellular level or in the tissues, and this circumstance is of crucial importance in relation to the chemical composition of industrially processed food products (Macheix *et al.*, 1990). At the subcellular level, these compounds are deposited mainly in the cell wall, where lignin and certain simple molecules (flavonoids and ferulic acid esters) accumulate, and in the vacuoles, where soluble phenolic compounds and their derivatives are stored (Towers, 1964; Macheix and Fleuriet, 1986; Monties, 1989; Ibrahim and Barron, 1989). In the tissues, accumulation of soluble phenolic compounds is greater in the outer tissues (epidermal and subepidermal layers) than in the inner tissues (mesocarp and pulp). For instance, in many fruits, flavonol glycosides are chiefly located in the outer portion or in the epicarp (Pérez-Ilzarbe *et al.*, 1991; Fernández de Simón *et al.*, 1993). Anthocyanins may be present throughout the fruit, as in the case of strawberries, cranberries, or raspberries, whereas in other fruits, they are located mainly in the skin. Grape seeds are particularly rich in procyanidins. The upshot is that juices manufactured from fruits from which the skin or peel has been removed will have a different phenolic composition than juices made from whole fruit, even though the content of other components, such as sugars and amino acids, may be similar in both types of juice.

At the present time, characterization of the intermediate products used in the manufacturing of fruit juices is of considerable interest both to governmental authorities and to manufacturing companies themselves, with a goal of detecting possible adulterations and fraudulent

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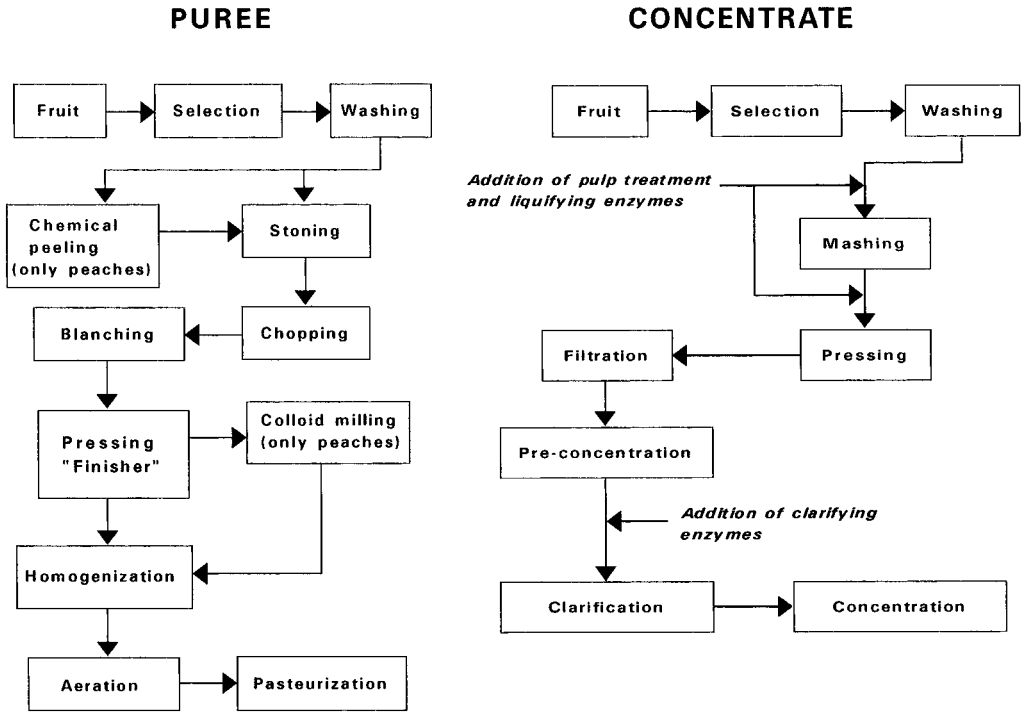


**Figure 1.** Schemes of technological processes for obtaining purées and concentrates from peach and apple fruits.

products. There are very few references in the literature dealing with intermediates, and for that reason, the object of the present study was to characterize intermediate purées and concentrates used in the industrial manufacturing of peach and apple juice. To that end, the phenolic composition was established and quantified in a large number of samples of peach and apple purées and concentrates. Samples were supplied directly by a company located in northeastern Spain. They were manufactured from fruit coming from different localities within a single geographic area at different times of the season.

## MATERIALS AND METHODS

**Samples.** All samples of purées and concentrates were supplied by Indulérida, S. A. (Lérida, Spain). A total of 10 peach purées, 10 apple purées, 3 peach concentrates, and 10 apple concentrates were analyzed. The fruit from which purées and concentrates were manufactured was from different localities of northeastern Spain and was harvested at different times (depending on the variety) during July–October 1993 and 1994 for peaches and during August–October 1993 and 1994 for apples. Samples arrived at the laboratory within 3 days of being manufactured at the plant and were kept at −18 °C until they were analyzed. The low number of peach concentrates available was due to the fact that this product is not in high demand and is only manufactured at certain times.

**Sample Extraction.** Extractions of phenolic compounds and furfural derivatives were performed as follows.

*Concentrates.* Samples (15 mL) were homogenized in 15 mL of methanol/hydrochloric acid (1000:1, v/v) with a blender (1 min). Methanol was evaporated under vacuum, and the residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with ethyl acetate (25 mL). The organic fractions were combined, dried for 30 min with anhydrous sodium sulfate, filtered through a Whatman-40 filter (Whatman International Ltd., Kent, England), and evaporated to dryness in a rotary evaporator, always keeping the bath temperature under 35 °C. The residue was redissolved in 2 mL of methanol/water (50:50, v/v) and 10 $\mu$L was injected into the HPLC apparatus. Samples were analyzed in duplicate.

*Purées.* Samples (50 mL) were homogenized in 50 mL of methanol/clorhidric acid (1000:1, v/v) with a blender (1 min). After centrifugation (3500 rpm, 20 min), supernatants were evaporated to dryness in a rotary evaporator. The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with ethyl acetate (25 mL). The organic fractions were combined, dried for 30 min with anhydrous sodium sulfate, filtered through a Whatman-40 filter (Whatman International Ltd., Kent, England), and evaporated to dryness in a rotary evaporator, always keeping the bath temperature under 35 °C. The residue was redissolved in 2 mL of methanol/water (50:50, v/v) and 10 $\mu$L was injected into the HPLC apparatus. Samples were analyzed in duplicate.

**HPLC Analysis.** The following equipment was obtained from Waters (Milford, MA): a model 600 E pump system controller, a U6K universal injector, and a model 991 photodiode array detector. The column was a reverse phase Nova-Pak $C_{18}$ column (300 × 3.9 mm inside diameter) with 4 $\mu$m packing. The gradient elution conditions used were described previously (Bengoechea *et al.*, 1995). Solvent A was water/acetic acid (98:2, v/v), and solvent B was water/acetonitrile/acetic acid (78:20:2, v/v/v). The concentration of solvent A was decreased from 100 to 20% over a 55 min period, using a flow rate of 1.0 mL/min; then, it was rapidly changed to 10% with a flow rate of 1.2 mL/min in 2 min, and it was kept under these conditions for 70 min. Detection was performed by scanning from 210 to 360 nm, with an acquisition speed of 1 s.

**Identification and Quantification of Components.** Identification was carried out by comparing retention times and spectral data with those of standards (Bartolomé *et al.*, 1993) from Sigma (Deisenhofen, Germany), Fluka (Buchs, Switzerland), and Aldrich (Steinheim/Albuch, Germany). Procyanidins $B_1$, $B_2$, $B_3$, and $B_4$, for which no standard is available, were identified by acid hydrolysis of pure compounds and analysis of their phoroglucinol derivatives (Pérez-Ilzarbe *et al.*, 1992). The number of units that form unknown procyanidins were elucidated by spectral data according to Bartolomé *et al.* (1996). The phenolic moiety of hydroxycinnamic acid derivatives was identified by spectral data according to Bengoechea *et al.* (1995). A sample of previously identified phloretin-2′-xyloglucose was kindly provided by F. A. Tomás-Barberán (Tomás-Barberán *et al.*, 1993).

Quantitative determinations were carried out with height measurements, using calibration curves of the standards. Procyanidins were quantified as (+)-catechin. Phloretin glycosides were quantified as phloridzin. Neochlorogenic and chlorogenic isomer acids were quantified as chlorogenic acid.

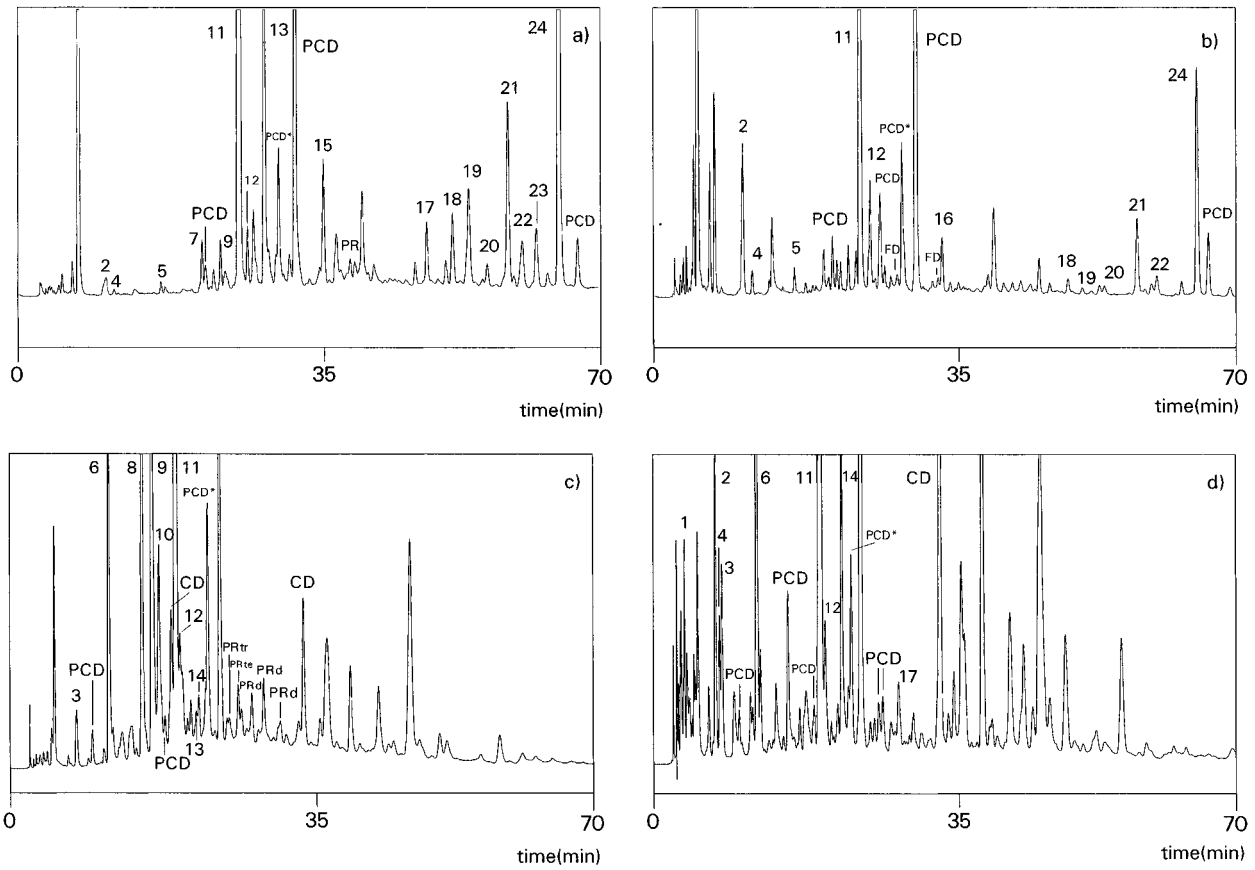


**Figure 2.** Chromatograms recorded at 280 nm of an apple purée (a), an apple concentrate (b), a peach purée (c), and a peach concentrate (d): (1) gallic acid, (2) HMF, (3) protocatechuic acid, (4) 2-furanoic acid, (5) protocatechuic aldehyde, (6) neochlorogenic acid, (7) $B_1$ [epicatechin(4$\beta$→8)catechin], (8) $B_3$ [catechin(4$\beta$→8)catechin], (9) (+)-catechin, (10) $B_4$ [catechin(4$\beta$→8)epicatechin], (11) chlorogenic acid, (12) caffeic acid, (13) $B_2$ [epicatechin(4$\beta$→8)epicatechin], (14) chlorogenic isomer, (15) (−)-epicatechin, (16) *p*-coumaric acid, (17) unidentified phloretin derivative, (18) quercetin-3-galactose, (19) quercetin-3-glucose, (20) quercetin-3-rutinose, (21) phloretin-2′-xyloglucose, (22) quercetin-3-arabinose, (23) quercetin-3-rhamnose, (24) phloretin-2′-glucose, (PCD) unidentified *p*-coumaric acid derivatives, (PCD*) unidentified *p*-coumaric acid derivative common to peach and apple purées and concentrates, (PRd) unidentified procyanidin dimer, (PRtr) unidentified procyanidin trimer, (PRte) unidentified procyanidin tetramer, (FD) unidentified ferulic acid derivative, and (CD) unidentified caffeic derivative.

**Table 1. Ranges of the Values for °Brix Grade and pH of Purées and Concentrates Industrially Manufactured from Peach and Apple Fruits**

| | peach purées[a] | apple purées[a] | peach concentrates[b] | apple concentrates[a] |
|---|---|---|---|---|
| °Brix | 11.2−13.0 | 12.1−15.9 | 60.8−65.6 | 70.4−70.7 |
| pH | 3.84−4.11 | 3.78−4.10 | 3.67−3.86 | 3.63−4.06 |

[a] $n = 10$. [b] $n = 3$.

## RESULTS AND DISCUSSION

Concentrates of peach and apple showed °Brix values at least 4-fold higher than those of respective purées, but no differences were found in pH values (Table 1). Different benzoic acids and aldehydes, cinnamic acids and derivatives, flavonoids, and furfural derivatives were identified in the HPLC chromatograms of purées and concentrates from peach and apple fruits (Figure 2).

Qualitative and quantitative differences in the phenolic composition were recorded in the intermediates manufactured from peaches and apples (Table 2). Caffeic acid was the cinnamic acid present in higher concentrations in the concentrates than in the purées for both types of fruit. *p*-Coumaric acid was only detected in the concentrates. In fruit juices made in the laboratory, the presence of free cinnamic acids has been attributed to the action of the different enzymes (Spanos *et al.*, 1990; Hernández *et al.*, 1997). In the technological manufacturing of concentrates (Figure 1), two types of enzymes were employed, pulp treatment and liquifying enzymes, chiefly pectinases and cellulases that break down pectins, thereby improving yields during crushing and pressing (Joshi *et al.*, 1991; Di Cesare *et al.*, 1993); and clarifying enzymes, pectinases, and amylases, used in the removal of suspended particulate matter and in clarification of the concentrates. Some of these enzymes may hydrolyze cinnamic derivatives, thereby giving rise to or increasing the levels of free acids observed in the concentrates.

The concentration of cinnamic derivatives was higher in the concentrates than in the purées (Table 2). Chlorogenic acid (5′-caffeoylquinic acid) was the main phenolic compound in all the samples and was present at higher concentrations in the peach than in the apple concentrates. In addition, two other isomers of chlorogenic acid, 3′-caffeoylquinic acid (neochlorogenic acid) and 4′-caffeoylquinic acid (chlorogenic isomer), were detected in the peach intermediates, along with another derivative of caffeic acid (CD in Figure 2) that was not in the apple purées and concentrates (Figure 2). Different *p*-coumaric acid derivatives (PCD) were present in the two fruits, though only one derivative (designated PCD* in Figure 2) was common to both.

Another significant difference between the two product types was the total absence of flavan-4-ols and procyanidins in the concentrates (Table 2). This may have been due to the differences in the heat treatments applied during processing of the purées and concentrates (Figure 1). At the high temperatures and acidic

**Table 2. Ranges of the Content of Phenolic Compounds (Milligrams per Liter) in Purées and Concentrates Industrially Manufactured from Peach and Apple Fruits**

| | peach purées[a] | apple purées[a] | peach concentrates[b] | apple concentrates[a] |
|---|---|---|---|---|
| 1. gallic acid | nd[c] | nd | tr | nd |
| 2. HMF | nd | 0.01−0.48 | 0.23−0.71 | 0.38−2.48 |
| 3. protocatechuic acid | 0.78−1.85 | nd | 27.67−30.24 | nd |
| 4. 2-furanoic acid | nd | 0.20−1.07 | 5.06−14.34 | 2.57−13.32 |
| 5. protocatechuic aldehyde | nd | tr−0.20[d] | nd | 0.31−0.78 |
| 6. neochlorogenic acid | 5.44−11.82 | nd | 46.65−68.15 | nd |
| 7. $B_1$ [epicatechin(4$\beta$→8)catechin] | nd | 1.57−7.60 | nd | nd |
| 8. $B_3$ [catechin(4$\beta$→8)catechin] | 7.24−13.66 | nd | nd | nd |
| 9. (+)-catechin | 12.43−25.39 | 1.58−6.82 | nd | nd |
| 10. $B_4$ [catechin(4$\beta$→8)epicatechin] | 2.08−8.92 | nd | nd | nd |
| 11. chlorogenic acid | 26.43−50.60 | 25.08−61.47 | 145.5−220.2 | 38.85−81.28 |
| 12. caffeic acid | 0.57−1.35 | 0.39−4.91 | 5.12−6.22 | 5.57−15.34 |
| 13. $B_2$ [epicatechin(4$\beta$→8)epicatechin] | 0.13−1.73 | 13.98−35.51 | nd | nd |
| 14. chlorogenic isomer | 0.10−1.07 | nd | 15.28−21.62 | nd |
| 15. (−)-epicatechin | 0.34−2.08 | 7.40−21.16 | nd | nd |
| 16. *p*-coumaric acid | nd | nd | 5.77−7.91 | 0.92−5.38 |
| 17. phloretin derivative | nd | 0.78−2.51 | nd | nd |
| 18. quercetin-3-galactose | nd | 1.17−9.88 | nd | 1.08−7.97 |
| 19. quercetin-3-glucose | nd | 0.48−2.23 | nd | 0.45−2.42 |
| 20. quercetin-3-rutinose | nd | 0.24−1.14 | nd | 0.44−3.25 |
| 21. phloretin-2′-xyloglucose | nd | 2.98−7.37 | nd | 4.14−11.75 |
| 22. quercetin-3-arabinose | nd | 1.22−5.60 | nd | 3.67−19.83 |
| 23. quercetin-3-rhamnose | nd | 1.44−3.73 | nd | nd |
| 24. phloretin-2′-glucose | nd | 10.39−29.02 | nd | 10.04−27.70 |

[a] $n = 10$. [b] $n = 3$. [c] nd, not detected. [d] tr, trace.

conditions used in concentrate manufacturing, the procyanidins could polymerize to a form not detectable by the analytical method used in this study. Those same polymerized forms might also precipitate out, yielding lees or sediment, as a consequence of, or even without, the action of proteins (Lea, 1984). In juice made in the laboratory, the loss of procyanidins and procyanidin monomers has been associated more closely with storage than with processing *per se* (Spanos *et al.*, 1990). However, in a different laboratory study (Hernández *et al.*, 1997) in which analyses were performed with minimal storage times, these same components were found to decrease when natural juices underwent heat treatment. (+)-Catechin and (−)-epicatechin and procyanidin dimers $B_1$ and $B_2$ were identified in the apple purées, mainly (−)-epicatechin and the $B_2$ dimer [epicatechin(4$\beta$→8)epicatechin]. This is in agreement with the findings reported by other researchers (Spanos *et al.*, 1990), who detected these same compounds in apple juice. Peach purées had high concentrations of (+)-catechin, followed by the dimers $B_3$, $B_4$, and $B_2$. This was also in agreement with the findings for peaches published by other workers (Lee *et al.*, 1990). Three dimers, one trimer, and one tetramer of procyanidin were also detected in the peach purées (respectively designated PRd, PRtr, and PRte in Figure 2) on the basis of their spectral characteristics (Bartolomé *et al.*, 1996).

The principal difference between apple-based and peach-based products is the fact that the latter are completely devoid of flavonol and dihydrochalcone derivatives (Table 2). Since peaches have a downy peel, the skin is removed chemically with alkalis; in addition, the central stone is removed during manufacturing of both purées and concentrates (Figure 1), which may explain the absence of flavonol derivatives, which accumulate mainly in the skin. As was previously noted, phloretin derivatives are characteristic of apples. Quercetin glycosides (quercetin-3-galactose, quercetin-3-glucose, quercetin-3-rutinose, quercetin-3-arabinose, and quercetin-3-rhamnose) and phloretin glycosides (phloretin-2′-glucose and phloretin-2′-xyloglucose, together with an unidentified derivative of phloretin) were detected in the apple purées and concentrates, though neither the unidentified phloretin derivative nor quercetin-3-rhamnose was present in the concentrates.

5-(Hydroxymethyl)furfural (HMF) is widely used as an index of nonenzymatic browning and is taken to be a measure of the intensity of the heat treatment applied during juice manufacturing (Toribio and Lozano, 1987; Garza *et al.*, 1996; Hernández *et al.*, 1997). HMF was not detected in the peach purées but was present in the peach concentrate at a defined concentration, indicating that the heat treatment employed in processing the concentrates was much more intense. In addition to the intensity of the heat treatments, the amount of HMF detected in the apple purées can be explained by the high content of reducing sugars in the fruit which tends to increase the formation of that compound (Pollard and Timberlake, 1970). The glucose content in apples is 3 times that in peaches, and the fructose content may be up to 8 times higher (Kline *et al.*, 1970; Wills *et al.*, 1987). The behavior of 2-furanoic acid was similar to that of HMF. It was present in higher concentrations than HMF in the concentrates of both fruits. It was present in the apple purées in small amounts but was not detected in the peach purées (Table 2). The higher concentrations of 2-furanoic acid in the concentrates raise the question of whether it would be a better index of the heat treatment employed than HMF and thus highlight the need for further study of this compound as a potential index.

In conclusion, the phenolic composition of intermediates used in the industrial manufacturing of fruit juices yields information about the different processing treatments applied to the fruit. The high concentrations of free cinnamic acids demonstrated that enzymes were used during industrial processing. The absence of certain flavonoid compounds in these products can possibly be attributed to chemical or physical removal of the skin of the fruit. The high concentrations of 2-furanoic acid and HMF reflected the thermal treatments used. In addition to their utility in the characterization of the products, phenolic compounds may also prove helpful in the detection of adulterations in the manufacturing of commercial fruit juices from intermediates. The presence of catechin and procyanidin monomers confirms that the juice was manufactured

from a purée. Juices identified as peach juice should not contain flavonoid glycosides unless they have been adulterated by the addition of apple juice or the juice of some other fruit.

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