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Effect of Processing Techniques at Industrial Scale on Orange Juice Antioxidant and Beneficial Health Compounds

ANGEL GIL-IZQUIERDO, MARIA I. GIL, AND FEDERICO FERRERES*

Departamento Ciencia y Tecnología de Alimentos, CEBAS-CSIC, P.O. Box 4195, 30080 Murcia, Spain

Phenolic compounds, vitamin C (L-ascorbic acid and L-dehydroascorbic acid), and antioxidant capacity were evaluated in orange juices manufactured by different techniques. Five processes at industrial scale (squeezing, mild pasteurization, standard pasteurization, concentration, and freezing) used in commercial orange juice manufacturing were studied. In addition, domestic squeezing (a hand processing technique) was compared with commercial squeezing (an industrial FMC single-strength extraction) to evaluate their influences on health components of orange juice. Whole orange juice was divided into soluble and cloud fractions after centrifugation. Total and individual phenolics were analyzed in both fractions by HPLC. Commercial squeezing extracted 22% more phenolics than hand squeezing. The freezing process caused a dramatic decrease in phenolics, whereas the concentration process caused a mild precipitation of these compounds to the juice cloud. In pulp, pasteurization led to degradation of several phenolic compounds, that is, caffeic acid derivatives, vicenin 2 (apigenin 6,8-di-*C*-glucoside), and narirutin (5,7,4′-trihydroxyflavanone-7-rutinoside) with losses of 34.5, 30.7, and 28%, respectively. Regarding vitamin C, orange juice produced by commercial squeezing contained 25% more of this compound than domestic squeezing. Mild and standard pasteurization slightly increased the total vitamin C content as the contribution from the orange solids parts, whereas concentration and freezing did not show significant changes. The content of L-ascorbic acid provided 77−96% of the total antioxidant capacity of orange juice. Mild pasteurization, standard pasteurization, concentration, and freezing did not affect the total antioxidant capacity of juice, but they did, however, in pulp, where it was reduced by 47%.

KEYWORDS: Orange juice; industrial processing; phenolics; flavanones; hydroxycinnamates; flavones; vitamin C; ascorbic acid; dehydroascorbic acid; antioxidant capacity

INTRODUCTION

Recent nutritional studies recommend the regular consumption of fruits and vegetables to favor a healthy quality of life. In fact, epidemiological studies have shown that these foods may reduce the risk of death from coronary heart diseases (*1*, *2*) and cancer (*3*). Citrus fruits are important sources of health-promoting constituents (*4*). Particularly, the intake of orange juice shows a positive effect against cardiovascular diseases (*5*). Orange juice shows an important content of flavanones, especially hesperidin and naringenin (104−584 and 18−84 mg L^{-1}, respectively) (*6*). High concentrations of flavanone glucosides are rare in other fruits and vegetables. Only similar citrus fruits and juices (lemon, lime, mandarin, and grapefruit) contribute concentrations of these compounds similar to those found in oranges and their juice (*6*). Flavones and hydroxycinnamic acids are present in this juice at a lower concentration than flavanones (*7*−*9*). Extensive in vivo and in vitro experiments of these flavanones showed beneficial health activities as protective agents against cancer (*10*) and cardiovascular (*11*), inflammatory (*12*), and allergic disorders (*13*). All of these properties have led to a commercialization of hesperidin and other citrus flavonoids as a medicine (Daflon 500 mg = 450 mg of diosmin and 50 mg of hesperidin per tablet, Servier S.A., Madrid, Spain). Hesperidin had demonstrated therapeutic effects on microcirculation in chronic venous insufficiency (*14*). Furthermore, the occurrence of these flavanones in plasma and urine has been reported in humans (*15*−*17*) and also described in rats (*18*). On the other hand, humans are unable to synthesize L-ascorbic acid (L-AA) and are thus entirely dependent upon dietary sources. Evidence suggests that the plasma levels of L-AA in large sections of the population are suboptimal for the protective health effects of this vitamin. For adults, dietary needs are met by a minimum intake of 60 mg per day (*19*), and the recommended dietary allowance (RDA) of vitamin C is 75−90 mg per day. Fruit tends to be the best food source of this vitamin. In the U.K. diet, fruit juices contribute 13% of L-AA to the general daily intake (*19*). Indeed, a serving of orange juice (240 mL) would cover the reference daily intake (RDI) amount for vitamin C (*20*). It is generally accepted that free

* Corresponding author (telephone +34968396324; fax +34968396213; e-mail federico@cebas.csic.es).

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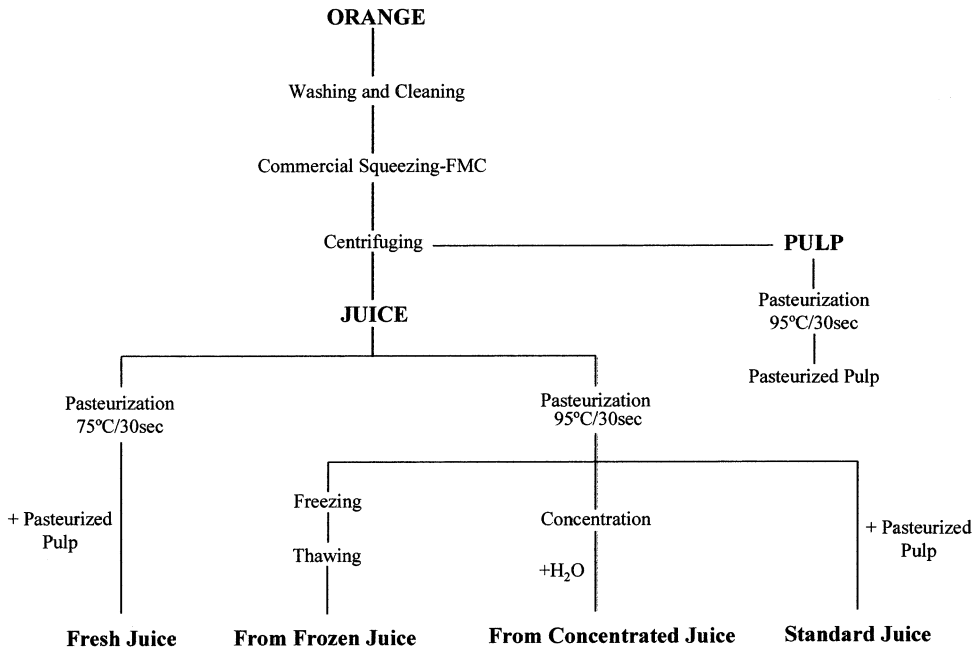

Figure 1. Scheme of the commercial orange juice processing plant.

radicals, a group of unstable and very active chemical species generated in the human body, cause oxidative disorders in lipids, proteins, and nucleic acids. Oxidative attacks on these vital biological compounds could be the cause of several diseases related to these disorders. Antioxidant compounds could prevent them by neutralizing the free radicals (*21*). The beneficial activities of orange juice components for human health are primarily attributed to their antioxidant capacity (*4*, *22*). Recent studies correlate 66% of the potential antioxidant capacity to vitamin C (*23*). However, the antioxidant efficiency of orange juices may also be attributed, in significant part, to the content of total phenols (*24*).

There are only a few studies which report that processing techniques could affect orange juice compounds (*6*). Researchers have a common aim to find the best conditions and processing techniques able to improve the above-described health-promoting and nutritional properties of orange juice. New techniques such as high-pressure and pulsed electric field have been analyzed, but their industrial use is quite limited at the moment (*25*, *26*). Furthermore, the influence of each current processing technique (pasteurization, freezing, and concentration) on phenolic compounds, vitamin C, and antioxidant capacity has been mainly described at pilot or experimental plant level or with the final commercial product but not at industrial scale (*24*, *27*, *28*). In a previous study with commercial orange juices, we observed quantitative differences in phenolic compounds (*29*). In that case, it was not possible to evaluate the effect of each individual processing step. The objective of our investigation was to determine the effects of individual orange juice processing techniques at industrial scale (pasteurization, concentration, and freezing) and two juice squeezing methods, with respect to the phenolic compounds, vitamin C, and antioxidant capacity. The effect of pasteurization on the pulp added to the final orange juice was also evaluated.

MATERIALS AND METHODS

Materials. All of the orange juices used in the analyses were obtained from Navel oranges grown in Murcia (Spain). The maturity stage of the oranges corresponded to a soluble solids content of 11−13 °Brix. The commercial processing techniques were carried out at a commercial juice manufacturing company (Juver Alimentación S.A., Murcia, Spain). The different types of juice were obtained during a standard in-line manufacturing process of commercial orange juice. However, the same type of orange juice was not used for every processing step because our study had to adapt to the production needs of the company. Therefore, the results of the orange juices were different depending of the batch of oranges used, but, this fact did not affect the development of the experiment because we evaluated individual techniques and the obtained results are independent of the different techniques.

Processing Techniques and Sampling. Orange fruit was subjected to two juice extraction methods: domestic squeezing (hand squeezing) and commercial squeezing (industrial squeezing). Both methods were compared using the same batch of oranges. A squeezer (model Citromatic, Braun Española S.A., Spain) was used to obtain the domestic squeezed juice. Three replicates of 10 oranges per replicate were squeezed for the domestic orange juice samples. To ensure the reliability and reproducibility of the squeezing of domestic orange juice, the oranges were squeezed carefully in order to obtain the juice from only the edible part of the fruit without reaching the albedo. A commercial squeezer (FMC Food Technology) was used for commercial extraction. The FMC squeezer is designed to separate the fruit into three different parts: (1) peel and seeds; (2) peel oil and washing water; and (3) juice. The commercial orange juice was collected immediately after commercial squeezing and centrifuging (**Figure 1**).

Juice samples at industrial scale were directly taken from the commercial orange juice production line. The major processing steps currently used in the manufacturing of different commercial orange juices existing in the Spanish market were analyzed. The study of each processing step was carried out on different days according to the production needs of the company. Therefore, the initial juices were obtained from different batches of oranges, and it has been represented as "B" in the figures. Orange juice was pasteurized using a plate pasteurization system (Alpha-Laval). Two pasteurization techniques, mild and standard, were carried out at the juice manufacturing plant. For the mild pasteurization, the equipment was set at 75 °C during 30 s, whereas the standard pasteurization was at 95 °C during 30 s. These two pasteurization techniques led to two clearly different commercial juices. Mild pasteurization results in fresh juices (i.e., ready-to-serve juice, which must be kept refrigerated during distribution and sale to the consumer), and standard pasteurization produces juices that can be stored at room temperature (**Figure 1**). After the pasteurization process, juices were rapidly refrigerated to 4 °C. Samples were taken before and after pasteurization and refrigeration (**Figure 1**).

The commercial concentration system was equipped with double-effect plate concentrators, two evaporators, and a thermocompressor pump (APV Baker, Ibérica, S.A., Madrid, Spain). Initially, juice was kept in a refrigerated tank at 4 °C. Prior to the concentration process, it was pasteurized at 95 °C during 30 s. The concentration process consisted of two steps. With the first one, orange juice reached 20 °Brix at 78 °C and with the second one, 60 °Brix at 64 °C. At the end of the process, the product was refrigerated at 4 °C. The vacuum pressure was adjusted at 7.2 kPa. Orange juice samples of the concentration processing technique were taken after the standard pasteurization and after the final concentration process (**Figure 1**). Concentrated orange juice was reconstituted with 5 volumes of purified water (Milli-Q purification system, Millipore Corp., Bedford, MA) using a precision refractometer (Atago) to reconstitute the original orange juice before analyses.

Freezing is a technique used in the industry to preserve the orange juice when production exceeds market demand. This commercial freezing system was equipped with a tunnel adjusted to -40 ± 5 °C, a compressor (Sabroe, H¢jbjerg, Denmark), an evaporator (Alfa-Laval), and an evaporative condenser (model Baltimore, Schier Co.). This process lasted 24−48 h depending on the initial load of the product. Frozen orange juice was maintained for 1 month (according to the production needs of the company) until thawing following a pasteurization (95 °C for 30 s) and aseptic commercial packaging. The two comparative samples involved in the freezing process were obtained after standard pasteurization and refrigeration at 4 °C and after commercial thawing of the frozen juice (**Figure 1**).

As **Figure 1** shows, the pulp was obtained after FMC squeezing and centrifuging of the orange juice. Then, it was pasteurized at 95 °C during 30 s by a plate pasteurization system (Alpha-Laval). After pasteurization process, the pulp was refrigerated at 4 °C. Pulp samples were collected prior to and after pasteurization and refrigeration at 4 °C (**Figure 1**). The pasteurized pulp was added to standard and fresh orange juices (**Figure 1**).

Analysis of Phenolic Compounds by HPLC. Sample preparation was carried out following the method described by Gil-Izquierdo and co-workers (*29*). The whole juice corresponded to the juice obtained after commercial FMC squeezing and centrifuging (**Figure 1**). For analysis, the whole juice was divided in two fractions: soluble and cloud fractions. Orange juice cloud (insoluble fraction) was separated by centrifugation of 1 mL of juice at 10500*g* in a Eppendorf centrifuge (model Sigma 1-13, B. Braun Biotech International, Osterode, Germany) for 5 min at room temperature. The supernatant (soluble fraction) was filtered through a 0.45 μm polyethersulfone filter and analyzed by HPLC. The pellet obtained after centrifugation was treated with 1 mL of dimethyl sulfoxide (DMSO) and sonicated for 2 min to extract the phenolic compounds. The solution was centrifuged at 10500*g* as described above. The supernatant was then filtered through a 0.45 μm polyethersulfone filter and analyzed by HPLC. The HPLC analyses were performed with a gradient liquid chromatograph (Merck-Hitachi, Darmstadt, Germany) with a pump (model L-6200) and a UV−vis detector (model L-7420). Separations were achieved on a Lichrocart C_{18} column (Merck, Darmstadt, Germany) (12 × 0.4 cm; 5 μm particle size), using as mobile phase water/formic acid (95:5, v/v) (A) and methanol (B). The solvent flow rate was 1 mL min^{-1}, and a linear gradient starting with 10% B in A to reach 35% B in A in 25 min was used. Sample aliquots of 50 μL were injected, and soluble phenolic compounds were identified and quantified by comparison of peak areas with external standards. Hydroxycinnamic acid derivatives, flavones, and flavanones (hesperidin, narirutin, and didymin) were determined. Chromatograms were recorded at 290 nm for flavanone quantitation and at 340 nm for hydroxycinnaminc derivatives and flavones. Hydroxycinnamic compounds were quantified as chlorogenic acid (5-caffeoylquinic acid) (Sigma, St. Louis, MO), flavones as vicenin 2 (previously isolated in our laboratory from lemon peel), and flavanones as hesperidin (Merck). The method recovery was 95%. The results were expressed as milligrams per serving of juice. A serving of juice corresponds to 240 mL according to the U.S. FDA (*30*).

Analysis of L-Ascorbic Acid and L-Dehydroascorbic Acid (L-DHAA). Vitamin C was evaluated in the supernatant fraction of orange juice after centrifugation of 1 mL of juice at 10500*g* in an Eppendorf centrifuge (model Sigma 1-13 B, Braun Biotech International) for 5 min at room temperature. The supernatant was filtered through a 0.45 μm polyethersulfone filter and adjusted at pH 2.2−2.4 with 1 M HCl. This solution (5 mL) were adsorbed onto a previously activated (with methanol, water, and finally air to remove the remaining water into the cartridge) C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA). A volume of 750 μL of this water soluble solution (rejecting the first 3 mL) was added to 250 μL of 18.8 mM 1,2-phenylenediamine dihydrochloride (OPDA) (Fluka Chemika, Neu-Ulm, Switzerland). The derivatization reaction was maintained during 37 min in the dark prior to analysis by HPLC. OPDA enhances the UV absorptivity of L-DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-*b*]quinoxaline-1-one (DFQ). Vitamin C was analyzed as the addition of L-AA and L-DHAA measured following the Zapata and Dufour method (*31*). The analyses were performed in an HPLC system (Merck-Hitachi). It consisted of an isocratic pump (model L-4000), an injection valve with a 20 μL sample loop (Rheodyne, Cotati, CA), and a UV variable-wavelength detector (model L-6000). Separations were achieved in a Kromasil 100 C_{18} column (250 × 4 mm, 5 μm particle size) (Teknokroma, Barcelona, Spain) protected with a guard C_{18} precolumn (Teknokroma). The eluent was methanol−water (5:95, v/v) containing 5 mM cetrimide (hexadecyltrimethylammonium bromide) (Sigma, Steinheim, Germany) and 50 mM potassium dihydrogen phosphate (Merck). The final pH of the eluent was 4.59, and the flow rate was 0.9 mL min^{-1} at room temperature. Prior to analyses, the column was equilibrated by pumping the eluent for 24 h at a reduced flow of 0.3 mL min^{-1}. Detector wavelength was initially set at 348 nm, and after elution of DFQ, it was manually shifted to 261 nm for L-AA detection. Sample aliquots of 20 μL were injected, and L-AA and L-DHAA were identified and quantified by comparison of peak areas with authentic markers. The results were expressed as milligrams per serving of orange juice.

Analysis of the Antioxidant Capacity. One milliliter of juice was centrifuged at 10500*g* in an Eppendorf centrifuge (model Sigma 1-13, B. Braun Biotech International) for 5 min at room temperature. The supernatant was analyzed according to the technique reported by Brand-Williams and co-workers (*32*). Briefly, 50 μL of sample was added to 950 μL of 0.094 mM 2.2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) (Sigma, Steinheim, Germany) in methanol. The free radical scavenging activity using the free radical DPPH$^{\bullet}$ reaction was evaluated by measuring the absorbance at 515 nm after 20 min of reaction at 20 °C in a spectrophotometer (model Anthelie Graphic, Secomam, France). The reaction was carried out in closed Eppendorf tubes shaken at 20 °C. The results were expressed as milligrams of L-AA equivalent per serving of orange juice. L-AA was used as antioxidant reference compound because it is one of the main components in orange juice and its antioxidant activity is similar to that of Trolox (*33*).

Data Analysis. Data were obtained as a mean of three values. Standard deviations were also calculated.

RESULTS AND DISCUSSION

Effect of Squeezing and Commercial Processing Techniques on the Phenolic Compounds of Orange Juice. The whole orange juices analyzed ranged from 52 to 175 mg per serving of flavanones (14−35, 24−120, and 9−17 mg per serving of narirutin, hesperidin, and didymin, respectively), from 17 to 20 mg per serving of flavones (3−4 and 14−16 mg per serving of luteolin derivative and vicenin 2, respectively), and from 16 to 20 mg per serving of hydroxycinnamic acid derivatives (11−14 and 5−6 mg per serving of caffeic acid derivatives and *p*-coumaric acid derivatives, respectively). When the squeezing methods were compared for the whole juice (supernatant and cloud fraction), the commercial squeezing technique extracted 22% more phenolics than the domestic squeezing (**Table 1**). This increase was due to a higher concentration of phenols in the cloud fraction of the commercial juices, which was 2.75-fold higher than the cloud content in the domestic juices (**Table 1**). The phenolic compounds content

Table 1. Total Phenolics (Whole Juice, Soluble Fraction, and Cloud Fraction), Dehydroascorbic Acid (L-DHAA), Ascorbic Acid (L-AA), and Vitamin C Contents and Antioxidant Capacity of Orange Juice and Pulp Following Domestic and Commercial Processing Techniques[a]

processing technique	sampling	phenols in whole juice[b]	phenols in soluble fraction[b]	phenols in cloud fraction[b]	vitamin C[b]	L-DHAA[b]	L-AA[b]	antioxidant capacity[c]
			In Orange Juice					
squeezing methods	domestic squeezing	172.0 (7.4)	141.3 (2.2)	30.7 (6.2)	114.7 (16.3)	13.3 (3.3)	101.4 (19.4)	131.0 (11.2)
	commercial squeezing	210.7 (2.0)	126.0 (2.2)	84.7 (3.4)	143.5 (10.8)	11.6 (0.4)	131.9 (10.4)	126.8 (4.2)
mild pasteurization	before pasteurization	210.7 (2.0)	126.0 (2.2)	84.7 (3.4)	143.5 (10.8)	11.6 (0.4)	131.9 (10.4)	126.8 (4.2)
(75 °C/30 s)	after pasteurization	219.8 (8.3)	130.5 (3.1)	89.3 (7.0)	160.5 (9.1)	17.7 (7.9)	142.8 (1.2)	135.3 (4.2)
standard pasteurization	before pasteurization	219.1 (0.5)	130.5 (0.5)	88.6 (0.2)	131.2 (12.6)	7.8 (0.5)	123.4 (12.7)	150.1 (3.0)
(95 °C/30 s)	after pasteurization	219.3 (7.1)	134.8 (1.4)	84.5 (8.2)	155.7 (4.3)	9.6 (0.2)	146.1 (4.2)	143.7 (7.3)
concentration	before concentration	84.5 (3.3)	79.5 (2.6)	5.1 (0.9)	101.0 (6.3)	11.5 (0.4)	89.5 (6.2)	99.3 (5.3)
	after concentration	81.0 (1.6)	71.4 (0.4)	9.6 (1.4)	98.5 (0.7)	4.6 (0.7)	93.9 (1.1)	97.4 (0.7)
freezing	before freezing	210.4 (7.2)	127.3 (1.3)	83.1 (8.2)	101.0 (6.3)	11.5 (0.4)	89.5 (6.2)	105.0 (3.6)
	after freezing	136.1 (16.3)	63.5 (14.3)	72.6 (9.2)	98.5 (15.8)	14.5 (0.1)	84.0 (15.7)	111.9 (17.9)
			In Orange Pulp					
standard pasteurization	before pasteurization	63.9 (1.2)			10.8 (0.9)	1.9 (0.4)	8.9 (0.6)	39.5 (4.9)
(95 °C/30 s)	after pasteurization	50.8 (6.0)			4.5 (0.4)	2.6 (0.2)	1.9 (0.2)	21.1 (0.0)

[a] Values are the mean of three replicates. Standard deviations are given in parentheses. [b] Total phenolics, L-DHAA, and L-AA are in mg per serving of orange juice. [c] Antioxidant capacity is in mg L-AA equivalents per serving of orange juice.

in the soluble fraction of the domestic squeezed juice was 11% more abundant than in the same fraction of the commercial juice (**Table 1**). Mild and standard pasteurization techniques did not show changes in the total content of phenolics in either the soluble fraction or the cloud fraction (**Table 1**). Therefore, neither of these techniques themselves nor temperature or pressure affected the content of phenolic compounds. When orange juice was subjected to the concentration process, the total phenolic content in the whole juice did not change from the initial one but underwent a mild precipitation of phenolics from the soluble fraction to the cloud (**Table 1**). No loss of these compounds was detected, despite the high grade of manipulation of the juice during the concentration process (**Table 1**). The lower content of phenolics in this juice, compared to the phenolic content of other juices, was due to the low quality of the oranges used at the end of the season. In the case of the freezing technique, the decrease of the phenolic compounds was dramatic in relation to its content before this process (loss of 35%) (**Table 1**). This degradation was associated with the soluble fraction (from 127.3 to 63.5 mg per serving after the freezing step) (**Table 1**). The phenolic precipitation to the cloud fraction could be due to the storage of the frozen juice for 1 month as suggested by previous results (*29*) and the thawing process.

Flavanones were the major phenolic compounds in orange juice. By comparison of the two juice squeezing methods, commercially squeezed juice provided more flavanones than domestic squeezing (**Figure 2**). This difference was due to an increase of the flavanone content in the cloud fraction (**Figure 2**). This major presence of flavanone in the cloud of the commercial juice favors the precipitation of these compounds during the storage according to the previous studies of Gil-Izquierdo and co-workers (*29*). HPLC analyses of orange juice showed the presence of narirutin (5,7,4′-trihydroxyflavanone-7-rutinoside), hesperidin (4′-methoxy-3′,5,7-trihydroxyflavanone-7-rutinoside), didymin (4′-methoxy-5,7-dihydroxyflavanone-7-rutinoside), and two other unidentified minor flavanones. Particularly, the higher increases belonged to hesperidin and didymin, with 30 and 27% increases, respectively, compared to the flavanones extracted by domestic squeezing (**Figure 2**). It is of note that the hesperidin content in the cloud provided by the commercial squeezing was 4-fold higher than that obtained by domestic squeezing (**Figure 2**). This could be due to the commercial squeezing method, which extracts flavanones from the albedo where the amount of these compounds is much higher than in the juice and flesh (*6*). Mild and standard

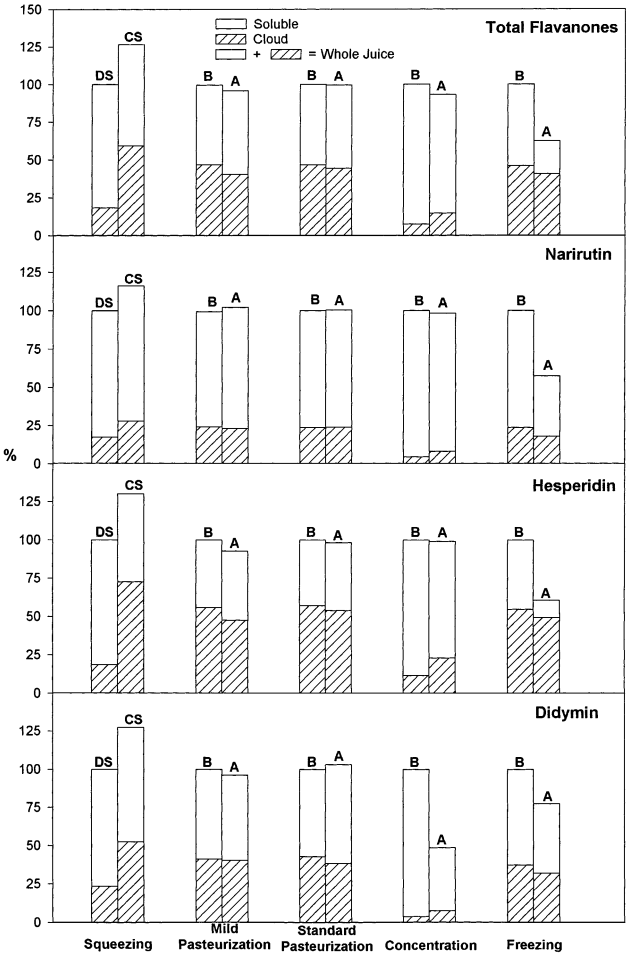

Figure 2. Changes in the total flavanones, narirutin, hesperidin, and didymin content (percent) of orange juice subjected to two squeezing methods (DS, domestic squeezing; CS, commercial squeezing) and four commercial processing techniques (mild pasteurization, standard pasteurization, concentration, and freezing): B, sample before processing step, which was referred to 100%; A, sample after processing step. The values of the commercial squeezing juice were referred to 100% of the domestic squeezing juice. Standard deviation was <11%.

pasteurization processes did not influence the flavanone content of the juice prior to and after the process in both fractions (**Figure 2**). Therefore, these flavanones in the whole juice were

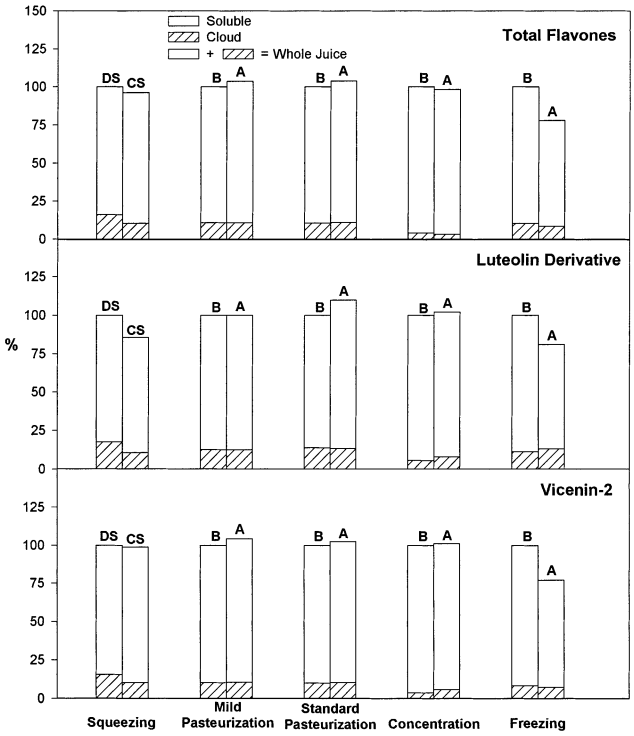

Figure 3. Changes in the total flavones, luteolin derivative, and vicenin 2 contents (percent) of orange juice subjected to two squeezing methods (DS, domestic squeezing; CS, commercial squeezing) and four commercial processing techniques (mild pasteurization, standard pasteurization, concentration, and freezing): B, sample before processing step, which was referred to 100%; A, sample after processing step. The values of the commercial squeezing juice were referred to 100% of the domestic squeezing juice. Standard deviation was <14%.

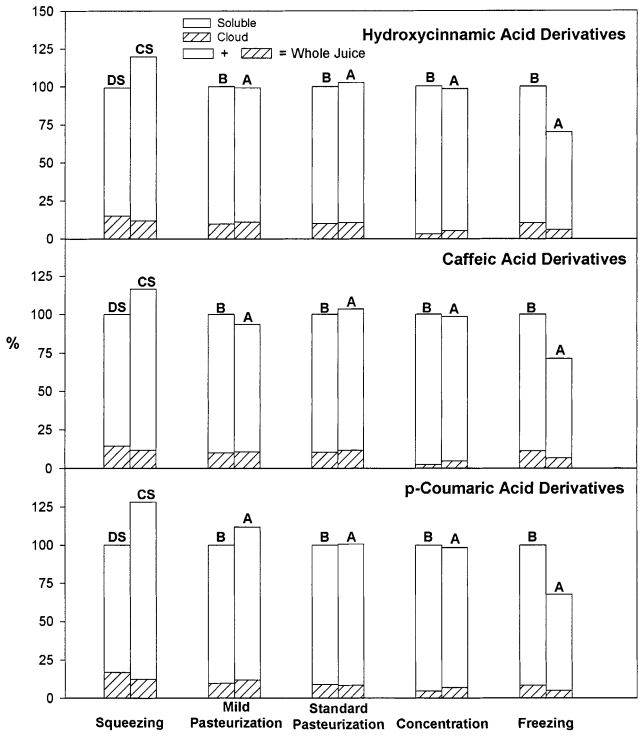

Figure 4. Changes in the hydroxycinnamic acid derivatives, caffeic acid derivatives, and *p*-coumaric acid derivatives content (percent) of orange juice subjected to two squeezing methods (DS, domestic squeezing; CS, commercial squeezing) and four commercial processing techniques (mild pasteurization, standard pasteurization, concentration, and freezing): B, sample before processing step, which was referred to 100%; A, sample after processing step. The values of the commercial squeezing juice were referred to 100% of the domestic squeezing juice. Standard deviation was <16%.

stable at the pasteurization temperatures. On the other hand, when the orange juice was subjected to the concentration process, didymin decreased 52% in the soluble fraction with respect to the content before concentration (**Figure 2**). The rest of the flavanones in the soluble fraction showed a slight decrease. In addition, concentration increased the total flavanone content in the cloud fraction due to the precipitation from the soluble fraction (**Figure 2**). In fact, the flavanone content in the cloud fraction was 2-fold higher in narirutin, hesperidin, and didymin with regard to the content before concentration (**Figure 2**). The freezing process caused a dramatic decrease of the flavanones in the soluble fraction (**Figure 2**). Didymin, hesperidin, and narirutin decreased 23, 39, and 43%, respectively, in relation to the content previous to the freezing step (**Figure 2**). Thus, didymin was the most stable flavanone and narirutin the least stable flavanone during this process. It is of note that frozen orange juice was kept during 1 month until thawing and final commercial packaging. The storage of orange juice favors the precipitation of the flavanones to the cloud fraction (*29*). The loss in the total and individual flavanone content could be due to the thawing process.

Two types of flavones, vicenin 2 (apigenin 6,8-di-*C*-glucoside) and a luteolin derivative, were detected in orange juice. Vicenin 2 (UV spectrum maxima at 272 and 335 nm) had been previously described in orange juice (*29*). Flavone content was different depending on the squeezing method used. This content was affected by the freezing technique but not by pasteurization and concentration processes (**Figure 3**). In a previous study, it was suggested that orange juice flavones decreased as a result of the pasteurization process (*29*). It must be taken into account that this previous study was performed with commercial orange juices and no individual processing techniques were evaluated. The content of luteolin derivative was lower in commercially squeezed juice than in domestic squeezing (15% less), both in the soluble fraction and in the cloud fraction (**Figure 3**). Vicenin 2 was found in the same proportion in the juice obtained by domestic and commercial squeezing, and its contents were also similar in both fractions (**Figure 3**). Freezing caused decreases of 19 and 22% of initial luteolin derivative and vicenin 2 contents (respectively) compared to the contents prior to freezing (**Figure 3**). This change was noticeable only in the soluble fraction, whereas the flavone content remained unchanged in the cloud fraction (**Figure 3**). This loss in the total and individual flavone content could be due to the thawing process.

Ten chromatographic peaks with hydroxycinnamic derivative spectra were detected such as sinapic, *p*-coumaric, ferulic, and caffeic acid derivatives. One peak showed a *p*-coumaric acid derivative spectrum (maximun at 312 nm and shoulder at 298 nm), and another peak showed a UV spectrum similar to that of a sinapic acid derivative (maximum at 329 nm and shoulder at 305 nm). The other eight peaks showed UV spectra as caffeic acid or ferulic acid derivatives (maximum at 325 nm with a shoulder at 298 nm). These UV values were in agreement with previously reported data for these compounds (*34*). Hydroxycinnamic acid derivatives were separated in two broad groups: caffeic acid derivatives and *p*-coumaric acid derivatives. Their contents were 16 and 28% higher in the commercial squeezing than in the domestic squeezing, respectively (**Figure 4**). This increase was exclusively detected in the soluble fraction. Pasteurization and concentration processes did not affect the initial hydroxycinnamic content of orange juice in agreement

Table 2. Loss of Total and Individual Flavanones, Flavones, and Hydroxycinnamic Acid Derivatives after Standard Pulp Pasteurization[a]

phenolic compound	%
total flavanones	19 (2)
narirutin	28 (3)
hesperidin	19 (2)
didymin	1 (0)
total flavones	29 (4)
luteolin derivative	20 (3)
vicenin 2	31 (4)
total hydroxycinnamic acids	29 (4)
caffeic acid derivatives	35 (5)
p-coumaric acid derivatives	15 (2)

[a] Values are the mean of three replicates. Standard deviations are given in parentheses. The percentage is calculated taking as reference the 100% of the corresponding phenolic compound just before pasteurization step.

with previous results (*29*) (**Figure 4**). However, freezing caused a dramatic decrease of hydroxycinnamic derivatives in the juice with losses of 29 and 32% of caffeic and *p*-coumaric derivatives, respectively, with respect to the contents before processing (**Figure 4**). This loss was evident in the soluble fraction as well as in the cloud fraction (**Figure 4**). As in flavones, part of the hydroxycinnamic acid derivatives could have been lost during the thawing.

Pulp was pasteurized separately from the juice at 95 °C during 30 s. Total phenolic compounds suffered losses during pulp pasteurization (**Table 1**). With regard to flavanones, a global loss of 19% was detected in comparison with the pulp content before pasteurization. Narirutin was the most labile flavanone due to its highest loss (28%), and didymin the most stable with a loss of only 1% (**Table 2**). Flavones and hydroxycinnamic acid derivatives showed higher losses than flavanones (29 and 29%, respectively). Vicenin 2 was less stable than luteolin derivative during pasteurization conditions (**Table 2**). A loss of 35% in caffeic derivatives demonstrated that this group of compounds was the most susceptible to degradation by the pasteurization process (**Table 2**). The degradation of *p*-coumaric compounds was, however, moderate (**Table 2**). The commercial squeezing as well as concentration and freezing had an important role in the phenolic content of orange juices. In the case of orange juice pulp, the pasteurization significantly affected the phenolic compounds, overall, caffeic acid derivatives, and the flavanone, narirutin.

Effect of Squeezing and Commercial Processing Techniques on the L-AA, L-DHAA, and Total Vitamin C of Orange Juice. Vinson and Bose (*35*) emphasized the importance of L-AA as a natural product in orange juice where flavonoids, proteins, and carbohydrates increase the bioavailability of this acid. In our case, in the orange juice tested, L-AA was shown to be the major compound in the total vitamin C content (~90% L-AA and ~10% L-DHAA). Juice produced by commercial squeezing contained 25% more vitamin C than domestic squeezing (**Table 1**). This could be due to the contribution of vitamin C from the solid parts of the orange by the commercial squeezing. This increase was particularly important in the L-AA content because L-DHAA remained unchanged for both juices. Mild and standard pasteurization treatments slightly increased the total vitamin C, L-AA, and L-DHAA contents, possibly due to the contribution from the solid parts as a consequence of the heat treatments (**Table 1**). However, these increases must be viewed with caution due to the high standard deviation values. Our data obtained at industrial scale are in disagreement with the degradation of orange juice L-AA at pilot plant level reported by Naim and co-workers (*28*). In our case, pasteurization did not play an important role in L-AA and L-DHAA contents. The concentration process favored the transformation of L-DHAA to L-AA, which is a compound with major antioxidant capacity. However, no degradation of the total vitamin C content was detected between juice samples before and after concentration (**Table 1**). When the orange juice was analyzed before and after the freezing, the main difference was detected in the increase of L-DHAA (26%) reciprocal to the L-AA content (**Table 1**). This process, however, did not affect the total vitamin C content (**Table 1**). In addition, little information has been found that describes the behavior of vitamin C during the freezing of orange juice.

The pasteurization of the pulp caused a loss of 58% in total vitamin C compared with the content prior to pasteurization (**Table 1**). L-AA showed a dramatic degradation, and only 21% remained of its initial content; L-DHAA increased 2-fold from its initial value (**Table 1**). However, these negative effects did not greatly influence the total content of vitamin C in the whole juice. It must be taken into account that in the commercial process, pulp is present at a low concentration (maximum of 10%) providing a small proportion of the total vitamin C content. In conclusion, there was no vitamin C degradation detected in the juice. It should be emphasized that commercial juice equipment is an efficient technique for vitamin C extraction. Concentration was seen as a positive technique because it favored the conversion of L-DHAA to L-AA. Pasteurization and freezing techniques mildly increased the presence of L-DHAA, but they did not seriously affect L-AA content. In pulp, vitamin C degradation was dramatic, overall, in terms of the L-AA content.

Effect of Squeezing and Commercial Processing Techniques on the Antioxidant Activity of Orange Juice. In the orange juice, L-AA provided at least 77% of the antioxidant capacity (comparing milligrams of L-AA with milligrams of L-AA equivalent) in agreement with Gardner and co-workers (*23*) (**Table 1**). They reported that vitamin C provides 65−100% of the antioxidant potential of beverages derived from citrus fruit. Furthermore, the contribution to the cited antioxidant potential of carotenoids was negligible. Juice antioxidant capacity did not show significant changes due to the high stability of L-AA during the different processing techniques (**Table 1**). Phenolic compounds were not so relevant for the antioxidant capacity contribution in the orange juice compared to L-AA. On the other hand, the L-AA content of the pulp supplied only 23% of the total antioxidant capacity because L-AA was a minor compound compared to the total phenolic content (**Table 1**). The pulp showed a loss of 47% in the antioxidant capacity, overall, due to phenolic compounds and, to a lesser degree, L-AA degradation (**Table 1**). It is important to note the major contribution of L-AA to the total antioxidant capacity of orange juice. In juice, processing techniques did not seriously affect this parameter due to the stability of L-AA. However, in pulp, where L-AA was detected as a minor compound, total antioxidant capacity decreased dramatically overald due to the phenolic compound degradation.

The juice extraction method is a critical point because oranges, apart from their sections, possess albedo and peel, where a broad group of compounds with health beneficial effects can be found, some of them (phenolic compounds and, particularly, flavanones) in much higher concentration than in the part considered edible by the consumers (*6*). In practice, domestic squeezing yielded orange juices richer in soluble phenolic compounds than that obtained from commercial

squeezing. On the other hand, commercial squeezing produced juices with a higher concentration of phenolic compounds and vitamin C than domestic squeezing. These results are due to the more vigorous juice extraction in the commercial squeezing technique. According to previous nutritional studies (*17*, *18*), orange juices from domestic squeezing may provide higher phenolic compounds available for absorption at the small intestinal level. On the other hand, orange juices from commercial squeezing may provide higher phenolic content available to absorption after fermentation by the gut microflora than orange juices obtained from domestic squeezing.

Juice pasteurization techniques did not modify the initial nutritional and antioxidant content of the orange juices. However, pulp pasteurization is a restrictive technique that could influence the final content of vitamin C and phenolic compounds. Orange juice made from concentrated and frozen orange juice provided lower phenolic content than the initial juice.

ABBREVIATIONS USED

DPPH•, 2,2-diphenyl-1-picrylhydrazyl radical; L-AA, ascorbic acid; DFQ, 3-(1,2-dihydroxyethyl)furo[3,4-*b*]quinoxaline-1-one; DMSO, dimethyl sulfoxide; L-DHAA, L-dehydroascorbic acid; OPDA, 1,2-phenylenediamine dihydrochloride; RDA, recommended daily allowance; RDI, reference daily intake.

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