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Influence of Industrial Processing on Orange Juice Flavanone Solubility and Transformation to Chalcones under Gastrointestinal Conditions

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Orange juice manufactured at industrial scale was subjected to digestion under in vitro gastrointestinal conditions (pH, temperature, and enzyme and chemical conditions) to evaluate the influence of individual industrial processing treatments on flavanone solubility, stability, and ability to permeate through a membrane under simulated physiological conditions. Four industrial processes including squeezing, standard pasteurization, concentration, and freezing were evaluated. Hand squeezing was compared with industrial squeezing. After in vitro gastrointestinal digestion of the orange juices, the flavanones able to permeate through a dialysis membrane, and those remaining in the retentate were evaluated by HPLC as were those present in the insoluble fraction. In all of the assayed orange juices, a high content of precipitated chalcones (≈70% of the total flavanones) was formed under the physiological conditions of the gastrointestinal tract. Hand squeezing provided a higher concentration of flavanones in the permeated fraction and lower transformation to chalcones than industrial squeezing. Standard pasteurization did not influence the solubility and permeability of the orange juice flavanones and chalcones. Industrial concentration did not affect the amount of flavanones able to permeate but decreased the chalcones produced. Juices produced from frozen orange juice contained considerably smaller amounts of both soluble flavanones and insoluble chalcones.

KEYWORDS: Orange juice; processing; flavanone; chalcone; solubility; stability; chemical transformation; gastrointestinal digestion

INTRODUCTION

Orange juice is highly and regularly consumed in Western countries. In 1998, the United States, Canada, and the European Union were the major consumers at 42.9, 40.6, and 36.7 kg/year of orange juice per capita (*1*). Furthermore, over the period of 10 years beginning in 1988, orange juice consumption grew 4.2% per annum (*2*).

Previous studies have shown the beneficial properties of orange juice for human health. The administration of orange juice to subjects with hypercholesterolemia improved their blood lipid profile (*3*). One of the main characteristics of orange juice is its high content of the flavanones hesperidin and narirutin (*4*). These compounds have shown potential beneficial properties against several diseases, as they have shown biological activity on experiments with animals, cell lines, and in vitro assays associated with carcinogenic (*5*), cardiovascular (*6*), inflammatory (*7*), allergic (*8*), and bleeding (*9*) disorders.

Recent studies have shown that orange juice flavanones are absorbed in humans at a higher rate than other flavonoids (*10*−*13*). After juice ingestion, hesperetin and naringenin glucuronides are detected in plasma with maximal concentrations after 4−7 h of ingestion (*10*, *11*), although these compounds are sometimes detected as early as 1 h after ingestion (*10*). These studies have shown that the flavanones present in orange juice, hesperetin and naringenin rutinosides, have to be hydrolyzed in the gastrointestinal tract due to the physiological conditions (*14*) or the microbial enzymes present in the gut (*10*−*14*), before absorption, and that the aglycons are then metabolized to the corresponding glucuronides that are detected in plasma. Pharmacokinetic studies suggest that flavanones are absorbed in the distal part of the small intestine or in the colon, although absorption at proximal parts of the intestinal tract can also be possible, albeit at lower rates.

Flavanones in orange juice are both soluble and precipitated to form crystals that interact with proteins, pectins, or other carbohydrates to be included in the cloud (*15*, *16*). Flavanone precipitation and/or transformation into other compounds (aglycons and chalcones) under the physiological conditions of the gastrointestinal tract (*14*, *17*) can have an effect in the absorption rate of these compounds, as precipitated flavanones

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included in the cloud particles can be less accessible to the action of the intestinal microbial enzymes than the soluble ones.

In a recent study, an in vitro gastrointestinal digestion method was used to simulate physiological conditions of the stomach and small intestine (pH, temperature, and enzyme conditions) (*18*) and to evaluate the extraction of phenolics from the food matrix, their stability, and chemical transformations under these conditions. In the case of orange juice this method allows the evaluation of the flavanones that remain soluble under physiological conditions and that are able to permeate through a membrane, those that, although soluble, interact with macromolecules (proteins, pectins, etc.) preventing their permeation, and those that precipitate to the cloud. In addition, it allows possible chemical transformations due to the enzymes and pH conditions of the stomach and small intestine (hydrolysis and other chemical transformations) to be monitored. Previous in vitro gastrointestinal studies have shown that soluble orange juice flavanones were partially transformed to chalcones which precipitate rapidly to the cloud (*17*, *18*).

Commercial processing techniques used to manufacture the orange juice such as extraction, concentration, and freezing affect the flavanone content and their solubility and precipitation to the cloud (*17*, *19*, *20*).

The present study focuses on the effect of the extraction method and processing techniques including pasteurization, concentration, and freezing on the solubility, stability, and ability to permeate through a membrane of the flavanones in orange juice when it is subjected to in vitro gastrointestinal conditions.

MATERIALS AND METHODS

Materials. All of the orange juices used in the analyses were obtained from navel oranges grown in Murcia (Spain). The maturity state corresponded to a soluble solids content of 11−13 °Brix. The commercial processing techniques were carried out in a commercial juice manufacturing company (Juver Alimentación S.A., Murcia, Spain). Juices were obtained during a standard in-line manufacturing process of commercial orange juice.

Processing Techniques and Sampling. The scheme of the commercial processing techniques can be found in a previous paper (*20*). Orange fruit was subjected to two juice extraction methods: domestic squeezing (hand squeezing) and industrial squeezing. Both methods were compared using the same batch of oranges. A squeezer (model Citromatic, Braun Española S.A.) was used to obtain the domestic squeezed juice. Oranges were squeezed carefully to obtain the juice only from the edible part of the fruit without reaching the albedo. Three replicates of 10 oranges per replicate were squeezed for the domestic orange juice samples. A commercial squeezer (FMC Food Technology) was used for extraction, and the obtained juice was collected immediately from the production line.

The major processing steps (pasteurization, concentration, and freezing) currently used in the manufacture 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.

Orange juice was pasteurized using a plate pasteurization system (Alpha-Laval) at 95 °C during 30 s. After that, juices were rapidly refrigerated to 4 °C. Samples were taken before and after pasteurization and refrigeration. Standard pasteurization produces juices that can be stored at room temperature.

The industrial 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. Orange juice reached 20 °Brix at 78 °C with the first one 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. Samples from the concentration process were taken after the standard pasteurization and at the end of the process. Concentrated orange juice was reconstituted with 5 volumes of purified water (Milli-Q purification system, Millipore Corp., Bedford, MA) using a precision refractometer (Atago).

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, Højbjerg, Denmark), an evaporator (Alfa-Laval), and an evaporative condenser (model Baltimore, Schier Co.). The orange juice underwent a first pasteurization at 95 °C for 30 s before its introduction into the freezing tunnel. 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 second pasteurization (95 °C for 30 s) and aseptic commercial packaging. The two comparative samples involved in the freezing process were obtained after the first standard pasteurization and refrigeration at 4 °C and after the frozen juice had been thawed.

In Vitro Simulation of Gastrointestinal Digestion. This method allows the orange juice phenolic compound release and their behavior and stability upon gastrointestinal conditions to be simulated. It consisted of a pepsin−HCl digestion for 2 h and a pancreatin digestion with bile salts for 2.5 h, both at 37 °C. For the pepsin−HCl digestion, to samples of orange juice (50 mL) was added 15750 units of pepsin (EC 3.423.1; Sigma, Steinheim, Germany). The pH was adjusted to 2, and the samples were incubated in a 37 °C shaking water bath (Selecta, Barcelona, Spain). The pepsin digests (20 mL) was transferred to a polyethylene tube containing a cellulose dialysis tubing (molecular weight cutoff of 12000 Da; Sigma) filled with 25 mL of water and the amount of $NaHCO_3$ (Sigma) equivalent to the titratable acidity (equivalents of $NaHCO_3$ required to titrate the combined pepsin-digest pancreatin−bile extract mixture to pH 7.5) (*18*). Five milliliters of pancreatin−bile extract mixture was added when the pH value reached 5.0 and was left for 2 h to allow the enzyme action and to reach an equilibrium between dialyzed (permeate) and nondialyzed (retentate) fractions. The phenolics present in both fractions were analyzed by HPLC (*18*), and their volume was recorded. In all cases the permeate volume was 21 ± 1 mL and the retentate volume was 27 ± 1 mL.

Analysis of Phenolic Compounds by HPLC. Sample preparation was carried out as reported previously (*17*). The whole juice was divided after centrifugation into two fractions: supernatant (soluble fraction) and cloud (insoluble fraction). The supernatant was filtered and directly analyzed by HPLC (*20*). The cloud fraction was treated with dimethyl sulfoxide (DMSO), sonicated, and centrifuged. The supernatant was then filtered and analyzed by HPLC. The analyses were performed with a Merck-Hitachi gradient liquid chromatograph with a pump model L-6200 and a diode array detector Merck-Hitachi model L-3000. Separations were achieved on a Lichrocart column (Merck, Darmstadt, Germany) (RP-18, 25 × 0.4 cm; 5 μm particle size), using a mobile phase of 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. Aliquots of 50 μL were injected, and soluble phenolic compounds were identified and quantified by comparison of peak areas with external standards. Flavanones such as hesperidin, narirutin, and didymin and the corresponding chalcones were determined according to a previous work (*17*). Chromatograms were recorded at 290 nm for flavanone quantitation and at 340 nm for chalcones. Flavanones were quantified as hesperidin (Merck) and chalcones as hesperidin chalcone (obtained in our laboratory by isomerization with NaOH at pH 14 from hesperidin and identified by mass spectrometry with an electrospray ionization system). 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 FDA (*21*).

RESULTS

The flavanone contents in the soluble and cloud fractions are shown in **Table 1**. Domestic squeezed juice had a higher soluble

Table 1. Total and Individual Flavanones and Chalcones before and after Gastrointestinal Digestion Following Domestic and Industrial Orange Juice Squeezing[a]

		domestic squeezing				industrial squeezing			
before or after digestion	fraction	total flavanones	narirutin	hesperidin	didymin	total flavanones	narirutin	hesperidin	didymin
before	soluble	120.9 ± 0.1 (79.2)[b]	25.2 ± 0.2 (82.0)	78.6 ± 0.1 (79.4)	10.4 ± 0.0 (70.2)	79.1 ± 0.5 (52.0)	20.3 ± 0.0 (76.1)	44.7 ± 0.4 (42.9)	7.8 ± 0.0 (57.3)
	cloud	31.8 ± 0.2 (20.8)	5.6 ± 0.0 (18.0)	20.4 ± 0.2 (20.6)	4.4 ± 0.3 (29.8)	72.9 ± 0.2 (48.0)	6.4 ± 0.1 (23.9)	59.5 ± 0.1 (57.1)	5.9 ± 0.0 (42.7)
after	permeate	24.7 ± 2.5 (16.2)	7.1 ± 0.7 (23.1)	12.6 ± 1.1 (12.7)	2.9 ± 0.7 (19.6)	17.0 ± 0.4 (11.2)	5.6 ± 0.1 (21.0)	8.6 ± 0.3 (8.3)	2.1 ± 0.3 (15.3)
	soluble retentate	47.0 ± 1.1 (30.8)	13.3 ± 0.2 (43.2)	23.5 ± 0.2 (23.7)	6.0 ± 1.2 (40.5)	29.7 ± 2.6 (19.5)	10.0 ± 1.0 (37.5)	14.8 ± 1.3 (14.2)	4.3 ± 0.3 (31.3)
	cloud	13.7 ± 3.4 (9.0)	1.3 ± 0.5 (4.2)	11.8 ± 3.0 (11.9)	nd	19.0 ± 3.8 (12.5)	1.6 ± 0.4 (6.0)	17.4 ± 3.5 (16.7)	nd
	fraction	chalcone[c]	narirutin chalcone	hesperidin chalcone	didymin chalcone	chalcone	narirutin chalcone	hesperidin chalcone	didymin chalcone
	cloud	53.9 ± 3.8 (35.3)	5.3 ± 0.6 (17.2)	43.7 ± 2.8 (44.1)	4.9 ± 0.3 (33.1)	75.3 ± 5.6 (49.5)	6.4 ± 0.7 (24.0)	61.8 ± 4.5 (59.3)	7.0 (51.1)

[a] Mean ($n = 3$) ± standard deviation in mg/serving. [b] Percentages of flavanones and chalcones relative to the initial content are given in parentheses. [c] Chalcones were detected only in the cloud part of the nondialyzed fraction.

flavanone content, whereas industrial squeezing decreased the content of soluble flavanones and increased the flavanone content of the cloud fraction, suggesting a precipitation of soluble flavanones to the cloud. This finding is in agreement with previous reports which suggested that the extent of flavanone crystallization in the juice is much influenced by technological treatments such as extraction pressure that increases this crystallization as is the case of industrial processing (*15*, *16*). Industrial squeezing extracted more flavanones, although less soluble, than domestic squeezing according to previous results (*20*). Hesperidin was the major flavanone in both juices. After the in vitro simulation of the gastrointestinal digestion, in the permeate fraction, hesperidin was quantitatively the most abundant flavanone (**Table 1**). However, narirutin and didymin permeated at higher rates than hesperidin in relation to the content before digestion for both squeezing methods. Therefore, narirutin and didymin permeated more easily than hesperidin, suggesting that this last should interact more strongly with orange juice polymers that are unable to permeate through the membrane. According to previous studies (*17*), chalcones were found only in the juice cloud. It has been suggested that these compounds are mainly formed under gastrointestinal conditions by conversion of soluble flavanones to chalcones and that then they precipitate rapidly to the cloud due to their high insolubility at pH 7.5. It is important to emphasize the high percentage of flavanone conversion to chalcone relative to the flavanone content before digestion that reaches some 70% of the flavanones. After an in vitro incubation of hesperidin in aqueous solution at pH 7.5, only 1% of the flavanone was transformed to the corresponding chalcone (unpublished results). Under gastrointestinal conditions, some factors, such as bile salts, could favor the equilibrium between flavanone and chalcone toward the chalcone form. After gastrointestinal digestion, the domestic squeezed juice provided a higher content of soluble flavanones able to permeate than the industrially squeezed juice (**Table 1**). In the retentate a higher transformation to chalcones in the industrially squeezed juice than in the domestic squeezed juice was detected. Narirutin chalcone, hesperidin chalcone, and didymin chalcone were detected and quantified in the cloud. These three individual chalcones were found in higher concentration in the digested industrial juice than in the digested domestic juice (**Table 1**). Therefore, under in vitro gastrointestinal conditions, orange juice obtained by domestic squeezing provided more soluble flavanones and less insoluble chalcones than that obtained by industrial squeezing.

Pasteurization did not modify the content of soluble and cloud flavanones in orange juice as it has been reported in a previous study (*20*). In the present study, after the initial and pasteurized orange juices had been subjected to in vitro gastrointestinal digestion, no significant changes in the insoluble and soluble total and individual flavanones were detected (**Table 2**). Chalcones were found only in the cloud, and a slight increase in chalcone formation was detected after pasteurization. Therefore, pasteurization had little influence on the solubility, chemical transformation, and ability to permeate of orange juice flavanones under gastrointestinal conditions. This contrasts with previous results suggesting that juice pasteurization could affect cloud formation and flavanone precipitation (*15*).

Industrial freezing (after pasteurization) caused a dramatic decrease of the flavanones in the soluble fraction, affecting hesperidin, narirutin, and didymin (**Table 2**). This could be due to flavanone precipitation and sedimentation at the bottom of the tanks during juice reconstitution from the frozen juice. After the in vitro digestion, the soluble flavanones able to permeate in the juice obtained from frozen juice decreased 43.4% (from 17.6 to 9.9 mg per serving) (**Table 2**). Permeated hesperidin showed a decrease of 57%, whereas for dialyzed narirutin and didymin, this decrease was 42 and 30%, respectively. In the cloud the chalcone formation decreased 31.3% after freezing (**Table 2**). The same behavior was observed for individual chalcones, the decrease ranging between 19% for narirutin chalcone and 32.8% for hesperidin chalcone. The different chalcones were formed from the soluble flavanones of the orange juice under gastrointestinal conditions following precipitation to the cloud. Therefore, the freezing process did not favor the content of soluble flavanones in orange juice under in vitro gastrointestinal conditions; however, the amount of chalcones formed after freezing was smaller than that found before freezing (**Table 2**).

The orange juice used for the concentration treatment contained a smaller amount of flavanones than those used for the other treatments. This was due to the lower quality of the oranges available at the end of the season. The concentration treatment did not affect the total flavanone content of the whole

Table 2. Total and Individual Flavanones and Chalcones before and after Gastrointestinal Digestion of Orange Juice after Pasteurization and Freezing (Initial Juice Values Are Those Quoted in **Table 1** for Industrial Squeezing)[a]

		after pasteurization				after freezing			
before or after digestion	fraction	total flavanones	narirutin	hesperidin	didymin	total flavanones	narirutin	hesperidin	didymin
before	soluble	81.1 ± 1.4 (53.7)[b]	20.4 ± 0.1 (76.1)	46.1 ± 0.3 (45.1)	8.8 ± 1.1 (62.0)	32.2 ± 7.2 (34.7)	10.6 ± 2.1 (68.8)	11.6 ± 3.3 (18.7)	6.4 ± 1.0 (58.2)
	cloud	69.1 ± 7.5 (45.8)	6.4 ± 0.4 (23.9)	56.1 ± 6.5 (54.9)	5.3 ± 0.5 (37.3)	60.6 ± 7.0 (65.2)	4.8 ± 1.0 (31.2)	50.5 ± 5.3 (81.3)	4.6 ± 0.6 (41.8)
after	permeate	17.6 ± 0.5 (11.7)	5.9 ± 0.2 (22.0)	9.0 ± 0.3 (8.8)	2.0 ± 0.1 (14.1)	9.9 ± 1.3 (10.7)	3.4 ± 0.7 (22.1)	3.9 ± 0.4 (6.3)	1.4 ± 0.1 (12.7)
	retentate soluble	30.3 ± 1.0 (20.1)	10.0 ± 0.3 (37.3)	15.0 ± 0.4 (14.7)	4.5 ± 0.3 (31.7)	17.3 ± 1.8 (18.6)	5.3 ± 0.7 (34.4)	6.8 ± 0.8 (11.0)	3.4 ± 0.2 (30.9)
	cloud	14.5 ± 2.3 (9.6)	1.3 ± 0.4 (4.9)	13.2 ± 1.9 (12.9)	nd	13.0 ± 3.0 (14.0)	0.9 ± 0.2 (5.8)	12.0 ± 3.1 (19.3)	0.1 ± 0.0 (0.9)
	fraction	chalcones[c]	narirutin chalcone	hesperidin chalcone	didymin chalcone	chalcones	narirutin chalcone	hesperidin chalcone	didymin chalcone
	cloud	77.1 ± 8.5 (51.1)	6.3 ± 0.6 (23.5)	64.0 ± 7.2 (62.6)	6.8 ± 0.7 (47.9)	53.0 ± 23.6 (57.1)	5.1 ± 2.3 (33.1)	43.0 ± 19.2 (69.2)	4.9 ± 2.1 (44.5)

[a] Mean ($n = 3$) ± standard deviation in mg/serving. [b] Percentages of flavanones and chalcones relative to the initial content are given in parentheses. [c] Chalcones were detected only in the cloud part of the nondialyzed fraction.

Table 3. Total and Individual Flavanones and Chalcones before and after Gastrointestinal Digestion before and after Industrial Orange Juice Concentration[a]

		before concentration				after concentration[b]			
before or after digestion	fraction	total flavanones	narirutin	hesperidin	didymin	total flavanones	narirutin	hesperidin	didymin
before	soluble	41.6 ± 2.2 (92.4)[c]	11.8 ± 0.3 (95.4)	18.2 ± 1.2 (88.3)	7.7 ± 0.7 (96.4)	40.5 ± 0.3 (85.8)	11.1 ± 0.1 (91.7)	15.7 ± 0.2 (77.0)	3.3 ± 0.1 (84.6)
	cloud	3.4 ± 0.6 (7.6)	0.6 ± 0.1 (4.6)	2.4 ± 0.4 (11.7)	0.3 ± 0.0 (3.6)	6.7 ± 1.4 (14.2)	1.0 ± 0.1 (8.3)	4.8 ± 1.2 (23.5)	0.6 ± 0.2 (15.4)
after	permeated	9.9 ± 0.2 (22.0)	3.4 ± 0.1 (27.6)	4.5 ± 0.1 (21.8)	0.9 ± 0.1 (11.3)	10.3 ± 1.2 (21.8)	3.5 ± 0.4 (28.9)	4.1 ± 0.4 (20.1)	1.5 ± 0.5 (38.5)
	retentate soluble	25.8 ± 0.4 (57.3)	8.4 ± 0.0 (68.3)	11.5 ± 0.4 (55.8)	3.0 ± 0.0 (37.5)	23.8 ± 0.7 (50.4)	8.1 ± 0.1 (66.9)	10.0 ± 0.4 (49.0)	2.7 ± 0.1 (69.2)
	cloud	1.8 ± 0.1 (4.0)	0.2 ± 0.0 (1.6)	1.0 ± 0.1 (4.9)	0.5 ± 0.0 (6.3)	3.6 ± 0.9 (7.6)	0.3 ± 0.1 (2.5)	2.4 ± 0.6 (11.8)	0.7 ± 0.1 (17.9)
	fraction	chalcones[d]	narirutin chalcone	hesperidin chalcone	didymin chalcone	chalcones	narirutin chalcone	hesperidin chalcone	didymin chalconen
	cloud	7.0 ± 0.3 (15.6)	0.3 ± 0.0 (2.4)	5.9 ± 0.3 (28.6)	0.8 ± 0.0 (10.0)	3.8 ± 0.1 (8.1)	2.2 ± 0.1 (18.2)	1.3 ± 0.0 (6.4)	0.3 ± 0.0 (7.7)

[a] Mean ($n = 3$) ± standard deviation in mg/serving. [b] And subsequent reconstitution of the juice. [c] Percentages of flavanones and chalcones relative to the initial content are given in parentheses. [d] Chalcones were detected only in the cloud part of the nondialyzed fraction.

juice and increased slightly the content of the cloud fraction (**Table 3**). When the orange juice was subjected to in vitro gastrointestinal digestion before and after concentration, no changes in the total and individual flavanone contents were detected in the soluble and permeate fractions. In the retentate fraction, an increase of flavanone precipitation to the cloud was observed after concentration. However, the chalcone content of the cloud decreased 50% after concentration (**Table 3**). Hesperidin and didymin chalcones decreased 78 and 62%, respectively, whereas narirutin chalcone increased after concentration in relation to the content found before concentration. Therefore, industrial juice concentration did not influence orange juice flavanone solubility and permeability and decreased chalcone formation during the in vitro simulation of the gastrointestinal conditions.

DISCUSSION

These results confirm the transformation of a large part of orange juice flavanones to chalcones under the physiological conditions of the gastrointestinal tract. This transformation occurs at a higher rate than that produced when hesperidin is "dissolved" in water at pH 7.5 (it is very difficult to dissolve in water), that is, below 1% (unpublished results). These findings support that the soluble flavanones are transformed to chalcones that immediately precipitate to the cloud, where they are exclusively found.

No hydrolysis of the glycosidic moiety of the naturally occurring flavanones to render the corresponding aglycons was observed in the acidic pH of the stomach. This possibility had been previously suggested (*14*) and could explain the finding of flavanone derivatives in plasma only 1 h after orange juice ingestion (*10*).

Previous studies have reported that the use of urine flavanone concentration as a biomarker of their dietary intake is rather problematic (*10*) and contrasts with the case of the flavonol quercetin from onions in which there is a close correlation between intake and presence of metabolites in biological fluids (*22*). This could suggest that the form in which the flavanone

is present in the juice (soluble or precipitated in the cloud) and the interaction with other juice constituents (proteins, pectins, etc.) can have a marked effect on the orange juice flavanone absorption in vivo. In fact, only 3% of the pure (solid and therefore rather insoluble) hesperidin ingested was excreted in the urine, whereas this excretion was reported to be 24% in the case of hesperidin from orange juice (*14*), confirming that the form in which hesperidin is present in the gastrointestinal tract (soluble or precipitated) can be very relevant in absorption. Even if hesperidin and other flavanone rutinosides need to be hydrolyzed by bacterial enzymes in the distal part of the intestine to render the aglycons before absorption, the accessibility of enzymes to the flavanones can be higher when these compounds are soluble and free than when they are interacting with other juice constituents (proteins, pectins, etc.) or when they are precipitated as crystals in the cloud.

From a global view of the effect of orange juice manufacturing on soluble and cloud flavanones, which can affect their absorption, domestic squeezing provided a higher content of soluble and permeable flavanones than industrial extraction. Pasteurization and concentration did not affect the content of soluble flavanones, and freezing decreased flavanones solubility. With regard to the transformation to chalcones, commercial squeezing favored a higher conversion than domestic squeezing. However, freezing and concentration decreased the chalcone content, whereas pasteurization did not affect this transformation.

It would be necessary, however, to achieve in vivo experiments in which the absorption of flavanones from orange juices obtained by domestic and industrial squeezing is evaluated to prove if the large differences in flavanone solubility and potential transformation to chalcones have a direct effect on the final absorption of these metabolites.

ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; FDA, U.S. Food and Drug Administration; HPLC, high-performance liquid chromatography.

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