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Citrus Juice Extraction Systems: Effect on Chemical Composition and Antioxidant Activity of Clementine Juice

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ABSTRACT: Clementines are especially appreciated for their delicious flavor, and recent years have seen a great increase in the consumption of clementine juice. In previous decades, antioxidant compounds have received particular attention because of widely demonstrated beneficial health effects. In this work, the organoleptic, volatile flavor, and antioxidant quality of clementine juice were studied with regard to the influence on them by different juice extraction systems: plug inside fruit and rotating cylinders. The results showed that juice extracted by the former method presented higher yields and hesperidin content, which was related to higher antioxidant activity, demonstrated by ORAC and LDL assays. The organoleptic quality was not affected by the processing technique, whereas there were significant differences in the chemical flavor profile. There are important differences in chemical and functional quality between juice extraction techniques, which must be taken into account when employing processing systems to produce high-quality products.

KEYWORDS: *clementine juice, organoleptic quality, ascorbic acid, flavonoids, nutraceutical, volatile profile*

INTRODUCTION

Citrus fruits are widely consumed around the world and are significant sources of vitamin C, containing more than the minimum daily requirement of 60 mg of vitamin C in 240 mL of juice.[1] From a nutritional point of view, vitamin C is probably the most important compound found in citrus fruits, and its content is taken as an indication of fruit freshness, retention of other compounds, and a quality indicator for shelf life of citrus-derived products.[2]

Citrus products have received much attention in recent years, because of potential therapeutic benefits associated with high levels of flavonoids, reportedly having antiallergenic, antioxidant, anticancer, and anti-inflammatory properties.[3] Citrus species typically contain various flavonoids such as flavanones, flavones and their glycosides (i.e., hesperidin, neohesperidin, naringin, narirutin, diosmin), and polymethoxyflavones (i.e., tangeritin, nobiletin).[4] It is known that hesperidin improves vascular integrity and decreases capillary permeability, such that it is given as a supplement to patients with fragile blood vessels.[5] Furthermore, in association with naringin, hesperidin might reduce cholesterol levels.[3] It is also reported that hesperidin can inhibit copper-induced low-density lipoprotein (LDL) oxidation.[6] The key role played by oxidized LDL in the initial and advanced stages of atherosclerotic lesions has been well established.[7]

The citrus juice concentrate market is estimated to have an annual world production of 3 million tonnes, led by Brazil, the United States, and Japan. The citrus-processing industry ensures a continuous supply of citrus juices and related products in many parts of the world. In addition, juice provides adequate quantities of water, raw enzymes (when freshly squeezed), vitamins, and minerals, all necessary for health.

Wide varieties of methods are available or have been proposed for extracting juice from citrus fruits. Industrial methods commonly operate by crushing the fruit and thereafter draining the juice, such as the extracting system from FMC (Food Machine Co.). In small-scale operations, the interior parts of the fruit halves are pressed by using a plunger, reamer, or rotating burr, such as home use or the Zumex extractors.[8]

The importance of color, vitamin C, and aroma as quality parameters in citrus products has been highlighted by many authors.[9] Several studies have shown that citrus processing not only affects the chemical and sensorial properties of the juice[10,11] but also their functional properties.[12−14]

Nowadays, the market demands processed products with characteristics resembling the fresh product and, additionally, preventive health measures, such as diets with high fruit and vegetable content, are promoted intensively.[15] These developments require better knowledge of the nutritional quality of these products. Fresh clementines are prized for the flavor of their fruit, but relatively little information is available on the quality of that flavor and its functional properties when compared with other citrus fruits, such as the orange or lemon.

The objective of this work was to study the effects of two commonly used juice extraction techniques on the chemical composition and functional properties of clementine juice.

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Organoleptic parameters and total content of vitamin C, polyphenols, and hesperidin, as nutrients with well-described antioxidant properties in citrus fruits, were evaluated and correlated with antioxidant activity assayed by ORAC and LDL. In addition, the chemical flavor profiles of the juices were analyzed by HS-SPME-GC-MS.

MATERIALS AND METHODS

Chemicals. Methanol and acetonitrile of HPLC grade was obtained from Merck (Darmstadt, Germany). Hesperidin, Folin−Ciocalteu reagent, 2,6-dichloroindophenol, dimethyl sulfoxide (DMSO), Trolox, fluorescein, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), thiobarbituric acid, copper sulfate, Sudan black B, sodium hydroxide, sodium chloride, phenolphthalein, and phosphate salts were of reactive grade and purchased from Sigma (St. Louis, MO). A BCA (bicinchoninic acid) Protein Assay Kit was purchased from Pierce (Rockford, IL). Reference standards of flavonoids were purchased from ChromaDex Inc. (Irvine, CA). A mix of alkanes C7−C40 was obtained from Supelco (Bellefonte, PA). Italian mandarin oil (*Citrus reticulata*, Fluka W265713) FCC, Kosher, was purchased from Sigma-Aldrich (Milwaukee, WI).

Citrus Fruits and Juice Extraction Systems. Clementine fruits (*Citrus clementina* Hort. Ex. Tanaka) at commercial maturity index were harvested from healthy adult trees at Hacienda La Crystalina (Támesis, Colombia) (5° 42′ N, 75° 40′ W, and 775 m above sea level) in March 2010.

The fruits were carefully selected for size and the absence of physical damage at a processing plant (Medellín, Colombia). Clementine fruits were randomly divided in lots of 100 fruits, making three replicates for each technique. Each lot was submerged in a 150 ppm sodium hypochlorite solution for 3 min and rinsed with potable water. The fruits were squeezed using two machineries: Extractor A, a Zumex squeezer model Z-200 Versatile (Zumex Maquinas y Elementos, Valencia, Spain), cuts the fruit through the middle and passes the halves between two rotating cylinders that press the fruit and extract the juice. Extractor B, a Fresh'n Squeeze model (JBT FoodTech, Citrus System, Lakeland, FL), consists of a plug that makes a cut in the center of the fruit and then pushes a strainer up inside the fruit. A mechanical hand presses the juice and pulp against this strainer, keeping the juice away from the strongly flavored peel oils in the exterior of the fruit. After squeezing, the juices were refrigerated until analysis.

Physicochemical Analysis. The equatorial diameter was measured in 10 fruits per lot, and the results were expressed in millimeters. For each extraction method were processed 100 fruits for triplicate; total juice yield was determined and expressed as a percentage of fruit weight.

All chemical analysis was done using three juices from each replicate and three measures for each parameter. Total titratable acidity was analyzed by titration with 0.1 N NaOH, using phenolphthalein as indicator according to AOAC Official Method 942.15. Total soluble solids content (TSS) was determined with a digital refractometer (Atago Digital Refractometer PR-1, Atago Co., Ltd., Tokyo, Japan). pH was also determined by a potentiometer (Handylab pH 12, Schott-Geräte GmbH, Mainz, Germany).

Ascorbic acid analysis was carried out according to AOAC Official Method 967.21, by titration with 2,6-dichloroindophenol. Total phenolic content was assessed with Folin−Ciocalteu reagent using the method of Singleton adapted by us in a previous study.[16] The results were expressed as milligrams of gallic acid equivalents per liter of juice (mg GAE/L of juice).

Hesperidin analysis was carried out by HPLC-DAD. The HPLC analysis was performed using an Agilent 1200 series (Agilent Techniques, Santa Clara, CA) with a photodiode array detector at 280 nm. An Agilent (C18) 150 mm × 4.6 mm, 5 μm, column was used at 23 °C. The mobile phase consisted of 80% water and 20% acetonitrile, used in isocratic mode. The flow rate was 0.85 mL/min. Due to the low solubility of flavonoids from citrus juice, juice was centrifuged four times at 4000 rpm for 15 min at 4 °C using a refrigerated centrifuge (Sorvall-Thermo, Waltham, MA). Analysis was performed on supernatants and the precipitate, both previously dissolved in DMSO. Validation parameters of the method were evaluated: linearity, range, accuracy, reproducibility, limit of detection, and limit of quantification. The results were expressed as milligrams of hesperidin per liter of juice.

Antioxidant Activity. Antioxidant activity was determined using the ORAC-FL assay.[17] Juices obtained from the two techniques (tree lots, 100 fruits per lot) were diluted in a 10 mM phosphate buffer at pH 7.4. Trolox (0−200 μM) was used as the standard. The juice was centrifuged at 13000 rpm for 30 min at 4 °C using a refrigerated centrifuge. A mixture of the fluorescent probe FL (150 μL of a 1 μM solution) and juice supernatant (25 μL of a 1/10 dilution) was preincubated for 30 min at 37 °C. Then, 25 μL of a 250 mM AAPH solution in phosphate buffer was added. Fluorescence intensity was measured every 2 min during 120 min with excitation and emission wavelengths set at 485 and 520 nm, respectively. The results were reported as micromoles of Trolox equivalents per liter of juice (μmol TE/L of juice).

Antioxidant activity was also determined through the inhibition of low-density lipoprotein (LDL) oxidation assay. LDL was isolated through a discontinuous density gradient centrifugation procedure using a Beckman XL-100 ultracentrifuge. The protein concentration was determined by BCA Protein Assay. Juices obtained from the two techniques (tree lots, 100 fruits per lot) were centrifuged at 13000 rpm for 30 min at 4 °C, and the supernatant was tested at 1/10 dilution. The LDL (500 μg/mL) was incubated with $CuSO_4$ (40 μM) and juice for 9 h at 37 °C. The extent of lipid peroxidation was determined by the thiobarbituric acid reactive substances (TBARS) method, and the antioxidant activity was expressed as a percentage of lipid peroxidation inhibition. In all experiments controls were made by adding all reagents except lipids or compounds. Changes on charge of LDL particles were determined by electrophoretic mobility, using agarose gel electrophoresis operated at 120 V and 500 mA in barbital buffer (pH 8.7). Bands were stained with Sudan black B (0.1% w/v in ethanol of 95%).

Volatile Analysis. *Headspace Solid Phase Microextraction (HS-SPME) Procedure.* Juices obtained from the two techniques (tree lots, 100 fruits per lot) were subjected directly after thawing to HS-SPME. Conditions were optimized using 5.0, 7.5, and 9.0 mL of sample in a 15 mL vial, based on the sum of total peak areas. Clementine juice volatiles were extracted using two fibers: polydimethylsiloxane (PDMS) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fibers were conditioned in a GC injector port as indicated by the manufacturer (Supelco, North Harrison, PA). To determine the optimal fiber for analyte extraction, experiments were carried out at ambient temperature with a ratio of 1:1 for sample/headspace volumes, and both equilibrium and extraction times were fixed at 1 h with constant agitation speed (800 rpm). The equilibrium time for sample analysis was selected from a number of time scales (15 min−6 h). The time and temperature of extraction were determined using four different temperatures (20, 30, 40, 50 °C) and seven different durations (15, 30, 50, 100, 150, 200, 250, 300, 360 min). For salting-out effect, 100 μL of saturated NaCl solution was employed. After the SPME, the fiber was inserted into the GC injector at 260 °C for desorption of volatile components (1 min). Results are from three independent experiments analyzed twice and are presented as the mean ± SEM.

GC−Mass Spectroscopy (MS). GC-MS analysis was carried out using an Agilent 7890 GC apparatus (Wilmington, DE) equipped with mass selective detector (MSD) 5975C and automatic liquid sampler (ALS) 7683B, with two capillary columns: HP-1MS 1% phenyl polymethylsiloxane (PMS) (30 m, 0.25 mm i.d., film thickness = 0.25 μm, J&W Scientific, Folsom, CA) and HP-5MS 5% PMS (30 m, 0.25 mm i.d., film thickness = 0.25 μm, J&W Scientific). The use of both capillary columns with different polarities of stationary phases allowed (a) resolution of overlapping compounds that have the same retention times and (b) better component identification by calculating two retention indices for some compounds.

Table 1. Physicochemical Parameters of Juice Extracted with Different Techniques[a]

extraction system	diameter (mm)	total acidity (g of citric acid/100 mL)	TSS (°Brix)	TSS/acid ratio	juice (%)	pH	ascorbic acid (mg/100 mL)
A	58.8 a	0.93 a	12.5 a	13.9 a	37.0 a	3.5 a	47.6 a
B	57.9 a	0.90 a	12.4 a	13.9 a	52.1 b	3.6 a	49.7 a

[a]Data followed by the same letter in each column do not differ at the 5% significance level.

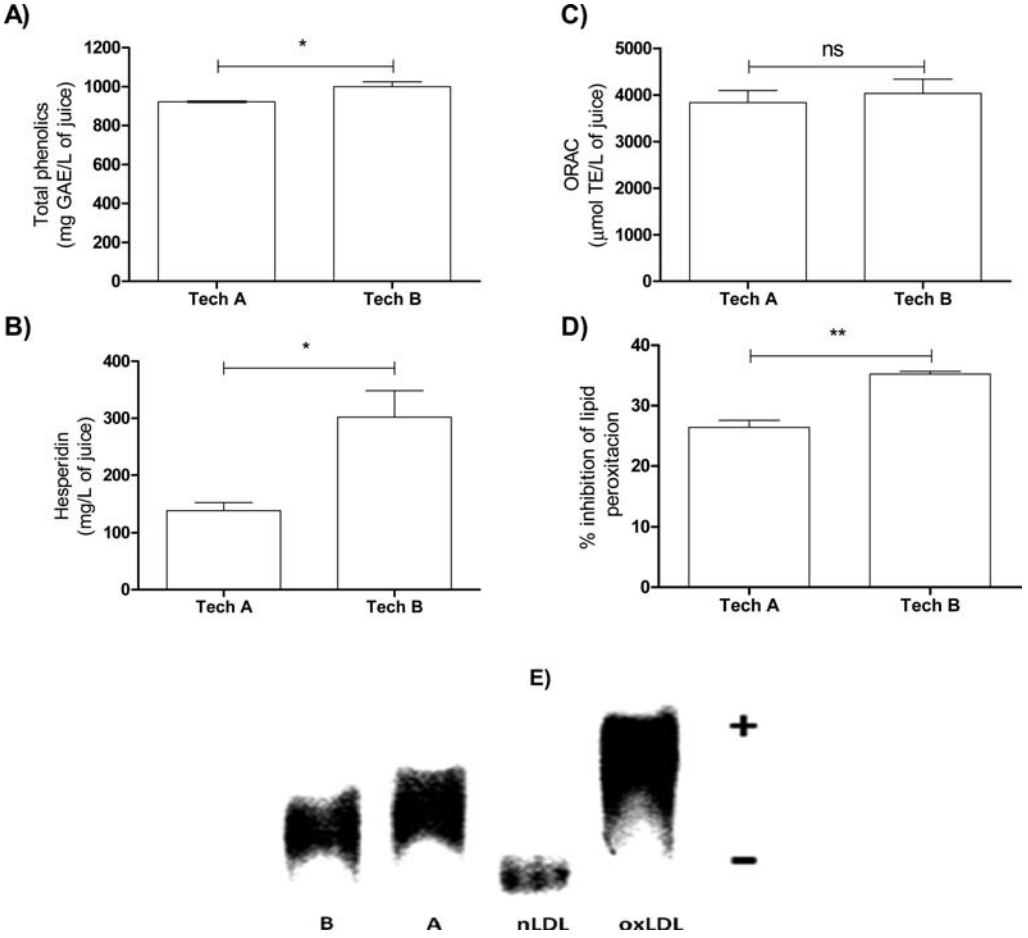

Figure 1. Antioxidant activity of juices obtained with different techniques (Tech A, technique A; Tech B, technique B): (A) total phenolic content (mg GAE/L juice) measured by Folin−Ciocalteu method; (B) hesperidin content (mg hesperidin/L juice) measured by HPLC; (C) antioxidant activity measured as ORAC value, expressed as Trolox equivalents; (D) antioxidant activity measured as TBARS, expressed as percentage of inhibition of lipid peroxidation; (E) antioxidant activity measured as inhibition of LDL oxidation, showing electrophoretic mobility from (−) to (+) on agarose gel. Bands correspond to B, juice from technique B; A, juice from technique A. nLDL, native LDL (no oxidized LDL); oxLDL, oxidized LDL (without antioxidant). Statistical differences correspond to p value < 0.05 (*), p value < 0.01 (**), p value < 0.001 (***), and p value > 0.05 (ns).

The oven temperature was programmed to be 40 °C (8 min), rising to 200 °C at 5 °C/min, to then held isothermally at 200 °C for 10 min. The injector temperature was established at 260 °C. Manual HS-SPME was performed in the split mode (150:1 with a SPME inlet linear, 0.75 mm, Agilent), using helium as carrier gas (1.3 mL/min). The MSD temperatures of the ionization chamber and MS Quad were set at 230 and 150 °C, respectively. Mass spectra and total ion currents (TIC chromatograms) were obtained by automatic scanning at 4.51 scan^{-1} with energy ionization 70 eV, in the mass range m/z 30−350. Chromatographic peaks were checked for homogeneity with the aid of the extracted ions of characteristic fragments to optimize resolution and peak symmetry.

Component Identification of Volatile Chemicals. Identification of the components was based on three methods. Method a compared the GC retention index (RI) on nonpolar columns with a series of *n*-alkanes (C8−C28) by linear interpolation. To calculate RI, we used Automatic Mass Spectral Deconvolution and Identification System software (AMDIS 2.68) compared with standard compounds or database (http://www.pherobase.com/database/kovats/kovats-index.php). Method b was based on computer matching with commercial mass spectral libraries (NIST/EPA/NIH, 2008) and method c on comparison of spectra with those of our laboratory library.

Sensory Evaluation. A trained sensorial panel evaluated clementine juices using a multidimensional approach. The judges, eight women between 25 and 50 years old, were trained in advance over 5 months to gain familiarity with citrus flavors. All sensory evaluations were conducted in individual booths under white illumination at 25 ± 2 °C and 50−75% relative humidity with continuous air flow in a laboratory certified according to the Colombian Technical Standard (NTC) 3884 and corresponding to the standard ISO 8589 Sensory Analysis—General Guidance for Design of Test Rooms. Mineral water was provided to rinse the mouth between evaluations. Juices were squeezed the day of testing, stored at 4 °C, and served at room temperature in transparent glasses labeled with random codes. The most representative sensorial descriptors were selected to establish a sensorial profile.[18] All descriptors were scored on a 6-point scale (0 =

none and 5 = strong) except for general quality, which was scored on a 3-point scale (1 = low and 3 = high). Three independent samples of each treatment were evaluated in triplicate.

Statistical Analysis. Data are presented as the mean ± SEM. The statistical significance of differences among groups was evaluated by *t* test using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA). The differences between the means were assessed, and significance was identified as p value < 0.05 (*), p value < 0.01 (**), p value < 0.001 (***), and p value > 0.05 (ns).

RESULTS AND DISCUSSION

Effect of Processing Technique on Organoleptic and Nutritional Juice Quality. The quality of citrus fruit for juice production is measured by the percentage of juice, soluble solids, and maturity index, which is recognized in international standards of quality, such as (CE) No. 1799/2001 of the European Community (http://eur-lex.europa.eu/LexUriServ/site/es/consleg/2001/R/02001R1799-20050106-es.pdf) and CODEX STAN 245-2004 and 247-2005 (http://www.codexalimentarius.net/web/standard_list.do?lang=en).

As can be seen in Table 1, juice extraction techniques used in this study did not significantly affect physicochemical parameters related to juice quality (Brix/acid ratio, pH, and ascorbic acid). However, juice yield was significantly reduced by technique A. With the squeeze technique A, we did not reach the 40% minimum yield required for clementine juice by European standards. This behavior may be explained by machinery design and its capability to adapt to fruits with differing sizes. We used clementines with diameters averaging 59 and 58 mm for extraction systems A and B, respectively, grade 4 in the European standard.

The contents of vitamin C found with both squeezing techniques (47.6 ± 2.9 and 49.7 ± 3.1 mg/mL for A and B, respectively) were similar to the values reported by other authors for clementines,[19] and in both cases, this was superior to the minimum established by the Experts Committee of the European Association of Juice Producers (AIJN) for orange and mandarin juices.[20] The extraction technique did not significantly affect ascorbic acid levels, as reported by other authors.[21]

Effect of the Extraction System on Phenolic Compounds. Total phenolics contents were reduced by extractor A (Figure 1A), which were 922.3 ± 4.6 and 999.5 ± 25.3 mg/L for systems A and B, respectively. These results are slightly higher than those reported by Gil-Isquierdo et al., who found a content of 839 mg/L phenolics in hand-squeezed navel orange juice.[13]

Folin−Ciocalteu's phenol reagent is nonspecific for phenolic compounds because it measures sample reducing capacity through electron transfer-based antioxidant capacity;[22] to avoid this, we also measured the content of hesperidin, the predominant flavanone glycoside in mandarins.

As shown in Figure 1B, hesperidin content was 139.25 and 302.02 mg/L for extraction systems A and B, respectively, concurrent to the behavior shown for total phenolics content. As shown, the value was in agreement with that found by Cano et al., who analyzed different citrus varieties and reported a hesperidin content of 285 mg/L in clementine cultivars.[23]

The clementine is taxonomically related to *Citrus reticulata* and probably to *Citrus sinensis*; the latter species has a high concentration of hesperidin (averaging 399 mg/L) as its main component, followed by narirutin (averaging 46.4 mg/L).[24] In fact, many cultivars of lemon, lime, tangerine, and sweet orange have been chemically characterized, showing that their flavonoid composition profile is mainly rutinosides (less bitter compounds such as hesperidin), whereas bitter orange mainly contains neohesperidin (bitter compounds such as naringin). For its part, grapefruit has a mixed profile of rutinosides and neohesperidin as it is a hybrid. This is important because the chromatographic profile of glycosylated flavonoids in citrus has become a useful tool for chemotaxonomy of hybrid materials, quality control, and detection of adulterated products.[25]

Effect of the Extraction System on Juice Antioxidant Activity. As can be seen in Figure 1, extractor B produces a juice with a higher ORAC value with respect to extractor A (Figure 1C) (3835 ± 443.0 and 4029 ± 532.7 as Trolox equivalent, for A and B techniques, respectively). Antioxidant activity (Figure 1D,E) was higher for juice from extractor B compared to juice from extractor A (26.41 ± 1.15 and 35.22 ± 0.42 as TBARS inhibition percentage for techniques A and B, respectively). It is known that oxidation of LDL generates conjugated dienes and peroxides, which lead to the development of TBARS. Oxidation of LDL can also result in the loss of positive charges from lysine residues on apolipoprotein B-100, resulting in changes in LDL electrophoretic mobility. These changes in mobility can also be used to evaluate the protection of apolipoprotein from oxidative modification.[26] It can be seen that the presence of antioxidants in juices during oxidative stress inhibited the formation of TBARS, thereby inhibiting the formation of conjugated dienes and peroxides, (Figure 1D). Antioxidants in juices also protected the lysine residues of LDL apolipoprotein B-100 from oxidation, which causes modified electrophoretic mobility (Figure 1E).

Antioxidant activity was correlated with a higher content of phenolic compounds (Figure 1A) and hesperidin concentration (Figure 1B). Other authors have reported the significant role hesperidin plays in the total antioxidant capacity of orange juices.[27]

The differences found between the two juice extraction systems may be explained because juice B has more suspended solids compared to juice A (as shown in sensory analysis), probably because extractor B exerts a higher pressure on fruits than extractor A by pushing a strainer up inside the fruit and then pressing the pulp against the strainer with the mechanical hand, producing in this way a juice with more solids suspended. Additionally, it is known that a higher content of antioxidant flavonoids has been found in albedo and membranes than in juice sacs.[28] In fact, hesperidin is insoluble in the acidic pH of juice, but soluble in the extraction solvent (DMSO). According to Gil-Isquierdo et al., the flavanones extracted by DMSO from the cloudy fraction account for 14% of the total in fresh juices.[13] Therefore, we anticipated that the precipitate from juice B would be richer in hesperidin than the precipitate from juice A. Indeed, hesperidin concentrations in the soluble fraction were found to be 80.2 ± 3.5 and 41.3 ± 2.8 mg/100 mL for extraction systems A and B, respectively.

Most flavonoid content values reported in the databases are pooled without considering the differences in sample preparation methods: this is very important because, whereas orange juice is a very rich source of flavanones, only a limited quantity is soluble, and this could affect availability for absorption. Gil-Isquierdo et al. reported availability of 11−36% for the soluble flavanones, depending on juice type. Moreover, flavanones precipitated in the cloud are not available for absorption and are partly transformed to the corresponding chalcones during the pancreatin−bile digestion.[13] Additionally, Vallejo et al. showed that the solubility of flavanones, and particularly that of hesperidin, in the juice, is a key factor for

Table 2. Volatile Flavor Compounds in Clementine Juice Extracted with Extractors A and B, Using Liquid Injection and HS-SPME-GC-EIMS (Relative Peak Area (%) ± SD (n = 6))

peak[a]	compound	I[b]	II[b]	A	B
1	tricyclene	899	923	0.01 ± 0.00	0.00 ± 0.00
2	α-thujene	924	932	0.15 ± 0.01	0.05 ± 0.00
3	1*R*-α-pinene	932	937	nc	9.65 ± 0.07
4	camphene	962	952	0.01 ± 0.00	0.00 ± 0.00
5	sabinene	967	978	0.17 ± 0.04	5.54 ± 0.06
6	β-pinene	973	980	nc	7.11 ± 0.24
7	*n*-octanal	977	986	0.31 ± 0.01	1.33 ± 0.01
8	β-myrcene	982	992	0.80 ± 0.02	5.95 ± 0.10
9	α-phellandrene	995	1005	0.01 ± 0.00	1.09 ± 0.03
10	Δ^3-carene	1000	1008	0.03 ± 0.00	0.27 ± 0.04
11	α-terpinene	1011	1021	0.02 ± 0.00	0.07 ± 0.01
12	*p*-cymene	1013	1030	0.05 ± 0.00	0.00 ± 0.00
13	limonene	1026	1032	97.50 ± 0.03	65.49 ± 0.13
14	β-phellandrene	1028	1032	nc	0.01 ± 0.00
15	*cis*-β-ocimene	1029	1039	0.01 ± 0.00	0.09 ± 0.00
16	*trans*-β-ocimene	1035	1049	nc	0.01 ± 0.00
17	γ-terpinene	1052	1064	0.04 ± 0.00	0.17 ± 0.02
18	*trans*-sabinene hydrate	1053	1069	nc	nc
19	*p*-mentha-3,8-diene	1064	1076	nc	nc
20	α-terpinolene	1080	1088	0.01 ± 0.00	0.12 ± 0.01
21	β-linalool	1082	1099	0.39 ± 0.01	0.89 ± 0.08
22	*cis*-sabinene hydrate	1088	1100	0.01 ± 0.00	0.00 ± 0.00
23	α-campholenal	1111	1130	nc	Nc
24	*trans*-limonene oxide	1121	1139	nc	0.00 ± 0.00
25	camphene hydrate	1131	1149	nc	0.00 ± 0.00
26	citronellal	1133	1152	nc	nc
27	β-pinene oxide	1138	1156	nc	0.00 ± 0.00
28	phellandral	1151	1159	nc	0.00 ± 0.00
29	thujan-4-ol	1159	1165	Nc	0.00 ± 0.00
30	L-4-terpineol	1168	1178	0.05 ± 0.01	0.14 ± 0.00
31	L-α-terpineol	1179	1190	0.02 ± 0.00	0.05 ± 0.00
32	phellandrene epoxide	1182	1191	nc	0.00 ± 0.00
33	*cis*-piperitol	1174	1193	nc	0.00 ± 0.00
34	myrtenol	1176	1194	nc	nc
35	methyl salicylate	1180	1200	nc	nd
36	*n*-decanal	1183	1205	0.29 ± 0.00	1.38 ± 0.00
37	*trans*-piperitol	1187	1210	nc	nc
38	citronellol	1203	1224	nc	0.00 ± 0.00
39	carvone	1220	1242	0.02 ± 0.00	nd
40	iPr-benzenemethanol	1250	1272	nd	0.00 ± 0.00
41	L-perillaldehyde	1255	1277	0.01 ± 0.00	nd
42	α-cubebene	1330	1351	0.01 ± 0.00	0.02 ± 0.00
43	α-copaene	1354	1376	0.01 ± 0.00	0.53 ± 0.01
44	β-cubebene	1374	1394	0.00 ± 0.00	nd
45	dodecanal	1391	1410	0.02 ± 0.00	nd
46	α-*cis*-bergamotene	1394	1415	nc	0.00 ± 0.02
47	*trans*-caryophyllene	1402	1420	0.00 ± 0.00	0.04 ± 0.00
48	α-*trans*-bergamotene	1417	1436	0.01 ± 0.00	nc
49	*cis*-α-bisabolene	1419	1438	0.01 ± 0.00	nd
50	α-caryophyllene	1439	1460	0.00 ± 0.00	nd
51	germacrene D	1463	1482	0.01 ± 0.00	nd
52	Δ-guaiene	1471	1490	nc	nd
53	β-ionone	1475	1494	nc	0.00 ± 0.00
54	α-nuurolene	1479	1501	0.00 ± 0.00	nd
55	(*E*,*E*)-α-farnecene	1492	1511	0.01 ± 0.00	nd
56	δ-cadinene	1513	1532	0.03 ± 0.00	0.00 ± 0.00
57	*trans*-nerolidol	1543	1564	nc	0.00 ± 0.00
58	β-sinensal	1673	1697	nc	0.00 ± 0.00
59	α-sinensal	1726	1750	nc	0.00 ± 0.00

Table 2. continued

[a]Peak numbering of signals in the chromatograms. [b]Relative retention index (Kovats retention index) of compounds in I (HP1) and II (HP5) capillary columns. Three relative percentages of compounds in the juice samples analyzed. nd, not detected; nc, not quantified.

bioavailability; in fact, the maximum concentration in plasma (C_{max}) achieved by flavanones correlates with the soluble flavanone concentration in the juice, whereas it has no correlation with the total flavanone intake.[29]

Other authors have found clear technological effects of the extraction system on the levels of the different types of flavonoids and antioxidant activity in citrus juices.[12] Commercial squeezing of navel orange extracted 22% more phenolics than hand squeezing. Freezing caused a dramatic decrease in polyphenols, whereas the concentration process caused only mild precipitation. Pulp pasteurization (conducted after squeezing and centrifugation) led to the degradation of several phenolic compounds but had no effect on juice.[14]

Volatile Composition of Juice Extracted with Different Extraction Systems. *Optimization of SPME Parameters.* The optimization of HS-SPME sampling parameters was performed using Italian mandarin oil and mandarin juices, and it was based on the sum of total GC peak areas. The triple fiber (DVB/CAR/PDMS) was chosen for the analysis of the volatile compounds of all juice samples because of its quantitative as well as qualitative capacity of wide metabolite extraction. The optimization of the equilibrium time was made at ambient temperature, and the maximal MSD response was measured after an equilibrium time of 120 min. Through combinations of time and temperature variations, the optimal extraction conditions were established as an extraction time of 120 min at 40 °C for juice sample analysis. Some researchers prefer to use a salt addition to extract volatile compounds;[30] we used a saturated solution of sodium chloride (400 μL) to obtain the salting-out effect, which shows comparatively better results than other salts (data not shown).

The coefficient of variation calculated on the basis of total area obtained from the MSD signal for the six samples (3.32% < CV < 4.45%) indicated that our equipment and the method were reliable. In the same way, the CV of the major compound (limonene) was always <3%. Quantities of the compounds and its standard deviations are reported in Table 2.

Volatile Flavor Composition. Many researchers have studied citrus volatile compounds using different analytical methods, and as a result, more than 200 components have been described as components of the flavor of citrus.[31] All volatile compounds found using both extraction techniques have previously been reported for citrus.[32] Nevertheless, the chemical flavor profile of the juices obtained had significant differences. HS-SPME-GC-MS analysis led to the identification of 59 representative compounds that represented 99.01% of the total volatile compounds for juice A and 99.52% for juice B (Table 2). For extractor A the principal volatile components were cyclic monoterpenes (97.41%), mainly sabinene and limonene; noncyclic monoterpenes (1.19%), mainly β-myrcene and β-linalool; nonterpenic compounds (0.61%), mainly *n*-octanol and *n*-decanal; and sesquiterpenes (0.29%), mainly α-copaene and α-sinensal.

For extraction system B the principal volatile components were cyclic monoterpenes (88.76%), mainly 1*R*-α-pinene, β-pinene, and limonene; noncyclic monoterpenes (6.95%), mainly β-myrcene; nonterpenic compounds (2.71%), mainly *n*-octanal and *n*-decanal; and sesquiterpenes (0.59%), mainly α-copaene, β-sinensal, and α-sinensal.

The limonene contents in the volatile fraction were 97.51 and 65.49% for juices obtained with extraction systems A and B, respectively. Other substances such as aldehydes, alcohols, and sesquiterpenes showed quantitative differences and, moreover, some compounds were not detected. The analysis of juice chromatograms revealed compositions similar to those previously reported by other authors,[33] in terms of most characteristic compounds for mandarins (decanal, octanal, methyl-*N*-anthranilate, thymol, α-sinensal, γ-terpinene, β-pinene).

By comparison of the average total chromatographic areas of the samples, juice from extractor B presented a very low volatile fraction in headspace conditions (juice with an 11.62% less volatile fraction) with respect to juice from extractor A. Furthermore, we observed that juice from extractor A had a higher content of essential oils (headspace ratio between (A) and (B) was approximately 9:1) than juice from extractor B, which may be related to the higher presence of compounds with spicy, oily, and green notes for juice A. In technique B the juice does not have contact with the peel oils of the fruit, and these are immediately washed down, producing a juice with much less content of essential oil.

Sensory Evaluation. In Table 3 can be seen the evaluation of sensory descriptors for juices from techniques A and B. In general, juice B had higher scores than juice A in some key descriptors such as aspect (yellow color and pulp), odor (fresh juice odor), taste (fruit taste and fresh juice taste), texture (pulp), and general quality. At the same time, juice B had the lowest score for the following undesirable descriptors: bitterness, peel oil taste, green taste, spicy, and astringency.

The essential oil of citrus peel contains high levels of limonene; therefore, high levels of peel oil could contribute to a bitter flavor.[34] Additionally, some authors considered that peel oil extraction in the industrial process could contribute to a high α-pinene content in juice, although this does not seem fully clear as others have reported that this compound adds a pine-like, citrusy odor and contributes to orange[35] and mandarin flavor.[33]

In conclusion, our results indicate that the extraction technique employed may influence the chemical composition and functional properties of clementine juice. Machinery A (rotating cylinders), compared with machinery B (plug inside fruit), produced a juice with lower yield, and it has fewer antioxidant flavonoids. In recent years, flavonoids have gained special relevance due to their functional properties, but care must be taken in the reporting of content values, and more studies should be done to determine their real availability in processed foods. Moreover, juice from extractor A contains a higher volatile fraction more complex than juice from extractor B. More studies are necessary to identify key aroma compounds and optimize juice-processing methods to improve the sensory quality of clementine juice. When employing processing systems to produce high-quality products, we must consider that the extraction method could determine important volatile flavors and functional quality parameters.

Table 3. Sensory Evaluation of Clementine Juices Extracted with Techniques A and B[a]

descriptors		juice A	juice B
aspect	yellow color	3.9	4.3
	color uniformity	4.6	4.3
	brightness	4.3	4.3
	pulp	2.6	3.2
odor	citric odor	4.2	3.9
	acid odor	3.3	3.3
	sweet odor	3	3
	fruit odor	4.5	4.6
	floral odor	1.9	1.9
	fresh juice odor	4.6	4.7
	rare odor	0	0
taste	acidity	3.4	3.1
	sweetness	3.3	3.4
	bitterness	2.7	1.6
	fruit taste	4.2	4.6
	peel oil taste	4.1	3
	floral taste	2	1.8
	green taste	2.4	1.6
	citric taste	4.1	4
	spicy somatosensation	2.6	1.5
	astringency somatosentation	2.9	2.7
	saline taste	0.9	0.7
	rare taste	0	0
	fresh juice taste	4.4	4.6
texture	liquid	4.3	4.4
	pulp	2.6	2.9
hedonic terms	general quality	1.8	2.7

[a]All descriptors were scored on a 6-point scale (0 = none and 5 = strong) except for general quality, which was scored on a 3-point scale (1 = low and 3 = high).

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REFERENCES

(1) COMA. Dietary reference values for food energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy, 1991.

(2) Pérez, A. G.; Olías, R.; Espada, J.; Olías, J. M.; Sanz, C. Rapid determination of sugars, nonvolatile acids, and ascorbic acid in strawberry and other fruits. *J. Agric. Food Chem.* **1997**, *45*, 3545−3549.

(3) Benavente-García, O.; Castillo, J. Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. *J. Agric. Food Chem.* **2008**, *56*, 6185−6205.

(4) Schijlen, E. G. W. M.; Ric de Vos, C. H.; van Tunen, A. J.; Bovy, A. G. Modification of flavonoid biosynthesis in crop plants. *Phytochemistry* **2004**, *65*, 2631−2648.

(5) Lyseng-Williamson, K. A.; Perry, C. M. Micronised purified flavonoid fraction: a review of its use in chronic venous insufficiency, venous ulcers and haemorrhoids. *Drugs* **2003**, *63*, 71−100.

(6) Cirico, T. L.; Omaye, S. T. Additive or synergetic effects of phenolic compounds on human low density lipoprotein oxidation. *Food Chem. Toxicol.* **2006**, *44*, 510−516.

(7) Witztum, J. L.; Steinberg, D. Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* **1991**, *88*, 1785−1792.

(8) Khazaei, J.; Massah, J.; Mansouri, G. H. Effect of some parameters of air-jet on pneumatic extraction of citrus juice and juice sacs. *J. Food Eng.* **2008**, *88*, 388.

(9) Meléndez-Martínez, A. J.; Vicario, I. M.; Heredia, F. J. Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices. *J. Agric. Food Chem.* **2003**, *51*, 7266−7270.

(10) Buettner, A.; Schieberle, P. Evaluation of aroma differences between hand-squeezed juices from Valencia late and navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 2387−2394.

(11) Perez-Cacho, P. R.; Rouseff, R. Processing and Storage effects on orange juice aroma: a review. *J. Agric. Food Chem.* **2008**, *56*, 9785−9796.

(12) Marín, F. R.; Martinez, M.; Uribesalgo, T.; Castillo, S.; Frutos, M. J. Changes in nutraceutical composition of lemon juices according to different industrial extraction systems. *Food Chem.* **2002**, *78*, 319.

(13) Gil-Izquierdo, A.; Gil, M. I.; Ferreres, F.; Tomás-Barberán, F. A. In vitro availability of flavonoids and other phenolics in orange juice. *J. Agric. Food Chem.* **2001**, *49*, 1035−1041.

(14) Gil-Izquierdo, A.; Gil, M. I.; Ferreres, F. Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds. *J. Agric. Food Chem.* **2002**, *50*, 5107−5114.

(15) Arts, I. C. W.; Hollman, P. C. H. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr.* **2005**, *81*, 317−325.

(16) Londoño, J.; Montoya, G.; Guerrero, K.; Aristizabal, L.; Arango, G. Citrus juice inhibits low density lipoprotein oxidation: relationship between free radical scavenger activity and electrophoretic mobility. *Rev. Chil. Nutr.* **2006**, *33*, 8.

(17) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.

(18) Carbonell, L.; Izquierdo, L.; Carbonell, I. Sensory analysis of Spanish mandarin juices. Selection of attributes and panel performance. *Food Qual. Pref.* **2007**, *18*, 329−341.

(19) Cano, A.; Bañón-Arnao, M. Actividad antioxidante hidrofílica y lipofílica y contenido en vitamina C de zumos de naranja comerciales. *Cienc. Tecnol. Aliment.* **2004**, *4*, 185−189.

(20) AIJN. Code of practice for evaluation of fruit and vegetables juices. Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (AIJN), Brussels, Belgium, 2006.

(21) Pérez, A. G. L. P.; Olmo, M.; Sanz, C.; García, J. M. Effect of intermittent curing on mandarin quality. *J. Food Sci.* **2005**, *70*, M65−M68.

(22) Huang, D.; Ou, B.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841−1856.

(23) Cano, A.; Medina, A.; Bermejo, A. Bioactive compounds in different citrus varieties. Discrimination among cultivars. *J. Food Compos. Anal.* **2008**, *21*, 377.

(24) Gattuso, G.; Barreca, D.; Gargiulli, C.; Leuzzi, U.; Caristi, C. Flavonoid composition of citrus juices. *Molecules* **2007**, *12*, 1641−1673.

(25) Rouseff, R. L.; Martin, S. F.; Youtsey, C. O. Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus. *J. Agric. Food Chem.* **2002**, *35*, 1027−1030.

(26) Esterbauer, H.; Gebicki, J.; Puhl, H.; Jürgens, G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biol. Med.* **1992**, *13*, 341−390.
(27) Dhuique-Mayer, C.; Caris-Veyrat, C.; Ollitrault, P.; Curk, F.; Amiot, M.-J. Varietal and interspecific influence on micronutrient contents in citrus from the Mediterranean area. *J. Agric. Food Chem.* **2005**, *53*, 2140−2145.
(28) McIntosh, C. A.; Mansell, R. L. Three-dimensional distribution of limonin, limonoate A-ring monolactone, and naringin in the fruit tissues of three varieties of *Citrus paradisi*. *J. Agric. Food chem.* **1997**, *45*, 2876−2883.
(29) Vallejo, F.; Larrosa, M.; Escudero, E.; Zafrilla, M. P.; Cerdá, B.; Boza, J.; García-Conesa, M. T.; Espín, J. C.; Tomás-Barberán, F. A. Concentration and solubility of flavanones in orange beverages affect their bioavailability in humans. *J. Agric. Food Chem.* **2010**, *58*, 6516−6524.
(30) Mirhosseini, H.; Salmah, Y.; Nazimah, S. A. H.; Tan, C. P. Solid-phase microextraction for headspace analysis of key volatile compounds in orange beverage emulsion. *Food Chem.* **2007**, *105*, 1659−1670.
(31) Högnadóttir, Á.; Rouseff, R. L. Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *J. Chromatogr., A* **2003**, *998*, 201−211.
(32) Obenland, D.; Collin, S.; Mackey, B.; Sievert, J.; Arpaia, M. L. Storage temperature and time influences sensory quality of mandarins by altering soluble solids, acidity and aroma volatile composition. *Postharvest Biol. Technol.* **2011**, *59*, 187−193.
(33) Elmaci, Y.; Altug, T. Flavor characterization of three mandarin cultivars (satsuma, bodrum, clementine) by using GC/MS and flavor profile analysis techniques. *J. Food Qual.* **2005**, *28*, 163−170.
(34) Yu Qiao, B. J. X.; Zhang, Y.; Zhang, Y,; Gang, F.; Xiao Lin, Y.; Si Yi, P. Characterization of aroma active compounds in fruit juice and peel oil of Jinchen sweet orange fruit (*Citrus sinensis* (L.) Osbeck) by GC-MS and GC-O. *Molecules* **2008**, *13*, 1333−1344.
(35) Moshonas, M. G.; Shaw, P. E.; George, C. Fresh orange juice flavor: a quantitative and qualitative determination of the volatile constitutents. In *Developments in Food Science*; Elsevier: Amsterdam, The Netherlands, 1995; Vol. *37*, pp 1479−1492.