JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effect of Orange Juice's Processing on the Color, Particle Size, and Bioaccessibility of Carotenoids

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ABSTRACT: This study was aimed at assessing the differences between industrially processed and hand-squeezed orange juices (OJs) in relation to their color, particle size, carotenoid content, and carotenoid bioaccessibility. Specifically, industrial samples of fresh squeezed OJs after the finishing steps (FISO) and the same OJs after pasteurization (PISO), as well as hand-squeezed OJs (HSO) were studied. The results showed that the HSO and PISO were different ($p < 0.05$) in terms of color (darker and more reddish vs brighter, more yellowish and colorful), particle size (volume and surface area mean diameter), and total carotenoid content (29 ± 5 and 22 ± 3 mg/L, respectively). On the other hand, the industrial extraction of OJs reduced the particle size distribution, and accordingly, the relative bioaccessibility of bioactive carotenoids increased ($p < 0.01$). Independently of the type of OJ, the bioaccessibility of carotenoids in decreasing order was the following: α-carotene > β-cryptoxanthin > β-carotene > zeaxanthin > lutein.

KEYWORDS: *pasteurization, orange juice, bioaccessibility, particle size, color, carotenoids*

INTRODUCTION

Consumption of fruit juices is increasing worldwide, probably due to public perception of juices as a healthy natural source of nutrients together with the increased public interest in health issues. Juices are also more convenient to consume and have in general a longer shelf life than fresh fruit. Orange juice (OJ) is the most popular fruit juice in the world market, due to its attractive color, appealing sensory properties, and nutritional value. Moreover, it is a good source of vitamin C, carotenoids, flavanones (hesperidin, naringin), and other nutrients as thiamine and folate.

Carotenoids are the compounds responsible for the attractive color of OJ and also for some of their healthy properties. The complex carotenoid profile of OJs comprises carotenes as well as free and esterified xanthophylls.[1] Some of these compounds (β-carotene, α-carotene, and β-cryptoxanthin) exhibit provitamin A activity and also attract interest because they may exhibit other biological properties, like antioxidant or anticarcinogenic activity. Others, like the xanthophylls lutein and zeaxanthin, have been the subject of much research lately due to their relationship with eye health.[2]

A critical feature in the assessment of the role of any food as a dietary source of carotenoids is evaluating their bioavailability from that source. The bioavalability of carotenoids is related to different factors, such as the physicochemical properties (i.e., trans vs cis isomers), the food matrix (subcellular localization), the type of food processing (raw vs processed food), the presence or absence of compounds that promote or inhibit their absorption (fat, protein, and fiber), the pathophysiological status of gut, and the nutritional status of the individual.[3] In order to be available for absorption, nutrients need to be released from the food matrix, what is referred to as bioaccessibility.

It can be a difficult task to accurately ascertain bioaccessibility in vivo; for that reason, several in vitro digestions models have been developed.[4−6] The advantages offered by these methods are that they are simple, inexpensive, rapid, and reproducible. Bioaccessibility is defined as the amount of an ingested compound that becomes available for absorption in the gastrointestinal tract.[6,7]

Bioaccessibility of carotenoids from fruits and vegetables has been assessed in the literature.[6,8] It has been shown that bioaccessibility of xanthophylls (lutein, zeaxanthin, and β-cryptoxanthin) from fruits is better than from green vegetables.[9] Few works have studied the effect of mechanical processing on carotenoid bioaccessibility of carrots, mango, and tomatoes,[10] but none on OJ processing.

As far as the industrial processing is concerned, OJ is commonly marketed in three forms: as a frozen concentrate, which is diluted with water after purchase; as a reconstituted liquid, which has been concentrated and then diluted prior to sale; or as a single strength, unconcentrated beverage called "not from concentrate OJ" (NFC). The latter two types are also known as "ready to drink" (RTD) and remain as the most common product in Europe and the United States. For elaborating the "fresh-squeezed" type of commercial orange juices, after the extraction, the juice is passed through a finisher for separating juice from pulp and seeds and then undergoes a thermal treatment that extends its shelf life but may deteriorate the color, flavor, and aroma quality of the juice and also may promote a substantial decrease in vitamin and phytochemical (carotenoids) content.[11,12] For that reason it is usually

Received: October 27, 2011
Revised: January 9, 2012
Accepted: January 15, 2012
Published: January 17, 2012

dx.doi.org/10.1021/jf2043949 | J. Agric. Food Chem. 2012, 60, 1447−1455

considered that the best form of orange juice remains when it is fresh squeezed at the moment. However, it has been reported that the food processing which modifies the matrix structure by mechanical homogenization or heat treatment may have a beneficial impact on the bioavailability of carotenoids from different foodstuffs.[3,10,13] The mechanical and chemical disruption of the food matrix improves the extractability of carotenoids, making them more accessible for absorption, increasing the bioavailability. In this sense, recent studies have emphasized the relevance of the particle size reduction for improving the β-carotene bioaccessibility from carrot (*Daucus carota* L) using in vitro digestion models.[14,15] In citrus juice production, the homogenization pressures have been reported to affect the particle size distribution and color but not the flavonoid content.[16]

In previous studies on different types of commercial OJs, quantitative and qualitative differences in carotenoid composition and color have been observed,[17,18] pointing out the relevance of each individual processing step on the color and composition of the OJs.

The aims of this study were first to assess the effect of the processing stages (extraction and pasteurization) of orange juice on particle size, color, and carotenoid content on an industrial scale in comparison to domestic hand-squeezed juice and second to evaluate the effect on the bioaccessibility of bioactive carotenoids. For this purpose an in vitro digestion method simulating the human digestion system was used.

MATERIALS AND METHODS

Chemicals. Extraction solvents were analytical-grade methanol, acetone, and dichloromethane from Carlo-Erba (Milan, Italy). Analytic solvents were HPLC-grade methanol and methyl *tert*-butyl ether (MTBE) from Merck (Darmstadt, Germany). Purified water was obtained from a NANOpure Diamond system (Barnsted Inc.). Mineral salts (KCl, NaCl), sodium bicarbonate, chlorhydric acid, pepsin (porcine gastric mucosa), pancreatin (porcine pancreas), bile salt, β-carotene, β-cryptoxantin, and zeaxanthin were purchased from Sigma-Aldrich (Steinheim, Germany). Other carotenoids standards were either isolated from appropriate sources or semisynthesized in accordance to standard procedures as explained elsewhere.[19]

Samples. Oranges and juice samples were directly taken from the commercial orange juice production line at the firm "Zumos Pascual" (Palma del Rio, Cordoba, Spain) at different times during the 2009 season (from May to August). Each sample (six in total) consisted of about 3 kg of fresh Valencia late (*Citrus sinensis* L.) oranges, the corresponding fresh industrially squeezed juice (FISO), and the pasteurized juice (PISO), all from the same batch.

Valencia late oranges in an appropiate stage of maturity, corresponding to a soluble solid content of 11−13 °Bx, were mechanically extracted with an FMC in line premium juice extractor (FMC Food Tech Citrus System). The extracted juice was then conveyed to a finisher to separate juice sacs from the juice. Juices undergo two finishing operation. The FMC juice extractor performs the primary finishing operation in the orifice tube during extraction followed by a secondary external finisher. The first finisher had openings of 0.040 in. in diameter and the second 0.020 in. The FISO samples were taken at this stage, although it is to be noted that this is not commercially available. Previous to pasteurization the OJ was preheated and deaerated at 60 °C/0.90 bar and then the pasteurization was carried in out at 99 °C for 15 s and then rapidly cooled to 1.8 °C. The PISO samples were taken at this stage. The orange fruits taken from the production line were hand-squeezed (HSO) in our laboratory. Three replicates of five oranges per replicate were squeezed with a domestic squeezer (Clatronic model ZP3066, International GMBH). To ensure the reliability and reproducibility of the domestic squeezing, the oranges were carefully squeezed in order to obtain the juice from the edible part of the fruit only without reaching the albedo and sifted through a domestic sieve. All the samples were processed on the day of reception.

Ascorbic Acid, Tritable Acidity, and pH. Ascorbic acid was measured by the titration method based on the reduction of the sodium salt of the dye 2,6-dichlorophenolindophenol by ascorbic acid.[20] The titratable acidity expressed as citric acid was assessed by standard procedures. pH was measured with a GLP-21 GRINSON pHmeter.

Total Phenolic Compound Analysis. Total soluble phenols in ethanol extracts were determined with Folin−Ciocalteau reagent.[21] The results were expressed as milligrams of galic acid equivalents per 1 L of juice. All analyses were made in triplicate.

Total Pulp Content. Total pulp content was measured by a centrifugal method.[22] Juice was centrifuged for 15 min at 3200*g* using an Allegra X-12R centrifuge (Beckman Coulter) to separate pulp and supernatant. The pulp content was expressed as a percentage (v/v).

Particle Size Distribution. The particle size of orange juice was analyzed by a Mastersizer (Malvern Instruments, Inc., Worcs, U.K.) based on laser diffraction analysis. A 5 mL aliquot of orange juice was diluted with 500 mL of distilled water and circulated in the Mastersizer at 2000 rpm. A computer equipped with Mastersizer Microplus 2.15 (Malvern Instruments, Inc.) recorded distributions of the particle size of orange juice. This method is based on laser diffraction analysis. When a parallel beam of a laser passes through the suspension, the diffracted light is focused onto a detector. The detector senses the distribution of scattered light intensity. Particles of a given size diffract light through a given angle, which increases with decreasing particle size. Particle size distribution was calculated and expressed as $D_{[4,3]}$, which is the volume-weighed mean diameter, and defined by the following equation, where d is the diameter of one unit.

$$D_{[4,3]} = \frac{\sum d_4}{\sum d_3} \quad (1)$$

$D_{[3,2]}$, which is the surface area weighed mean diameter, and determined as

$$D_{[3,2]} = \frac{\sum d_3}{\sum d_2} \quad (2)$$

$d(0.1)$ is the size of particle for which 10% of the sample is below this size. $d(0.5)$ is the median of the particle size distribution on the basis of volume. $d(0.9)$ gives a size of particle for which 90% of the sample is below this size.

Color Measurement. The reflectance spectra were obtained by means of a CAS 140 B spectroradiometer (Instrument Systems) fitted with a Top 100 telescope optical probe, a Tamron zoom model SP 23A (Tamron USA, Inc., Commack, NY), and as external light source a white light 150 W metal halide lamp (Phillips MHN-TD Pro) (12 900 lm, 4200 K color temperature). Blank measurements were made with distilled water against a white background.

The entire visible spectrum (380−770 nm) was recorded with a bandwidth of 1 nm, and the Illuminant D65 and the 10° Observer were taken as references.[23] The color parameters of the uniform color space CIELAB, L^*; a^*; b^*; C^*_{ab}, and h_{ab}, were obtained directly from the apparatus. The color data obtained were averages of three measurements. The color differences (ΔE^*_{ab}) between two points in the CIELAB space are defined as the Euclidean distance between their locations in the three-dimensional space defined by L^*, a^*, and b^*. This was calculating using the formula

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

where ΔL^*, Δa^*, and Δb^* are differences between the color of fresh and pasteurized orange juice, and orange juice squeezed manual and industrially.

In Vitro Digestion and Bioaccessibility. The in vitro gastrointestinal digestion protocol used in this study was a combination of the methods proposed by Garret et al.[4] and Liu et al.[24] The former method has been widely used in carotenoid research in different

Table 1. Physicochemical and Colorimetric Parameters (including color differences ΔE^*_{ab}) for the Different Orange Juices Analyzed[a]

physicochemical parameters	HSO	FISO	PISO
pH	3.68 ± 0.11 a	3.68 ± 0.10 a	3.62 ± 0.08 a
acidity %[b]	0.77 ± 0.06 a	0.82 ± 0.04 ab	0.90 ± 0.09 b
ascorbic acid[c]	448.01 ± 27.48 a	449.59 ± 44.96 a	433.78 ± 56.58 a
total phenol content[d]	684.85 ± 69.62 a	725.64 ± 75.35 a	723.52 ± 67.94 a
colorimetric parameters			
L^*	74.84 ± 1.68 a	77.93 ± 1.43 b	77.04 ± 0.43 b
a^*	20.53 ± 1.07 a	13.79 ± 0.78 b	13.94 ± 0.53 b
b^*	67.66 ± 1.48 a	73.37 ± 1.48 b	73.52 ± 0.88 b
C^*_{ab}	70.71 ± 1.32 a	74.65 ± 1.55 b	74.84 ± 0.87 b
h_{ab}	73.11 ± 1.03 a	79.36 ± 0.47 b	79.27 ± 0.41 b
color differences	HSO/FISO	FISO/PISO	HSO/PISO
ΔE^*_{ab}	9.47 ± 1.41	1.84 ± 1.35	9.19 ± 1.43

[a]HSO, hand squeezed; FISO, fresh industrially squeezed; and PISO, pasteurized industrially squeezed. Different letters within the same row indicate statistically significant differences ($p < 0.05$). [b]Grams of citric acid/100 mL. [c]Milligrams of ascorbic acid/L. vjnmk [d]Milligrams of galic acid/L.

laboratories, and some interesting variations having also been proposed.[6] Briefly, the method consisted of a first pepsin-HCl digestion for 1 h (to simulate gastric digestion) and a pancreatic digestion with bile salts for 2 h at 37 °C (to simulate small intestine). For the gastric digestion, 1 mL of OJ sample was added to 1.8 mL of saline solution (140 mM NaCl/5 mM KCl) and 0.2 mL of pepsin solution (160 mg of pepsin in 4 mL of 0.1 M HCl), the pH was adjusted to 2 by addition of HCl 0.1 M, and it was incubated in a shaker Max Q5000 (Thermo Fisher Scientific Inc., Waltham, MA) at 95 rpm and 37 °C for 1 h. For pancreatic digestion, the pH of the partially digested mixture was raised to 6.9 by adding 0.1 M $NaHCO_3$, followed by the addition 0.25 mL of a mixture of bile extract and pancreatin (containing 2 mg/mL pancreatin and 12 mg/mL bile extract in 5 mL of 0.1 M $NaHCO_3$ solution). Samples were incubated in a shaker Max Q5000 (Thermo Fisher Scientific Inc., Waltham, MA) (95 rpm, at 37 °C) for 2 h to complete the intestinal phase. Transfer from the duodenal digesta to the aqueous-micellar phase was estimated by calculating the proportion of carotenoids in the supernatants after low-speed centrifugation (5000*g* for 20 min). The supernatants were used for carotenoids analysis. The "relative bioaccessibility" was estimated by considering the carotenoid content in the supernatant of the digest. The percentage of "relative bioaccessibility" for each carotenoid was calculated as follows:

$$\%\text{bioaccessibility}_{\text{carotenoid}} = \frac{\text{mg/L}_{\text{carotenoid.digest}}}{\text{mg/L}_{\text{carotenoid.sample}}} \times 100 \quad (4)$$

Carotenoid Analysis. *OJs: Pigment Extraction and Saponification.* Five hundred microliter aliquots of OJ were gently mixed with 2 × 600 μL of the extracting solvent (dichloromethane/methanol/acetone, 50:25:25 v/v/v, containing 0.1% butylated hydroxytoluene) and centrifuged for 5 min at 18 000*g*. Upon centrifugation, the upper colored layers containing the carotenoid pigments were recovered and washed with water (2 × 500 μL) to remove any trace of acetone. To obtain saponified carotenoids, the extracts were treated with 600 μL of methanolic KOH (30% w/v) for 1 h under dim light and at room temperature, after which they were washed with water to remove any trace of base.

Supernatants from the in Vitro Digestion: Pigment Extraction and Saponification. The digestions of the six OJ samples corresponding to each processing (HSO, FISO, and PISO) were analyzed in triplicate. The supernatants (ca. 5 mL) were gently mixed with 1000 μL of the extracting solvent (dichloromethane/methanol/acetone, 50:25:25, v/v/v, containing 0.1% butylated hydroxytoluene) and centrifuged for 5 min at 3280*g*. The extraction was performed twice more. Upon centrifugation, the upper colored layers containing the carotenoid pigments were recovered and washed with water. To obtain the saponified carotenoids, the extracts were treated with 3 mL of methanolic KOH (30% w/v) for 1 h under dim light and at room temperature, after which they were washed with water to remove any trace of base.

The colored dichloromethane extracts obtained form both OJs and supernatants were concentrated to dryness in a rotary evaporator at temperature below 30 °C and dissolved in 60 μL of ethyl acetate prior to their injection in the HPLC system. The analyses were performed in triplicate.

HPLC Analysis of Carotenoids. The HPLC analysis was carried out on an Agilent 1100 system consisting of a quaternary pump, a photodiode array detector, a column temperature control module, and an autosampler, which was set to draw 20 μL from the samples (Agilent, Palo Alto, CA). The pigments were separated on an YMC C30 column (5 μm, 250 × 4.6 mm) (YMC, Wilmington, NC) kept at 20 °C.

Methanol (MeOH), methyl *tert*-butyl ether (MTBE), and water were used in the mobile phase. The linear gradient elution was 0 min, 90% MeOH + 5% MTBE + 5% water; 12 min, 95% MeOH + 5% MTBE; 25 min, 89% MeOH + 11% MTBE; 40 min, 75% MeOH + 25% MTBE; 50 min, 40% MeOH + 60% MTBE; 56 min, 15% MeOH + 85% MTBE; 62 min, 90% MeOH + 5% MTBE + 5% water. The mobile phase was pumped at 1 mL/min, and the chromatograms were monitored at 450 nm

The identification of carotenoids was made by comparison of their chromatographic and UV/vis spectroscopic characteristics with those of standards either isolated from appropriate sources or semi-synthesized in accordance with standard procedures, as explained elsewhere.[19]

The absolute concentration of orange juice carotenoids was worked out by external calibration performed in compliance with recommended guidelines[25] from calibration curves constructed with the corresponding standards, as explained elsewhere.[19] The total content of carotenoids was assessed as the sum of the content of individual pigments.

Assessment of Vitamin A Activity. The vitamin A activity of the OJ samples and the corresponding digest was expressed in terms of retinol activity equivalents (RAE).[26] The following formula was used for obtaining the RAE value, and the results were referred to 1 L of OJ

$$\text{RAE} = \frac{\mu\text{g}(\beta\text{-carotene})}{12} + \frac{\mu\text{g}(\beta\text{-cryptoxanthin}) + \mu\text{g}(\alpha\text{-carotene})}{24} \quad (5)$$

Statistical Analysis. Results were given as mean and standard deviation of six independent determinations. One-way analysis of variance (ANOVA) was used to compare the means. All statistical analyses were performed with Statistica v.8.0 software.[27]

RESULTS AND DISCUSSION

In this study, fresh hand-squeezed orange juice (HSO) was obtained from the same batch of oranges that were being processed in the industry, where the juice was squeezed with an FMC juice extractor and then passed through a finisher (FISO) before pasteurization (PISO). The fresh and industrial OJs were characterized by measuring the titratable acidity, pH, and ascorbic acid and phenolic content, as shown in Table 1. The titratable acidity values were within the recommended range (0.6−1.6 g/100 g) for all the samples;[28] however, the acidity mean value in the pasteurized juice was 17% over the value in HSO. It can be observed that neither the extraction method nor the pasteurization process affected the total phenolic content, the pH, or the vitamin C content.[29]

Color and Particle Size. The utility of some color parameters (L^* and C^*_{ab}) to classify different OJs (from concentrate, fresh squeezed, and ultrafrozen) according to the industrial processing has been previously reported.[18,19,30] These studies were conducted on commercially available OJs; thus it was difficult to draw specific conclusions about the influence of the industrial process (extraction and thermal conditions) on the final color. Factors that may affect this attribute, like the particular thermal conditions used or the orange variety, were not controlled. However, in this particular study, both the raw sample and the industrial samples have been analyzed, which give us the advantage of following the same batch of oranges through the process, assuring that the any changes observed were related to the particular process conditions and not to the orange characteristics (variety, stage of maturity, etc.).

Figure 1 shows the samples distributions in the a^*, b^* color diagram. The samples are gathered in two groups corresponding to the industrial samples (FISO and PISO) and the homemade sample (HSO). The summary of the objective color coordinates and color differences among samples are shown in Table 1. To determine the significant differences among the three types of juices, an analysis of variance was conducted. The extraction method (HSO vs FISO) had a significant effect ($p < 0.05$) on both the qualitative (L^*, C^*_{ab}) and quantitative (h_{ab}) color attributes. The hand-squeezed juices were darker, with lower L^* and h_{ab} values, which indicates that they were more reddish (higher a^* and lower b^* values), while the FISO samples were brighter, more yellowish (lower a^* and higher b^* values) and more colorful, since chroma, the quantitative component of colorfulness, showed higher values in these OJs (Figure 1). The comparison of the results obtained with those reported by other authors is not straightforward whatsoever, due to the marked differences in both the temperatures and length of the treatments, as well as in the instrumental assessments of the color. However, some similarities were found. For instance, our results are in accordance with those obtained by other authors,[29,31] who reported a slight increase in C^*_{ab} after the pasteurization (98 °C, 21 s), which could be related to a partial precipitation of unstable, suspended particles. It is important to point out that the pasteurization conditions (temperature and time) may vary from one industry to another, from mild pasteurization conditions (75 °C, 30 s), to standard pasteurization (95 °C, 30 s), which lead to clearly different orange juices.[32]

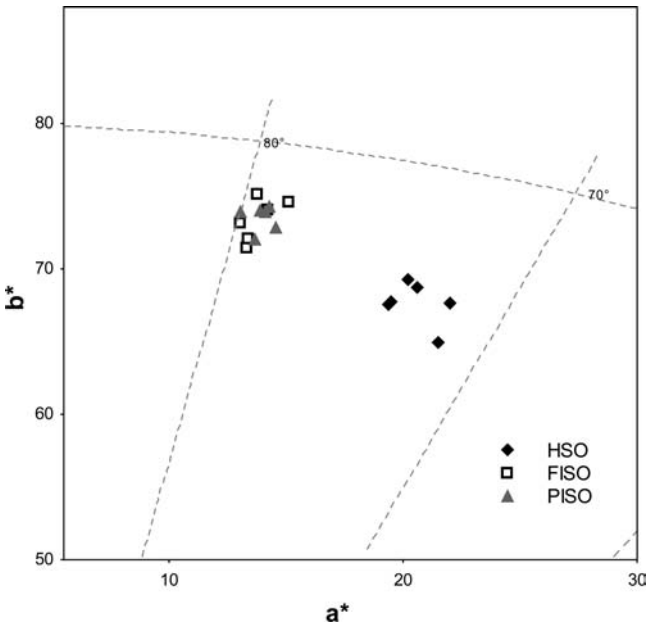

Figure 1. Location of the hand squeezed (HSO), fresh industrially squeezed (FISO) and pasteurized industrially squeezed (PISO) samples in the a^*b^* plane.

The color differences ΔE^*_{ab}, in relation to the extraction method (HSO/FISO), were in all cases higher than the visual discrimination threshold ($\Delta E^*_{ab} > 3$).[33] However, the particular pasteurization conditions (99 °C, 5 s) applied in this industry did not affect the color of the pasteurized juices in comparison with the fresh juices; moreover, the color differences (FISO/PISO) were below the discrimination threshold (range 0.12−3.84 CIELAB units, mean value $\Delta E^*_{ab} = 1.84 \pm 1.34$ CIELAB units). These results are in accordance with those published by Betoret et al.[16] but seem to be in contradiction with those reported by other authors,[12,34] who observed an increase in b^* values and a decrease in a^* after pasteurization.

According to the results above, it could be concluded that the main differences between the color of the homemade OJs and the industrial ones are more related to the extraction process than to the thermal treatment. It could also be inferred that the modification of the pulp structure can be related to this effect. Similarly, Arena et al.[35] pointed out the change of the pulp structure as one of the factors affecting the color modifications in concentrated juices.

In order to explore the effect that the changes on the pulp structure could have in the final color, the particle size distribution, volume mean diameter, and the surface area mean diameter were investigated. The three types of OJs had similar pulp contents, but they had clearly different particle size distributions, as shown in Figure 2. The detailed information related to the particle size distribution is shown in Table 2. According to the $d(0.5)$ values, 50% of the particles sizes in HSO, FISO, and PISO were smaller than 505.78, 406.62, and 381.30 μm, respectively. Similarly, the average $d(0.1)$ values for the samples were 55.59, 48.53, and 57.82 μm, and the $d(0.9)$ values were 1124.39, 921.70, and 871.61 μm, respectively, indicating that 10% and 90% of the samples were below these values. It could be concluded that the industrial OJs had significant smaller particles size than homemade OJs. The volume mean diameter, $D_{[4,3]}$, indicates the diameter of the average volume of a particle. The analysis of variance clearly showed significant differences ($p < 0.05$) between HSO and the industrial juices (FISO and PISO) for this parameter, but the pasteurization process seemed not to affect it. The industrial

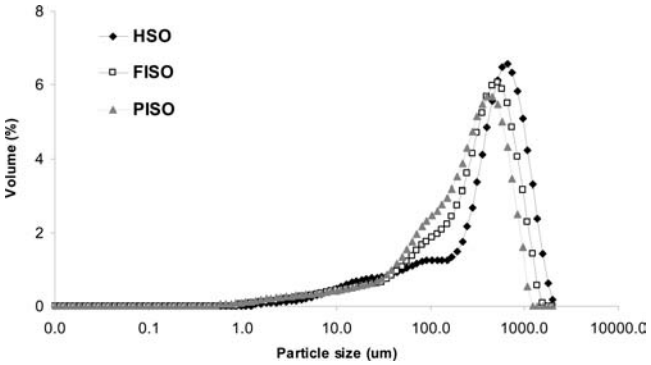

Figure 2. Particle-size distribution in the HSO, FISO, and PISO orange juice samples.

squeezing and finishing-steps decreased the volume mean diameter. These results are in concordance with those reported by Betoret et al.,[16] who found a decreased in the particle size when increasing the homogenization pressure. In the same way, the surface area mean diameter $D_{[3,2]}$ was significantly smaller in the pasteurized juices, in relation to the HSO.

The specific surface area (A_s), which is related to the density of each particle, was higher ($p < 0.05$) in the industrial juice PISO (0.08 mm^2/g) than in the homemade juice (0.06 mm^2/g) but not in FISO, indicating an adding effect of both process (extraction and pasteurization) on this parameter. According to this parameter, the commercial juices were finer than the domestic one. To sum up, the industrial processing reduced the particle size, increasing the surface area and viscosity, and as a consequence, there was a visually appreciable change of color, as reported previously. The juices became brighter and more yellowish and colorful.

Carotenoids and Color. The pigments responsible for OJ color are the carotenoids. Previous studies on the carotenoid profile of commercially available OJs (ultrafrozen, fresh-squeezed, and OJ from concentrate) have shown that the conditions of processing and storage do have an impact on their carotenoid profile.[1,19] However, other variables like variety and geographical origin among many others should also be considered.[36] As mentioned above, the homemade and industrial samples analyzed in this study were from the same variety and geographical origin and they were all processed in the same batch, so differences in the pigment content and profile should be exclusively related to the processing. Typical chromatograms of the carotenoids present in the three types of OJs analyzed are depicted in Figure 3. The industrial processing (extraction and pasteurization) affected negatively ($p < 0.05$) the content of carotenoids, which ranged between 35 and 18 mg/L, with the highest values found in HSO and the lowest in PISO (Table 3). As reported previously for Valencia late ultrafrozen orange juices, xanthophylls predominated over carotenes, and the 5,6-epoxycarotenoids (violaxanthin, antheraxanthin, and geometrical isomers) were the major ones followed by 5,8-epoxycarotenoids (luteoxanthin and mutatoxanthin).[19] The individual profile of carotenoids consisted of violaxanthin (38−44%), antheraxanthin (12−13%), luteoxanthin (9−11%), zeaxanthin (8−9%), mutatoxanthin (7−10%), β-cryptoxanthin (6−7%), lutein (5%), and β-carotene, zeinoxanthin, and α-carotene which accounted for about 3%, 2.5%, and 1% of the total carotenoid content. HSO resulted in a 15% higher content of carotenoids than FISO. The pasteurization reduced by 10% the carotenoid content in relation to FISO and 23.5% in relation to HSO ($p < 0.05$). Lee et al.[12] also reported a significant ($p < 0.05$) reduction in the carotenoid content after the pasteurization at 90 °C for 30 s. On the other hand, it was observed that the thermal processing did not affect the provitamin A content (β-carotene, α-carotene, and β-cryptoxanthin), in accordance with previous investigations.[12,37,38] Provitamin A activity in HSO was not significantly different ($p < 0.05$) from that of PISO.

The 5,6-epoxycarotenoids, (9Z)-violaxanthin, and antheraxanthin (peak 4) decreased ($p < 0.05$) in the industrially processed OJs: 29% in FISO and 54% in PISO. This decrease in the concentration of the 5,6-epoxides could be attributed to an isomerization into 5,8-epoxides (luteoxanthin, auroxanthin, and mutatoxanthin). As explained elsewhere,[1,19,39] violaxanthin is easily isomerized to luteoxanthin and afterward to auroxanthin in an acidic medium. The higher acidity value observed in PISO in relation to HSO (Table 1) could explain this fact.

The eye-health related carotenoids, zeaxanthin (peak 9) and lutein (peak 7), were affected neither by the extraction nor the pasteurization; only zeinoxanthin (peak 11) content was reduced ($p < 0.05$) in the pasteurized juice (20%), in comparison with the HSO.

With regard to the color differences detected between the three types of orange juices, it has been reported that both carotenoid content and structure influence the color of OJs. The pigments mainly related to the qualitative color attribute, h_{ab}, are zeinoxanthin, lutein, and a mixture of *all*-(*E*)-violaxanthin + (*Z*)-violaxanthin isomers, while those mainly related to the quantitative attribute, C^*_{ab}, are zeaxanthin, (9*Z*)- or (9′*Z*)-antheraxanthin, and zeinoxanthin.[17,19]

Table 2. Summary of the Particle-Size Distribution of the Orange Juices[a]

	mean ± SD[b]		
	HSO	FISO	PISO
% pulp content	7.65 ± 0.68 a	8.35 ± 1.18 a	7.05 ± 0.54 a
$D_{[4,3]}$	556.46 ± 71.63 a	451.23 ± 43.15 b	427.93 ± 65.60 bc
$D_{[3,2]}$	98.34 ± 11.95 a	82.08 ± 11.98 ab	74.86 ± 17.39 b
A_s	0.06 ± 0.01 a	0.07 ± 0.01 ab	0.08 ± 0.02 b
$d(0.1)$	55.59 ± 13.66 a	48.53 ± 7.50 a	57.82 ± 14.27 a
$d(0.5)$	505.78 ± 86.81 a	406.62 ± 46.06 ab	381.30 ± 65.75 b
$d(0.9)$	1124.39 ± 91.37 a	921.70 ± 79.50 b	871.61 ± 115.66 bc

[a] $D_{[4,3]}$, volume mean diameter; $D_{[3,2]}$, surface area mean diameter; A_s, specific surface area, $d(0.5)$, $d(0.1)$, and $d(0.9)$, standard percentile reading. [b] HSO, hand squeezed; FISO, fresh industrially squeezed; and PISO, pasteurized industrially squeezed. Different letters within the same row indicate statistically significant differences ($p < 0.05$).

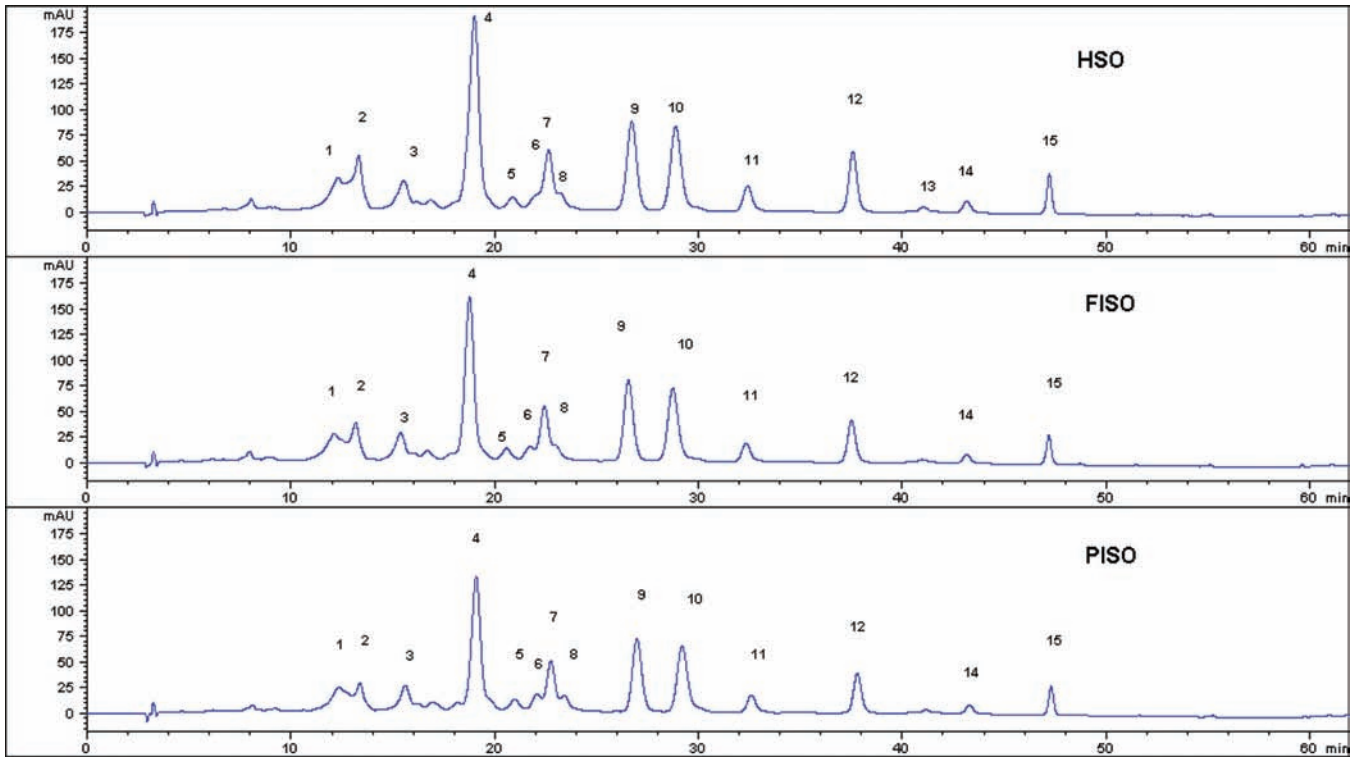

Figure 3. Chromatograms at 450 nm of the carotenoid extracts from OJs samples. Peak identification is in Table 3.

Table 3. Carotenoids Levels (mg/L) and Retinol Activity Equivalents (RAE) in the Orange Juices Analyzed

		mean ± SD[a]		
peak	identification	HSO	FISO	PISO
1	unidentified	not quantified	not quantified	not quantified
2	*all*-(*E*)-violaxanthin + (*Z*)-violaxanthin isomers	4.12 ± 1.02 a	3.36 ± 0.61 ab	2.83 ± 0.41 b
3	luteoxanthin + (*Z*)-antheraxanthin isomer	2.17 ± 0.50 a	1.99 ± 0.32 a	1.84 ± 0.23 a
4	(9*Z*)-violaxanthin + antheraxanthin	8.71 ± 1.50 a	6.76 ± 1.31 b	5.65 ± 0.82 bc
5	(*Z*)-luteoxanthin isomer	0.52 ± 0.17 a	0.58 ± 0.08 a	0.60 ± 0.10 a
6	mutatoxanthin epimer	0.93 ± 0.33 a	0.99 ± 0.14 a	1.11 ± 0.18 a
7	lutein	1.39 ± 0.21 a	1.26 ± 0.12 a	1.17 ± 0.08 a
8	mutatoxanthin epimer	1.06 ± 0.36 a	1.09 ± 0.21 a	1.15 ± 0.24 a
9	zeaxanthin	2.52 ± 0.33 a	2.19 ± 0.28 ab	1.93 ± 0.17 b
10	(9*Z*)- or (9′*Z*)-antheraxanthin	3.73 ± 0.66 a	3.23 ± 0.61 ab	2.78 ± 0.40 b
11	zeinoxanthin	0.70 ± 0.07 a	0.61 ± 0.08 ab	0.56 ± 0.04 b
12	*β*-cryptoxanthin	2.09 ± 0.42 a	1.66 ± 0.39 a	1.68 ± 0.24 a
13	(*Z*)-*ζ*-carotene isomer	not quantified	not quantified	not quantified
14	*α*-carotene	0.32 ± 0.04 a	0.29 ± 0.02 a	0.27 ± 0.02 a
15	*β*-carotene	0.88 ± 0.17 a	0.78 ± 0.13 a	0.72 ± 0.09 a
	total carotenoids	29.13 ± 4.57 a	24.79 ± 3.33 ab	22.29 ± 2.48 b
	RAE	173.32 ± 28.92 a	145.91 ± 24.97 a	140.98 ± 17.12 a

[a]HSO, hand squeezed; FISO, fresh industrially squeezed; and PISO, pasteurized industrially squeezed. Different letters within the same row indicate statistically significant differences ($p < 0.05$).

Visually perceived color differences were obtained between HSO and FISO and HSO and PISO (Table 1). In the first case the color differences seem to be only attributable to significant differences in peak 4 ((9*Z*)-violaxanthin + antheraxanthin). Meanwhile, the visible color differences (Table 1) detected between HSO and PISO can be related to significant differences ($p < 0.05$) in the contents of several carotenoids such as (9*Z*)-violaxanthin + antheraxanthin, zeaxanthin, (9*Z*)- or (9′*Z*)-antheraxanthin, and zeinoxanthin, which indicates a more complex effect of the thermal treatment.

To sum up, the extraction method affected ($p < 0.05$) the color parameters, the color differences between HSO and FISO being visually perceived as stated above, but only the (9*Z*)-violaxanthin + antheraxanthin content was affected by the industrial extraction process, thus suggesting that the color differences could be related not only to the carotenoid composition but also to the particle size. On the contrary, the thermal treatment affected neither the color nor the content of OJ carotenoids in comparison with the fresh industrially squeezed juice. When it comes to a comparison of a homemade

Table 4. Carotenoids Levels in the Digests (mg/L) and Retinol Activity Equivalents (RAE) in the Orange Juices Analyzed

peak	identification	mean ± SD[a] HSO	FISO	PISO
1	unidentified	not quantified	not quantified	not quantified
2	all-(E)-violaxanthin + (Z)-violaxanthin isomers	1.01 ± 0.28 a	1.31 ± 0.34 a	0.90 ± 0.19 a
3	luteoxanthin + (Z)-antheraxanthin isomer	0.64 ± 0.15 a	1.02 ± 0.26 b	0.69 ± 0.13 a
4	(9Z)-violaxanthin + antheraxanthin	2.19 ± 0.50 a	2.78 ± 0.83 a	2.08 ± 0.43 a
5	(Z)-luteoxanthin isomer	0.26 ± 0.06 a	0.41 ± 0.12 b	0.26 ± 0.07 a
6	mutatoxanthin epimer	0.51 ± 0.10 a	0.76 ± 0.24 a	0.55 ± 0.15 a
7	lutein	0.46 ± 0.12 a	0.60 ± 0.15 a	0.44 ± 0.07 a
8	mutatoxanthin epimer	0.49 ± 0.11 a	0.61 ± 0.17 a	0.44 ± 0.13 a
9	zeaxanthin	0.81 ± 0.19 a	1.08 ± 0.19 b	0.76 ± 0.14 a
10	(9Z)- or (9′Z)-antheraxanthin	1.12 ± 0.14 a	1.63 ± 0.45 b	1.17 ± 0.23 a
11	zeinoxanthin	0.25 ± 0.05 ab	0.32 ± 0.05 a	0.23 ± 0.03 b
12	β-cryptoxanthin	0.72 ± 0.23 a	0.92 ± 0.23 a	0.68 ± 0.17 a
13	(Z)-ζ-carotene isomer	not quantified	not quantified	not quantified
14	α-carotene	0.13 ± 0.02 ab	0.15 ± 0.02 a	0.12 ± 0.01 b
15	β-carotene	0.29 ± 0.12 a	0.40 ± 0.09 a	0.28 ± 0.09 a
	total carotenoids	8.87 ± 1.54 a	12.00 ± 2.49 b	8.61 ± 1.52 a
	RAE	59.47 ± 19.81 a	78.02 ± 16.85 a	56.90 ± 15.13 a

[a]HSO, hand squeezed; FISO, fresh industrially squeezed; and PISO, pasteurized industrially squeezed. Different letters within the same row indicate statistically significant differences ($p < 0.05$).

juice (HSO) with a commercial one (PISO), we find significant differences in the color and carotenoids. These differences could be related both to the decrease ($p < 0.05$) in the levels of some carotenoids, specifically those including oxygenated functions in their structure [*all*-(*E*)-violaxanthin + (*Z*)-violaxanthin isomers, (9*Z*)-violaxanthin + antheraxanthin, zeaxanthin, (9*Z*)- or (9′*Z*)-antheraxanthin, zeinoxanthin], and total carotenoids content as well as to a reduction in the particle size. Beyond the organoleptic quality, which has not been evaluated in this work, attending to the nutritional quality of the OJs, both the hand squeezed and the industrially processed juices (FISO and PISO) were not significantly different ($p < 0.05$) in provitamin A content (β-carotene, α-carotene, and β-cryptoxanthin).

Bioaccessibility of Carotenoids and Particle Size. The structure of the food matrix is one of the main factors related to the release of carotenoids to a solubilized form, thus affecting bioaccessibility. In the present paper we have evaluated the "relative bioaccessibility", since we have assessed the amount of carotenoids that are transferred from the orange juice to the supernatant obtained after a low-speed centrifugation.[8,13]

Table 4 shows the levels of the carotenoids detected in the digests of the three types of OJs after the in vitro digestion. Carotenoid epoxides, which are not found in human plasma and tissues, are considered not to be absorbed by humans.[40] From this point of view, their bioaccessibility is not relevant. However, considering that they are also affected by extraction/pasteurization and the digestion process, we have also included data on their contents in the digest. The transfer efficiency from the food to the digest fraction is known to be related to the structure of the food matrix. In this sense, it can be observed that the industrial extraction of OJ increased by 35% the total content of carotenoids in the digest ($p < 0.05$) in comparison with the hand-squeezing. On the contrary, pasteurization reduced the level by 39% ($p < 0.05$), and as a result, PISO digest was only 3% lower in carotenoid content than HSO digest. Provitamin A carotenoids and, consequently, the RAE values were not affected either by the extraction or the pasteurization process.

Figure 4 shows the relative bioaccessibility values, expressed as the percentage of carotenoids in the digest in relation to the initial content in the OJ (see formula 4). As stated above, a higher percentage of relative bioaccessibilty ($p < 0.01$) in the industrially extracted OJs was observed in comparison with the home squeezed ones for lutein (15%), zeaxanthin (18%), β-cryptoxanthin (22%), α-carotene (14%), and β-carotene (21%). On the other hand, the pasteurization reduced the bioaccessibility ($p < 0.05$) of provitamin A carotenoids (α-carotene and β-cryptoxanthin) compared to FISO (Figure 4). Similar results were reported by Tydeman et al.[15] in heated carrot. This could be due to a higher susceptibility of cells to rupture in fresh tissues compared to heated tissues, in which the carotenes remain encapsulated. As a final result, the HSO and PISO were equal in terms of bioaccessibility of bioactive carotenoids. Regardless of the type of OJ, the bioactive carotenoids that showed the highest percnetage of relative bioaccessibility on average were α-carotene (45.99 ± 7.63), followed by β-

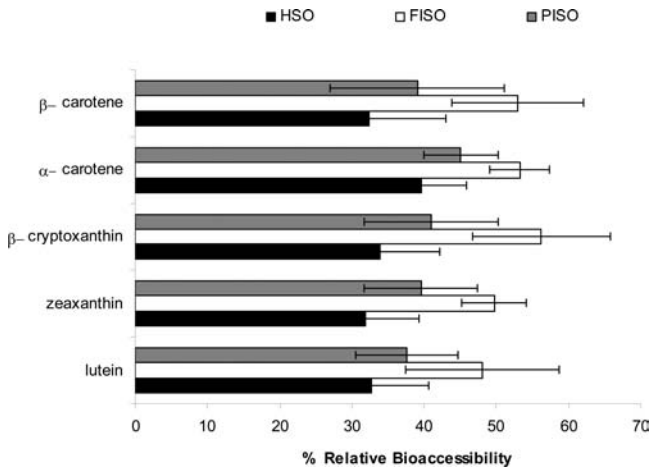

Figure 4. Relative bioaccessibility (%) of the bioactive carotenoids in the OJ samples.

dx.doi.org/10.1021/jf2043949 | J. Agric. Food Chem. 2012, 60, 1447−1455

cryptoxanthin (43.68 ± 12.84), β-carotene (41.52 ± 13.32), zeaxanthin (40.39 ± 9.65), and lutein (39.50 ± 10.45).

As discussed above, the industrial extraction (the squeezing and the finishing-steps) reduces the pulp particle size and enhances the carotenoid bioaccessibility. The higher specific surface area (A_s; see Table 2) in industrial juices in comparison with HSO and the decrease in the particle size increases the surface area available for the attack by digestive enzymes, thus increasing the overall digestion efficiency and the gastrointestinal absorption of nutrientsp; i.e., the extraction methods facilite transfer from the food matrix. These results are in agreement with those of Tydeman et al.,[15] who demostrated the relevance of particle size reduction on carotene bioaccessibility in carrot juice, and Hedrén et al.,[13] who reported an increase in the bioaccessibility of β-carotene after grinding raw and cooked carrots into small pieces.

In conclusion, home (HSO) and industrially extracted OJs (FISO) are significantly different in terms of color and particle size distribution but not in the total carotenoids content, including provitamin A carotenoids. However, the total industrial processing (extraction and pasteurization) (PISO) does have a reducing effect on the carotenoid content in comparison to the home squeezed juice. In terms of the relative bioaccessibility of carotenoids, the finishing steps in the industrial extraction reduce the particle size, improving the bioaccessibility. In fact, bioactive carotenoids are more bioaccessible from the industrially extracted OJs than from the homemade ones. Bioaccessibility of carotenoids from OJs seems to be more related to the mechanical processing than to the thermal treatment, since pasteurization reduces slightly the bioaccessibility of the bioactive carotenoids. Beyond the sensory quality, which is assumed to be higher in homemade juices, the nutritional quality concerning the provitamin A content and even the bioaccessibility of bioactive carotenoids is equal in home-squeezed and the pateurized OJs. More research is needed to advance our understanding of the carotenoids bioaccessibility and bioavailability problematic.

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Funding

This work was partially supported by the project P08-AGR03784 (Consejería de Innovación Ciencia y Empresa, Junta de Andalucía). C.M.S. holds a predoctoral research grant from the Universidad de Sevilla. A.J.M.M. acknowledges funding from the Spanish Government through the program Ramon y Cajal (ref RYC-2010-07115).

ACKNOWLEDGMENTS

The authors acknowledge the collaboration of Zumos Pascual (Palma del Rio, Spain) in this study.

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