Effect of orange juice's processing on the color, particle size and bioaccessibility of carotenoids.

or

Processing of Orange juice: impact on color, particle size and in vitro bioaccessibility of carotenoids

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Abstract

This study was aimed at assessing the influence of the industrial processing (extraction and pasteurization) in comparison with hand squeezing on the colour, particle size and carotenoid content of orange juice OJ (Citrus sinesis L. Var. Valencia Late). Six industrial samples including fresh oranges for obtaining hand-squeezed OJ (HSO), industrial fresh squeezed OJ after homogenization (in a single strength FMC extractor) (FISO) and the same OJ after pasteurization (PISO) (99 ºC, 5s) were analyzed. Bioaccesibility was also evaluated by an in vitro digestion method. The HSO and PISO were different ($p<0.05$) in color, particle size and total carotenoid content. The hand-squeezed OJs provided a higher ($p<0.05$) level of total carotenoids than FISO and PISO (29.13±4.57 vs 24.79± 3.33 and 22.29± 2.48 mg/L, respectively) and were darker and more reddish, while the industrial juices were brighter, more yellowish and colourful. However industrial processing reduced the particle size distribution what had a positive effect in terms of bioaccessibility. Industrial squeezing increased the bioaccesibility of total carotenoids (8.87± mg/L vs 12.00±2.49 in the digest of HSO and FISO respectively), but pasteurization had the opposite effect. Thus there were no differences between domestic juices (HSO) and commercial (PISO) in terms of carotenoids bioaccesibility (8.87±1.54 vs 8.61±1.52 mg/L in the digest of HSO and PISO respectively). Independently of the type of OJ, the bioaccessibility of carotenoids had the following decreasing order: β-cryptoxanthin > β-carotene, α-carotene > zeaxanthin > lutein.

Keywords: pasteurization, orange juice, bioaccesibility, particle size, color

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. Among them stands the orange juice (OJ) which certainly remains as 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 (Martí et al., 2009), carotenoids (Melendez et al., 2007) and other nutrients as thiamin, folate and flavanones (hesperidin, naringin) (McGill et al., 2004).

Carotenoids are the compounds responsible for the OJ´s attractive color and also for some of their healthy properties. The complex carotenoid profile of OJs comprises carotenes as well as free and esterified xanthophylls (Meléndez-Martínez et al., 2008). Some of these compounds (β-carotene, α-carotene and β-cryptoxanthin) exhibit provitamin A activity and are attracting interest because they may exhibit other biological properties, like antioxidant or anticarcinogenic activity. Others, like the xanthophylls lutein and zeaxanthin, are being the subject of much research lately due to their relationship with the eye health (Krinsky et al., 2004).

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 physico-chemical properties (ie., trans *vs* cis-isomers), the food matrix (subcelular 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), pathophysiological status of gut, and the nutritional status of the individual as reviewed by Castenmiller & West (1998) and Yeum et al., (2002). In order to be available for absorption, nutrients need to be released form the food matrix, what is referred to as bioaccesibility (Van Buggenhout et al. 2010). The bioaccesibility can be a difficult task to be accurately ascertained *in viv*o.For that reason, several *in vitro* digestions models have been developed (Garrett et al., 1999; Fernández-García et al., 2009). In the literature information concerning the bioaccesibility of carotenoids form raw fruits and raw/cooked vegetables can be found (Goñi et al. 2006; Granado-Lorencio et al. 2007), Thus, a better bioaccesibility of xanthophylls carotenoids (lutein, zeaxanthin and β-cryptoxanthin) from fruits than dark

green vegetables has been reported (O'Connell et al. 2007). However studies on the effects of the type of OJ's processing on the bioaccesibility of carotenoids are scant.

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 (NFC). The latter two types are also known as Ready To Drink (RTD) and remain as the most common product in Europe and USA. For elaborating the "fresh squeezed" type commercial orange juices, after the extraction, the stream of pulpy juice goes through a finisher for homogenization, the pulp and seeds are removed and, then undergoes a thermal treatment that elongate its shelf life, but also lower notably its vitamin activity and deteriorate somehow its flavor, aroma, and color (Farnworth et al., 2001; Lee et al., 2003; Lessin et al., 1997). For that reason it is usually considered that the best form of orange juice remains when it's 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 (Hedrén et al., 2002; Hornero-Méndez et al., 2007; Yeum et al., 2002). The mechanical and chemical disruption of the food matrix improve the extractability of carotenoids, making them more accessible for absorption (Furr et al., 1997) increasing the bioavailability (Mamatha et al., 2010). In this sense, recent studies have emphasized the relevance of the particle size reduction for improving the β-carotene bioaccessibility fom carrot (Daucus carota) using in vitro digestion models (Lemmens et al., 2010; Tydeman et al., 2010). Homogenization has also been reported to influence the particle size distributions in tomato juice (Miki et al., 1971). Similarly the homogenization pressures have been reported to affect the particle size distribution and colour, but not the flavonoid content in citrus juices (Betoret et al., 2009).

In a previous study with commercial orange juices, we have observed quantitative and qualitative differences in carotenoid composition and color in different types of commercial OJs (Melendez et al, 2010; Fernandez-Vázquez et al., 2010). However we analyzed commercial samples thus it was not possible to evaluate the effect of each individual processing step on the color and composition of the OJs. The objective of our investigation was to determine the effects of processing stages at industrial scale (extraction, finishing and pasteurization) with respect to home squeezed

OJs on particle size, color and carotenoid content. Also changes in bioaccessibility of carotenoids due to the different processing were evaluated by means of an in vitro digestion method simulating the human digestion system.

Materials

Samples

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

Valencia late oranges in an appropiatte stage of maturity, corresponding to a soluble solid content of 11-13 ºBrix, were mecanically extracted with an FMC in line Premium Juice Extractor (FMC Food Tech Citrus System, Lakeland, USA). The extracted juice was then conveyed to a finishers 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 sencondary external finisher. The first finisher had openings of 0.040 inches in diameter and the second 0.020 inches. The fresh industrial squeezed OJ (FISO) samples were take at this stage. It must be said that this is not a commercially availabel OJ. Previous to pasteurization the OJ was preheated and deaerated at 60 º C / -0.90 bars, then the pasteurization was carried in out at 99 º C for 15 sec and then rapidly cooled to 1.8 ° C. The pasteurized industrial OJ samples (PISO) were taken at this satage. The orange fruits taken in the production line were hand-squeezed (HSO) in our laboratory, Three replicates of 5 oranges per replicate were squeezed with a domestic squeezer (Clatronic Model ZP3066, International GMBH, Germany). 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.

Methods

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 (AOAC

International, 1995).Equal volumes (5 ml) of orange juice and metaphosphoric acid as an aqueous solution (3% w/v) were mixed and subsequently centrifuged at 5000 rpm for 5 min. Five-milliliter aliquots of the supernatant were recovered and further diluted with the metaphosphoric acid solution before titration, (Meléndez-Martínez et al., 2001). The titratable acidity expressed as citric acid was assessed by standard procedures. In brief, the samples of OJ were diluted with distilled water, a few drops of ethanolic phenolphthalein (1%) were added, and the juice mixture was subsequently titrated with aqueous NaOH 0.1 M until color change. 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 (Singleton et al., 1965). The results were expressed as mg of galic acid equivalents per 1 L of juice. All analyses were done in triplicate.

Total pulp content

Total pulp content, was measured by a centrifugal method (Kimball, 1999). Juice was centrifuged for 15 min at 3200 x g using Allegra X-12R Centrifuge (Beckman Coulter, USA) to separate pulp and supernatant. Pulp content was expressed as % 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 Micropulus 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-weighted mean diameter, and defined as the following equation, where d is the diameter of one unit.

$$D_{[4,3]} = \frac{\sum d_4}{\sum d_3}$$

D[3,2], which is the surface area weighted mean diameter, and determined as

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

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 (Malvern Instruments, 1995).

Color Measurement.

The reflectance spectra were obtained by means of a CAS 140 B spectroradiometer (Instrument Systems, Germany) fitted with a Top 100 telescope optical probe, a Tamron zoom mod. SP 23A (Tamron USA, Inc., Commack, NY), and an external incandescent lamp as source of illumination. 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 10º Observer were taken as references (CIE 1978). 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 CIE L*, a* and b* values were used to calculate the total colour differences (ΔE^*_{ab}), using the formula:

$$\Delta E^*_{ab} = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}$$

where ΔL*, Δa* and Δb* are differences between the orange juice colour of fresh and pasteurized juice , and orange juice squeezed manual and squeezed industrial

In vitro digestion and bioaccesibility

The *in vitro* gastrointestinal digestion protocol used in this study was a combination of the methods proposed by Garret et al., (1999) and Liu et al., (2004) which are based on the method previously developed by Miller et al.,(1981). Briefly the method consisted of a first pepsin-HCl digestion for 2 h (to simulate gastric digestion) and a pancreatin digestion with bile salts for 2 h at 37°C (to simulate small intestine). 1 mL of OJ sample

was added to 1,8 mL of saline solution (140mM NaCl / 5 mM KCl) and 0.2 ml pepsin solution (160 mg pepsin in 4 mL 0.1M 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 the pancreatin digestion, the pH of the partially digested mixture was raised to 6,9 by adding of 0,1M $NaHCO_3$, 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 mins). The supernatants were used for carotenoids analysis. The bioaccesibility was estimated considering the carotenoid content in the supernatant of the digest. The percentage of bioaccesibility for each carotenoid was calculated as follows:

$$\%Bio_{carotenoid} = \frac{mg/L_{carotenoid\_digest}}{mg/L_{carotenoid\_sample}} x100$$

Carotenoids Analysis.

Pigment Extraction and Saponification from Orange Juice and supernatants from *in vitro* digestion.

Five hundred microliters aliquots of the OJs were gently mixed with 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 14000 rpm. Upon centrifugation, the upper colored layers containing the carotenoid pigments were recovered and washed with water (2x 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.

The supernatants were gently mixed with L of the extracting solvent dichloromethane 0.1% butylated hydroxytoluene and centrifuged for 5 min at 3750 rpm. 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 3ml 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 coloured dichloromethane extracts obtained were concentrated to dryness in a rotary evaporator at temperature below 30 °C and redissolved in 60 μL of a ethyl acetate prior to their injection in the HPLC system. The analyses were performed in duplicate.

High-Performance Liquid Chromatography.

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 x 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 (Meléndez-Martínez et al. 2003).

Identification and Quantitative Analysis of Carotenoids.

The identification of the majority of the carotenoids detected was made by comparison of their cromatographic and UV/vis spectroscopic characteristics with those of standards either isolated from appropriate sources or semisynthesized in accordance to standard procedures as explained elsewhere (Meléndez-Martínez et al., 2005a; Meléndez-Martínez et al., 2005b; Meléndez-Martínez et al., 2005c; Meléndez-Martínez et al., 2007). The absolute concentration of orange juice carotenoids was worked out by external calibration performed in compliance with recommended guidelines (Kimura et al., 1999; Rodríguez-Amaya, 2001) from calibration curves constructed with the corresponding standards, as explained elsewhere (Meléndez-Martínez et al., 2007). 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 were expressed in terms of retinol activity equivalents (RAE). The following formula was used for obtaining the RAE value and the results were referred to 1 L of OJ:

$$RAE = \frac{\mu g\ \beta\ carotene}{12} + \frac{\mu g\ \beta\ cryptoxanthin + \mu g\ \alpha\ carotene}{24}$$

Statistical Analysis.

Results were given as mean (standard deviation of six independent determinations). One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered to be significant at ($p<0.05$). All statistical analyses were performed with Statistica v.8.0 software (StatSoft 2007).

Results and discussion

In this study we have produced fresh hand squeezed orange juice (HSO) from the same batch of oranges that were industrially processed. The industrial OJ samples were obtained with an FMC juice extractor and passed through a finisher for homogenization (FISO) and then submitted to pasteurization (PISO). The fresh and industrial OJs were characterized by measuring the tritrable acidity, pH, acorbic acid and phenolic content as shown in Table 1. The titratable acidity values were within the recommended range (0.6 to 1.6 g/100 g) for all the samples (Redd et al. 1986), however the pasteurization process increased in 17 % de acidity value of the OJs, as other authors had reported previously (Akinyele et al. 1990; Rivas et al. 2006). It can be observed that neither the extraction method nor the pasteurization process affected the total phenolic content nor the pH, as reported by Rivas (Rivas et al. 2006). The unthermally treated OJs (HSO, FISO), showed higher (but not significant) vitamin C values than pasteurized juices (PISO).

Color and particle size

In previous works we have characterized the color of different OJs (from concentrate , fresh squeeze and ultrafrozen) finding out that they could be classified according to the industrial processing, taking into account the colour coordinates L* and C^*_{ab} (Fernández-Vázquez R. et al. 2010; Meléndez-Martínez et al. 2001; Meléndez-Martínez et al. 2007; Meléndez-Martínez et al. 2010). These studies were conducted on commercially available OJs, thus it was difficult to draw conclusions about the influence of the industrial process (extraction and thermal conditions) on the final colour, since some factors that may affect this attribute eg. the particular thermal conditions used or the orange variety, were not controlled. However in this particular study, we have analyzed both the raw sample and the industrial samples, which give us the advantage of following the same batch of oranges through the process, assuring that the any changes observed are 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 home made sample (HSO).

The summary of the objective colour coordinates and colour 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 ($p < 0.05$) in both the qualitative and quantitative colour attributes. The hand-squeezed juices were darker, with lower CIE L* values and lower hue vales, which indicate that they were more reddish (higher a* and lower b* values) while the FISO were brighter and more yellowish (lower a* and higher b* values) and more colourful, since chroma, the quantitative component of chromaticity, showed higher values in these OJs. These results are in accordance with those obtained by other authors (Genovese et al. 1997; Lee et al. 1999; Rivas et al. 2006), that reported a slight not significant increase in C^*_{ab} after the pasteurization (98ºC, 21s), that 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 (75ºC, 30 s), to standard pasteurization (95ºC, 30s), which lead to clearly different orange juices (Gil-Izquierdo et al., 2002)

The color differences $\Delta E^*_{ab,}$ in relation to the extraction method (HSO/FISO) were in all cases higher than the visual discrimination threshold ($\Delta E^*_{ab}>3$) (Lozano 1979; Melgosa et al. 1997; Melgosa et al. 2001). However, the particular pasteurization conditions (99 ºC, 5 s) applied in this industry did not affect the color of the pasteurized juices in comparison with the fresh one, moreover the color differences (FISO/PISO) were bellow the discrimination threshold (range 0.12 to 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., (2009) but seems to be in contradiction with those reported by other authors (Lee et al. 2003; Sánchez-Moreno et al. 2005), who observed an increase in CIE b* values and a decrease in CIE a* after pasteurization.

According to the results above we could conclude that the main differences between the color of the home made OJs and the industrial ones are more related to the extraction process than to the thermal treatment. We can infer that the modification of the pulp structure could be related to this effect. Similarly, Arena et al., (2000) pointed out that the change of the pulp structure as one of the factors affecting the color modifications in concentrated juices.

To explore the effect that the changes on the pulp structure could have in the final color we investigated the particle size distribution and volume mean diameter and the surface area mean diameter. The three types of OJs had similar pulp contents but they were clearly different in the particle size distribution 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, d (0.1) values 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 home-made 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 did not affect it. The industrial squeezing and finishing steps had a decreasing effect on the volume mean diameter. These results are in concordance with those of Buslig (1974) and Betoret et al., (2009), who reported a decrease 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 (Aw), which is related to the density of each particle, was higher in the industrial juices, PISO and FISO, (0.08 and 0.07mm^2/g) than in the home-made juice (0.06 mm^2/g). This indicates that industrial juices were finer as compared with domestic ones. However these differences were significant ($p<0.05$) only for the pasteurized juices when compared with the home-made juices, indicating an adding effect of both process (extraction and pasteurization) on this parameter. To sum up the industrial processing reduces the particle size increasing the surface area and viscosity and, as a consequence, there is a visually appreciable change of color, as reported previously. The juices become brighter, more yellowish and colourful.

Carotenoids and color

The pigments responsible for OJs color are the carotenoids. We have deeply studied the carotenoid profile of commercially available OJs, including ultrafrozen, fresh squeeze and OJs from concentrate (Meléndez-Martínez et al., 2007; Meléndez-Martínez et al., 2008), concluding that the conditions of processing and storage do have an impact on their carotenoid profile. However other variables like variety and geographical origin should also be considered. As mentioned above, the home and industrials 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. The industrial processing (extraction and pasteurization) affected negatively ($p<0.05$) the content of carotenoids which ranged between 35 mg/L 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 (Meléndez-Martínez et al., 2007) xanthophylls predominated over carotene and the 5,6-epoxy carotenoids (violaxanthin, anteraxanthin and isomers) were the mayor ones followed by 5,8 epoxicarotenoids (luteoxanthin and mutatoxanthin), mono and dihidroxycarotenoids (zeaxanthin, β-cryptoxanthin and lutein) and finally β-carotene, zeinoxanthin and α-carotene. The individual profile of carotenoids was composed by violaxanthin (38-44%), antheraxanthin (12-13%), luteoxanthin (9-11%), zeaxanthin (8-9%) followed by mutatoxanthin (7-10%), β-cryptoxanthin (6-7%) and lutein (5%) and β-carotene, zeinoxanthin and α carotene which accounted for about 3%, 2.5% and 1% of the total carotenoid content. The levels of zeaxanthin were 1,7-fold higher relative to those of lutein in each type of juice, in agreement with observations by Dhuique-Mayer et al., (2005). Home made orange juices (HSO) resulted in a 15% higher content of carotenoids than the fresh industrial squeezed ones (FISO). Pasteurization reduced in 10 % the carotenoid content in relation to FISO and 23.5 % in relation to HSO ($p<0.05$). Lee et al. (2003) 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 (Lee et al., 2003; Sánchez-Moreno et al., 2005; Torres Gama et al., 2007). HSO showed the highest provitamin A activity, but due to the high standard deviation it was not significantly different from PISO. The epoxy-carotenoids, (9cis)-violaxanthin and all-(E)-antheraxanthin (peak 4), decreased ($p<0.05$) in the industrial 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-epoxyfuranoids (luteoxanthin, auroxanthin and mutatoxanthin) (Meléndez-Martínez et al., 2007). As explained elsewhere (Lee et al., 2003; Meléndez-Martínez et al., 2009; Rodriguez-Amaya, 1999), violaxanthin, is easily isomerized to luteoxanthin and afterwards to auroxanthin in an acid medium. The slight increase in acidy observed in PISO in relation to HSO (Table 1) could explain this fact.

In regard to the extraction methods, the levels of these compounds were slightly higher in FISO (3%, 1% and 11%, respectively) than in HSO. The eye-health related

carotenoids, zeaxanthin (peak 9) and lutein (peak 7), were not 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 home made.

In regard to the color differences detected between the three types of orange juices, we have previously reported that both, carotenoid content and structure influences the color of OJs (Meléndez-Martínez et al., 2007; Meléndez-Martínez et al., 2010). We also reported that the pigments mainly related to the qualitative color attribute, $h_{ab,}$ were zeinoxanthin, lutein and a mixture of violaxanthin isomers, whilst those mainly related to the quatitative attribute, $C^*_{ab,}$ were zeaxanthin, (9Z)- or (9′Z)-antheraxanthin and zeinoxanthin (Meléndez-Martínez et al., 2010).

In accordance, visually perceived colour differences were obtained between HSO and FISO and HSO and PISO (Table 1). In the first case the colour differences can only be attributable to significant differences in peak 4 (9-cis violaxanthin + antheraxanthin). Meanwhile, the visible color differences (Table 1) detected between HSO and PISO, can be related with significant differences ($p<0.05$) in the contents of the carotenoid several carotenoids as 9-cis violaxanthin + antheraxanthin, zeaxanthin, antheraxanthin and zeinoxanthin, which indicates a more complex effect of the thermal treatment. The isomerization of epoxycarotenoids, which occurs in the thermally treated orange juices, brought about colour changes discernible by the human eye (Meléndez-Martínez et al., 2009).

To sum up the extraction method affected ($p<0.05$) the color parameters, being the color differences between HSO and FISO visually perceived as stated above, but only the 9-cis violaxanthin + antheraxanthin content was affected by the industrial extraction process, thus confirming that the color differences could be more related to changes in particle size than to carotenoid composition. On the contrary the thermal treatment affected neither the color nor the content of OJ's carotenoids in comparison with the fresh industrial squeezed. When it comes to compare a home made juice (HSO) with a commercial one (PISO), we find significant differences in color and carotenoids. These differences could be related both to the decrease ($p<0.05$) in the levels of some carotenoids (violaxanthin isomers, 9 cis-violaxantin + antheraxanthin, zeaxanthin, antheraxanthin, zeinoxanthin) and total carotenoids content as well as to a reduction in the particle size.

Bioaccesibility 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 bioaccesibility. The processing of certain foods by mechanical homogenization and heat treatment have been reported to have a beneficial impact on this process (Yeum et al., 2002). We have evaluated bioccesibility by means of an in vitro model simmulating th Table 4 shows the levels of the bioactive carotenoids in the digests of the three types of OJs after the in vitro digestion. 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 in 39% ($p<0.05$). In this sense when comparing the HSO and the PISO, only a small decrease of 3% was observed. The industrial extraction increased ($p<0.05$) the level of the mixture of luteoxanthin and cis-antheraxanthin (peak 3), cis-luteoxanthin (peak5), zeaxanthin (peak 9) and antheraxanthin (peak10), with respect to the hand squeezed orange juice (59%, 58%, 33%, 45% respectively). It was noticed that neither violaxanthin and its isomers nor mutatoxanthin bioaccesibility were significantly affected by the extraction method or the pasteurization process. Also provitamin A carotenoids and consequently, the RAE values were not affected by the extraction method. Similarly, the pasteurization did not induced significant differences in the carotenoid provitamin A content, after digestion. FISO digest contained higher levels of α-□carotene than PISO, although RAE values in the digest were not affected by that difference between them. There was a clear decrease in the concentrations of lutein in the digest of PISO, nevertheless, these decrease was not statistically significant ($p > 0.05$).

Regardless of type of OJ, the bioactive carotenoids that showed the highest % of bioaccesibility w β-cryptoxanthin (34 – 56), followed by β-carotene (32 - 53), α-carotene (40-53), zeaxanthin (32 - 50) and lutein (32 - 48). The transfer efficiency from the food to the digest fraction is known to be related to the structure of the food matrix (Garrett et al., 1999). In the case of fruits, like oranges, the carotenoids occur as membrane-bound semicrystalline structures, which are a complex matrix for their extraction and analysis (Liu et al., 2004). In this sense the bioaccesibility, expressed as percentage of carotenoids in the digest in relation to the initial content in the OJ, improves with the industrial extraction. Thus we observed higher % of bioaccesibilty ($p<0.01$) in the industrial OJs in comparison with the home squeezed for Lutein (15%), zeaxanthin (18%), β-cryptoxanthin (22%), α-carotene (14%) β-carotene (21%).

On the other hand, the thermal treatment seems to have a negative impact in the bioaccesibility of provitamin A carotenoids, since lower values of bioaccesibility ($p < 0.05$) were observed for β-cryptoxanthin, α-carotene and β-carotene in comparison to FISO (Figure 3). However, when assessing both effects simultaneously (HSO *vs* PISO) no significant differences between the bioaccesibility of domestic *vs* industrial juices were observed.

As discuss above, the industrial extraction (squeezing and the homogenization) reduces the pulp particle size and enhances the carotenoid bioaccessibility. The higher specific surface area (Aw) in industrial juices in comparison with HSO and the decrease in the particle size enlarges the surface area available for the attack by digestive enzymes, thus increasing the overall digestion efficiency and the gastrointestinal absorption of nutrients , i.e. the extraction methods facilites transfer from the food matrix. The highest bioaccesibilities were obtained for the

Conclusion

Home and industrially extracted OJs are different in term of color and particle size, but not in terms of carotenoids content. The industrial pasteurization however, decreases the carotenoid content. When considering only the extraction method, the industrial extraction and homogenization reduces the particle size, improving the bioaccesibility of carotenoids. In fact, some carotenoids are more bioaccesible from the industrially extracted OJs than from the home made. Bioaccesibility of carotenoids from OJ is more related to the mechanical processing (extraction and homogenization) rather than to the thermal treatment, since pasteurization seems to reduce slightly the bioaccesibility of some carotenoids.

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Figure Legends

Figure 1. Location of the Hand Squeezed (HSO), Fresh Industrial Squeezed (FISO) and Pasteurized Industrial Squeezed in the a*b* plane.

Figure 2. Particle-size distribution in the Hand Squeezed (HSO), Fresh Industrial Squeezed (FISO) and Pasteurized Industrial Squeezed

Figure 3. Bioaccessibility in percentage of the bioactive carotenoids in the OJ samples (HSO: Hand Squeezed, FISO: Fresh Industrial Squeezed and PISO: Pasteurized Industrial Squeezed)