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Bioaccessibility, antioxidant activity and colour of carotenoids in ultrafrozen orange juices: Influence of thawing conditions

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ABSTRACT

Ultrafrozen orange juices are produced to preserve the nutritional and sensory quality of the fresh juices. However the effects of the thawing conditions on the nutritional quality have been scarcely studied. To gain insight into this subject we have assessed the impact of different thawing conditions (microwave, room and refrigeration temperature) on the carotenoids levels and bioaccessibility in ultrafrozen orange juices. Other related properties, such as colour, and antioxidant activity were also evaluated. The results demonstrated that the bioactive carotenoid content and the antioxidant activity were significantly affected by the microwave thawing. These juices showed the highest values for the % of relative bioaccessibility of provitamin A carotenoids, when compared to the other thawing conditions. On the other hand, thawing at room or refrigeration temperatures did not have a negative impact neither on the colour, provitamin A and macular carotenoid compounds nor in the antioxidant activity.

1. Introduction

Orange juice (OJ) is one of the juices most widely consumed worldwide. It is a good source of nutritionally important compounds such as carotenoids, vitamin C and flavonoids. The carotenoid profile of orange juices is one of the most intricate among fruits (Dugo et al., 2008; Meléndez-Martínez, Britton, Vicario, & Heredia, 2008). These compounds have been associated with a lower risk of degenerative diseases in humans. The health effects of carotenoids depend on the amount consumed and on their bioavailability, which can be estimated as bioaccessibility using in vitro digestions. A number of factors that affect the bioaccessibility/bioavailability are described in the literature, as the nature and contents of carotenoids, presence of fat and fiber, food matrix, nutrient status, genetics, and interactions among these variables, as well as the effect of the industrial processes (Ornelas-Paz, Failla, Yahia, & Gardea-Bejar, 2008).

To preserve the nutritional and sensory quality of the fresh OJ several preserving methods are used in the industry. Among the different kinds of commercial orange juices those undergoing pasteurization treatments and those obtained from concentrate are the most popular. In both cases, the juices are submitted to thermal treatments that elongate their shelf life but can affect negatively its vitamin activity, its flavour, aroma, and colour (Farnworth, Lagacé, Couture, Yaylayan, & Stewart, 2001; Lee & Coates, 2003; Lessin, Catignani, & Schwartz, 1997). Due to the growing demand of consumers for healthier and natural foods, new alternative technologies that meet the primary objective of ensuring the conservation of the product have been assayed such as high pressure (HPP) (Polydera, Stoforos, & Taoukis, 2003; Polydera, Stoforos, & Taoukisa, 2005), pulsed electric fields (PEF) (Cortés, Esteve, & Frígola, 2008; Cortés, Esteve, Rodrigo, Torregrosa, & Frígola, 2006), microwave energy (Cinquanta, Albanese, Cuccurullo, & Di Matteo, 2010; Villamiel, Castillo, San Martín, & Corzo, 1998) or ultrasound (Tiwari, Muthukumarappan, ÖDonnell, & Cullen, 2008), although the scaling-up of some of them at an industrial levels is not profitable. Freezing quickly at very low temperatures ensures the nutritional quality, as well as the flavour and texture of foods, due to formation of small and uniform ice crystals. Ultra-high freezing rates can be achieved using liquid nitrogen. Ultrafrozen orange juices (UFOJ), from the Valencia late variety, has deeper and more appealing colouration due to its higher content of carotenoid pigments, which is related to a higher quality (Meléndez-Martínez, Britton, Vicario, & Heredia, 2005; Meléndez-Martínez, Vicario, & Heredia, 2007). The amount of carotenoids is not affected by freezing, particularly quick freezing. However deteriorative process occurs, although at a

Abbreviations: FOJ fresh orange juice; Mw microwave; OJ orange juices; *R* refrigeration temperature; RAE retinol activity equivalent; *A* room temperature; TEAC Trolox Equivalent Antioxidant Capacity Assay; UFOJ ultrafrozen orange juice.

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very low rate, during storage (Thane & Reddy, 1997). Loss of nutritional value due to the freezing process has been extensively studied in fruits and vegetables, particularly in relation to vitamin C. According to Lee and Coates (1999) after 24 months of storage at −23 °C, frozen and un-pasteurized orange juice reduced 19.2% its vitamin C content.

Thawing is a complex process, involving heat transfer and possibilities of a series of physical and chemical changes which may greatly affect the quality of the product. Common thawing conditions are room temperature (*A*), refrigeration temperature (*R*) or microwave (Mw) thawing. The first method (*A*) allows a rapid thawing, but there is a risk of potential growth of pathogens if food temperatures rise into the danger zone. In the second method (*R*) the temperature of the food remains 'safe' so there is very little pathogen build-up. Disadvantages of this method are longer thawing times and the requirement of space in refrigerators. The last method (Mw) may be the fastest although localized overheating should be avoided as it can be detrimental, particularly because the product can suffer chemical deterioration. From this, it can be inferred that it is important to adjust the freezing–thawing variables in order to preserve and retain the nutritional and sensory quality of juices.

The aim of this work was to assess the impact of different thawing conditions (*A*, *R*, Mw) on the carotenoid content and bioavailability of UFOJ, other properties related to carotenoids such as antioxidant activity and colour were also evaluated.

2. Materials and methods

2.1. 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 Dlamond (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 semi synthesized in accordance to standard procedures as explained elsewhere (Meléndez-Martínez et al., 2007).

2.2. Samples

Orange juice samples were taken directly from the commercial production line at the firm "Zumos Pascual" (Palma del Rio, Cordoba, Spain) at different dates during the 2009 season (from May to August). Valencia late oranges in an appropriate stage of maturity, corresponding to a soluble solid content of 11–13 °Brix, were mechanically extracted with an FMC© in line Premium Juice Extractor (FMC Food Tech Citrus System, Lakeland, USA). The fresh industrial squeezed OJ samples (FOJ) were taken at this stage (six in total) and consisted of about 2 L.

Ultrafreezing was carried out by direct immersion in liquid nitrogen and posterior storage at −20 °C until analysis.

The thawing conditions used were: room temperature (*A*), refrigeration temperature (*R*) and microwave defrosting at maximum power (800 w), 20 s (Mw). These conditions were selected based on preliminary studies, in order to avoid overheating of the juice.

2.3. Colour 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 23 A (Tamron USA, Inc., Commack, NY, USA), and an external light source a white light 150 W-metal-halide lamp Phillips MHN-TD Pro (12,900 lumen, 4200 K colour temperature) 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 D_{65} and the 10° Observer were taken as references (CIE, 1978). The colour parameters of the uniform colour space CIELAB L^*; a^*; b^*; C^*_{ab} and h_{ab} were obtained directly from the apparatus. The colour data obtained were averages of three measurements. The colour 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 calculated using the formula:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

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

2.4. Titratable acidity and pH compound analysis

The titratable acidity expressed as citric acid was assessed by standard procedures. pH was measured with a GLP-21 GRISON pHmeter (Herisau, Switzerland). All analyses were made in triplicate.

2.5. Assessment of the in vitro antioxidant activity of lipophilic extracts by the TEAC method (Trolox Equivalent Antioxidant Capacity Assay)

The antioxidant activity of the lipophilic fraction of the OJs was assessed on aliquots of the same extracts used to determine carotenoids by HPLC.

The method used was based on the capture of the radical cation $ABTS^+$, generated in the reaction medium, compared to an antioxidant which produces the fall in absorbance at 734 nm (Re et al., 1999). The method measures the difference between the initial and the final absorbance due to the reduction experienced by the compound and can be used to assess the antioxidant capacity of the reducing substance.

The 2,2′-Azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) radical cation ($ABTS^+$) was produced by reacting a 7 mmol/l ABTS aqueous solution with 2.45 mmol/l potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm (30°). Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) was used as a standard for comparison for determination of the capacity of free radical scavenging. One mL of the ABTS radical solution was added to the cuvette and the absorbance was measured at time 0. Subsequently, 5 μL and 15 μL of the same extracts used to determine carotenoids by HPLC were added to the cuvette. It stirred and incubated at 30 °C. After 6 min, the absorbance was measured at 734 nm on an HP-8453 spectrophotometer equipped with temperature controller. The dose–response curve for Trolox consisted of plotting the absorbance at 734 nm as a percentage of the absorbance of the uninhibited radical cation (blank) and was based on triplicate determinations. The Trolox equivalent antioxidant activity (TEAC) was calculated by dividing the gradient of the curve of the sample and the gradient of the standard Trolox curve, taking into account the dilution used. The antioxidant activity of the lipophilic fraction was expressed in millimols of Trolox per L of orange juice.