Impact of minimal processing on orange bioactive compounds during refrigerated storage

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ABSTRACT

The effect of minimal processing on the health-related attributes of orange fruit was investigated. Oranges were prepared as whole fruits, hand-peeled fruits and manually separated segments, packed under air atmosphere and stored at 4 °C for 12 days. The stability of main bioactive compounds (carotenoids, flavanones and vitamin C) and antioxidant activity was evaluated. The total carotenoid content showed a significant increase for the whole samples during refrigerated storage, whereas no significant changes were observed for segments or peeled samples. A similar trend was found for vitamin A. With regard to vitamin C, at the end of refrigerated storage, some losses were observed although no significant differences were found among the different processed samples. The flavanone content showed a significant increase throughout refrigerated storage as response to cold stress. In general, the antioxidant activity remained stable in relation to the initial values. Hence, the health-related characteristics of minimally processed oranges were retained during refrigerated storage.

1. Introduction

Nowadays, consumers are more and more concerned about the impact of diet on health. As it has been shown, diets rich in fruits and vegetables and low in fat may prevent and reduce some chronic diseases (Dauchet, Amouyel, Hercberg, & Dallongeville, 2006; Mirmiran, Noori, Zavareh, & Azizi, 2009; Pitsavos et al., 2005). This protective effect has been mainly attributed to the high concentrations of bioactive compounds in fruits and vegetables, most of which possess antioxidant properties (García-Alonso, de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004; Wannamethee, Lowe, Rumley, Bruckdorfer, & Whincup, 2006). Among fruits, oranges are a good source of bioactive compounds, such as vitamin C, carotenoids or flavanones. It has been reported that the consumption of orange juice increases the vitamin C levels in plasma and reduces the markers of oxidative stress in humans (Sánchez-Moreno et al., 2003a). Also, De Pascual-Teresa et al. (2007) have reported that daily ingestion of minimally processed orange fruit increases the levels of hesperetin in plasma.

The consumption of ready-to-eat products has increased as a consequence of their convenience and freshness. Minimal process-ing of fruits involves the use of a combination of procedures, such as washing, peeling, and slicing or shredding that may cause an increase in respiration, biochemical changes and microbial spoilage and, therefore, detrimental effects on food quality (Allende, Tomás-Barberán, & Gil, 2006). To minimise or retard these effects and extend the shelf life, one of the most used methods is modified atmosphere packaging in combination with refrigerated storage (Del Caro, Piga, Vacca, & Agabbio, 2004; Habibunnisa, Baskaran, Prasad, & Shivaiah, 2001; Klaiber, Baur, Koblo, & Carle, 2005; Saxena, Bawa, & Raju, 2008). Oranges have appropriate morphological (structure in segments) and physiological (non-climacteric fruit) characteristics for the preparation of this kind of products. With regard to minimally processed oranges, most of the works have focused on the sensory and microbiological quality (Restuccia, Randazzo, & Caggia, 2006; Rocha, Brochado, Kirby, & Morais, 1995). For instance, Pretel, Fernández, Romojaro, and Martínez (1998) studied the effect of modified atmosphere packaging on the physical–chemical, microbiological and sensorial parameters of ready-to-eat oranges. However, knowledge on how minimal processing affects the nutritional quality and health-related properties of fruits becomes essential in order to satisfy the consumer requirements. Therefore, the aim of the present work was to study the influence of minimal processing on bioactive compounds (carotenoids, flavanones and vitamin C) and on the antioxidant activity of oranges during refrigerated storage.

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2. Materials and methods

2.1. Chemicals

Acetic acid was obtained from Hopkin & Williams (Essex, UK). L(+)-Ascorbic acid and *meta*-phosphoric acid were purchased from Merck (Darmstadt, Germany). Butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH·), DL-dithiothreitol, hesperetin and *trans*-β-apo-8′-carotenal were obtained from Sigma (St. Louis, MO, USA). Anhydrous sodium sulphate, chlorhydric acid 35%, citric acid monohydrate, *ortho*-phosphoric acid 85%, potassium hydroxide 85%, sodium chloride ($NaCl$) and sodium hydroxide ($NaOH$) 0.1 N were purchased from Panreac Química (Barcelona, Spain). Hoffman–La Roche (Basel, Switzerland) kindly provided β-carotene, β-cryptoxanthin, lutein and zeaxanthin. Sodium hypochlorite ($NaClO$) was purchased from Saeclor Sae (Madrid, Spain). Naringenin was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). Plate count agar (PCA) and ringer powder were purchased from Scharlau (Barcelona, Spain). Acetonitrile, dichloromethane, diethyl ether, methanol, sulphuric acid (H_2SO_4) and tetrahydrofuran (THF) were obtained from Labscan Ltd (Dublin, Ireland).

2.2. Plant material and processing

Oranges (*Citrus sinensis* L. Osbeck, cv. Navelina) (Valencia, Spain) were obtained from a local supermarket at commercial maturity. Fruits free of visual defects and uniform in colour and size were selected. Oranges were washed in sterile water (200 ppm $NaOCl$), rinsed and dried. Then, three batches of oranges were prepared: whole fruits, hand-peeled fruits and manually separated segments. Approximately 200 g of sample was placed on polystyrene trays, which were packaged in 20 × 30 cm plastic bags (AMCOR P-Plus premade bags, antimist coated oriented polypropylene film, AMCOR Flexibles Hispania S.A., Granollers, Barcelona, Spain) of medium oxygen permeability [5200 cm³/(m² 24 h bar) at 23 °C and 0% relative humidity (RH)] under air atmosphere and sealed by a Multivac vacuum machine (Wolferchweden, Germany). The headspace gas concentration of CO_2 (%) and O_2 (%) was stabilised at the second day of storage for peeled orange (19% O_2 and 1.60% CO_2) and orange segments (19% O_2 and 2.60% CO_2). Samples were stored at 4 °C in darkness for 12 days. The initial characteristics of orange fruit are shown in Table 1.

2.3. Colour, pH, titratable acidity, soluble and total solids, and microbiological analysis

Colour was measured using a tristimulus reflectance colorimeter (HunterLab, model D25 A9, Hunter Associates Laboratory, Inc.,

Table 1
Initial characteristics of orange fruit.

Characteristic	Value[a]
Titratable acidity (g citric acid/100 g fw[b])	0.67 ± 0.01
pH	3.55 ± 0.06
Soluble solids (°Brix at 20 °C)	10.62 ± 0.23
Total solids (g/100 g of fw)	10.60 ± 0.28
Firmness (N)	17.03 ± 3.95
Total microbial counts (log CFU/g fw)	1.45 ± 0.36
CIELab colour parameters	
L^*	27.07 ± 0.77
a^*	−4.30 ± 0.10
b^*	8.01 ± 0.82
$h[(\tan^{-1}(b^*/a^*))]$	61.62 ± 2.20
$C[(a^{*2} + b^{*2})^{1/2}]$	9.10 ± 0.75

[a] Values are the mean of three independent determinations ± standard deviation.
[b] fw, Fresh weight.

Reston, VA, USA), as described by Plaza et al. (2006). For pH and titratable acidity, 10 g of sample was blended with 20 ml deionised water in an ultrahomogeniser (Omni mixer, model ES-207, Omni International Inc., Gainesville, VA, USA). The mixture was heated to 100 °C, then 20 ml deionised water was added and the resulting mixture was cooled to 20 °C. The pH was measured at this temperature with a pH meter (Microph 2000, Crison, Barcelona, Spain). After the determination of pH, the solution was titrated with 0.1 N NaOH to pH 8.1 and the results were expressed as percentage of citric acid (grams of citric acid per 100 g fresh weight). The soluble solids were determined using a digital refractometer (ATAGO, Tokyo, Japan) at 20 °C and the results were reported as degrees Brix. The total solids were measured as described in Sánchez-Moreno, Plaza, De Ancos, and Cano (2003b), and the results expressed as grams of total solids per 100 g fresh weight. Firmness was determined using a texture analyser (Instron, model 1140, UK) and was expressed as maximum strength (N). For microbiological analysis, sample (25 g) was aseptically homogenised with 250 ml Ringer's powder solution for 2 min. From this, serial decimal dilutions were prepared with the same solvent, and 1 ml aliquots were inoculated. Dilutions of the samples were plated in duplicate for counting of the total aerobic mesophilic bacteria on PCA and incubated at 30 °C for 72 h. Results were reported as log colony-forming units (CFU) per gram of fresh weight.

2.4. Carotenoid analysis

Extraction, separation and identification of carotenoids were carried out according to a previous method with minor modifications (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003c). Triplicates of each sample (30 g) were extracted with 50 ml of THF stabilised with 0.01% BHT until the extracts became colourless. One millilitre of a solution of *trans*-β-apo-8′-carotenal (1 mg/ml), as internal standard, was added. The combined THF extracts were concentrated on a rotatory evaporator at 35 °C and partitioned between diethyl ether and a saturated solution of sodium chloride in water, and transferred to a separating funnel. The organic and aqueous layers were separated and the extraction process was repeated twice. The organic layers were combined and dried over anhydrous sodium sulfate. The ethereal solution was concentrated to approximately 30 ml. For saponification, 30% methanolic potassium hydroxide was added to the extract and the mixture was left under nitrogen atmosphere in darkness overnight. Then, the reaction mixture was repeatedly extracted with water until the neutralisation of the pH. The organic layer was dried over anhydrous sodium sulfate. The ethereal solution was evaporated to dryness and the residue dissolved in 2 ml of dichloromethane. All steps were performed under diminished light. Samples were filtered through a 0.45-μm membrane filter and duplicates of 20 μl for each extract were analysed by HPLC.

The HPLC system consisted of a Hewlett-Packard (Palo Alto, CA, USA) model 1050 quaternary solvent delivery pump, equipped with an autosampler (G1329A ALS) and a Hewlett-Packard 1040A rapid scanning UV–visible photodiode array detector. The separation of carotenoids was performed on a reverse phase C18 Hypersil ODS stainless steel column (250 mm × 4.6 mm, 5 μm) (Technochroma, Barcelona, Spain). The solvent system used was a gradient of methanol/water (75:25) (solution A) and acetonitrile/dichloromethane/methanol (70:5:25) (solution B). The flow rate was fixed at 1 ml/min and the runs were monitored with the UV–visible photodiode array detector set at 450 nm. Data were stored and processed using a Hewlett-Packard Chem Station and related software. Identification of the carotenoids was carried out by HPLC by comparing the retention time and UV–visible absorption spectrum with those of the standards previously referred to. Quantification was achieved according to the procedure described by

Hart and Scott (1995). Results were expressed as micrograms of the corresponding carotenoid per 100 g of fresh weight.

The vitamin A value was given as the retinol activity equivalents (RAE) per 100 g of fresh weight, according to the equation RAE = [μg of β-carotene/12] + [μg of other pro-vitamin A carotenoids (β- and α-cryptoxanthin, and α-carotene)/24] (Institute of Medicine, Food and Nutrition Board, 2001).

2.5. Vitamin C analysis

Ascorbic acid and total vitamin C (ascorbic acid plus dehydroascorbic acid) were determined by HPLC as described previously by our group (Sánchez-Moreno et al., 2003c). The procedure employed to determine total vitamin C was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as a reducing reagent, according to a modification of the procedure of other authors (Sánchez-Mata, Cámara-Hurtado, Díez-Marqués, & Torija-Isasa, 2000). Sample (15 g) was homogenised with 40 ml of extraction solution (3% meta-phosphoric acid plus 8% acetic acid). The resulting mixture was centrifuged, filtered and adjusted up to 100 ml with distilled water. Samples were filtered through a 0.45-μm membrane filter and duplicates of 20 μl for each extract were analysed by HPLC. The results were expressed as milligrams of ascorbic acid per 100 g of fresh weight.

An aliquot (0.5 ml) of the mixture was taken to react with 3 ml of a water solution containing 20 mg/ml DL-dithiothreitol for 2 h at room temperature and in darkness. During this time, the reduction of dehydroascorbic acid to ascorbic acid took place. Samples were filtered through a 0.45-μm membrane filter and duplicates of 20 μl for each extract were analysed by HPLC. The results were expressed as milligrams of total vitamin C per 100 g of fresh weight.

The separation of ascorbic acid was performed by HPLC using a reversed-phase C 18 Hypersil ODS stainless steel column (250 mm × 4.6 mm, 5 μm) (Technochroma, Barcelona, Spain). The solvent system used was an isocratic elution of a solution 0.01% H_2SO_4, adjusted to pH 2.5–2.6. The flow rate was fixed at 1 ml/min and runs were monitored at 245 nm. The identification of the ascorbic acid was carried out by HPLC by comparing the retention time and UV–visible absorption spectrum with those of the standard previously referred to. Quantification was carried out by the external standard method.

2.6. Flavanone analysis

The extraction of flavanones was based on the procedure of Sánchez-Moreno et al. (2003b). Sample (5 g) was homogenised with 15 ml 80% aqueous methanol and centrifuged. An aliquot of this extract was used for subsequent flavanone aglycons determination after acid hydrolysis. To 800 μl methanolic extract, 200 μl of 6 M HCl was added. Hydrolysis was carried out by refluxing on a water bath at 90 °C for 30 min. After hydrolysis, the sample was cooled and filtered through a 0.45-μm membrane filter and duplicates of 50 μl for each extract were analysed by HPLC.

Separation of flavanones was performed by HPLC using a reversed-phase Luna C18 (2) stainless steel column (150 mm × 4.6 mm, 5 μm) (Phenomenex, Torrance, CA, USA). The mobile phase used was deionised Milli-Q water adjusted to a pH 2.5 with 50 mM solution of ortho-phosphoric acid 85% (solution A) and acetonitrile (solution B). The gradient elution employed was the following: 0 min, 10% B; 5 min, 10% B; 12 min, 35% B; 20 min, 60% B; 27 min, 65% B; 30 min, 90% B; 35 min, 90% B; 40 min, 10% B. The flow rate was fixed at 0.5 ml/min and runs were monitored at 284 nm. Identification of the flavanones was carried out by HPLC by comparing the retention times and UV–visible absorption spectrum with those of the hesperetin and naringenin standards. Quantification of the flavanones was achieved by the absorbance

recorded in the chromatograms relative to the external standards of flavanones previously referred to. Results were expressed as milligrams of flavanone per 100 g of fresh weight.

2.7. DPPH· radical scavenging capacity

Triplicate aliquots of each sample (15 g) were extracted with 40 ml of a methanol:water (1:1) solution and centrifuged at 10 000×g for 10 min at 4 °C. The extraction was repeated twice. Supernatants were combined to yield an aqueous fraction. The free radical scavenging capacity was evaluated with the DPPH· stable radical. 0.1 ml of the diluted methanolic extract of sample was added to 3.9 ml of DPPH (0.030 g l⁻¹) in methanol. The method is described extensively elsewhere (Sánchez-Moreno et al., 2003b). The parameters EC_{50}, which reflect 50% depletion of the initial DPPH· radical, and the time needed to reach the steady state at EC_{50} concentration (t_{EC50}) were calculated. The antiradical efficiency (AE = 1/[$EC_{50} \times t_{EC50}$]), a parameter that combines both factors, was also calculated.

2.8. Statistical analysis

Results were given as mean ± standard deviation of three independent determinations. Significant differences between results were calculated by one-way analysis of variance (ANOVA). Differences were considered to be significant at $P < 0.05$ (95% confidence level). All statistical analyses were performed with Statgraphics Plus 5.1 (Statistical Graphics Corporation, Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Microbiological quality

The initial total mesophilic bacterial counts in minimally processed orange products (peeled and segments) were very low, i.e. 1.45 ± 0.36 log CFU/g. After 12 days at 4 °C, the population of aerobic bacterial counts was below 2 log CFU/g. The hygienic practices and technological procedures (packaging and refrigerated storage) appear effective to maintain the microbiological quality of these products during 12 days.

3.2. Carotenoid content

The main carotenoids with antioxidant and/or pro-vitamin A properties present in the saponified extract of oranges were α- and β-cryptoxanthin, zeaxanthin, lutein, and α- and β-carotene. Table 2 shows the carotenoid content of the minimally processed oranges during refrigerated storage at 4 °C. Lutein, β-cryptoxanthin and zeaxanthin were the major carotenoids, representing 39%, 27%, and 20%, respectively, of total carotenoids. This composition was similar to that reported by other authors for orange juice and pulp (Alquezar, Rodrigo, & Zacarías, 2008; Gama & Sylos, 2005).

During refrigerated storage at 4 °C, there were no significant changes in the total carotenoid content for segments and peeled samples, whereas the whole samples showed a significant increase. In general, the same pattern was observed for individual carotenoids. At the end of cold storage, the only individual carotenoid that presented a significant increase for all processed samples was β-carotene, whole samples showed the highest value. In the same way, related with their higher pro-vitamin A carotenoid content, whole samples showed the highest vitamin A content throughout the 12 days of cold storage (Fig. 1). Also, Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, and Martín-Belloso (2009) reported an increase in the β-carotene content of fresh-cut mango during refrigerated storage at 5 °C. It is well

Table 2
Carotenoid content of minimally processed oranges during storage at 4 °C (µg/100 g fresh weight).[a]

Sample	Storage days	Lutein	Zeaxanthin	α-Criptoxanthin (as β-criptoxanthin)	β-Criptoxanthin	α-Carotene (as β-Carotene)	β-Carotene	Total carotenoids
Segments	0	56.7 ± 5.89 a	28.7 ± 3.89 a	6.53 ± 0.34 a	39.1 ± 1.21 a	4.47 ± 0.14 a	7.32 ± 0.12 a	143 ± 11.6 a
	2	45.5 ± 4.90 aA	23.1 ± 2.20 aA	4.61 ± 0.56 aA	30.1 ± 8.50 aA	4.19 ± 0.60 aA	5.40 ± 0.17 aA	113 ± 16.9 aA
	5	58.1 ± 22.4 aA	34.0 ± 16.2 aA	4.95 ± 1.54 aA	62.4 ± 28.9 aA	3.96 ± 0.98 aA	6.89 ± 3.00 aA	170 ± 73.1 aA
	8	62.7 ± 3.69 aA	29.0 ± 2.80 aA	6.85 ± 0.68 abA	57.8 ± 15.4 aA	4.93 ± 0.07 abA	9.41 ± 2.33 abA	171 ± 25.0 aA
	12	58.2 ± 18.3 aA	30.8 ± 9.22 aA	9.75 ± 3.52 bA	62.1 ± 24.5 aAB	7.17 ± 2.50 bA	11.5 ± 3.29 bAB	180 ± 61.3 aA
Peeled	0	56.7 ± 5.89 ab	28.7 ± 3.89 a	6.53 ± 0.34 a	39.1 ± 1.21 a	4.47 ± 0.14 a	7.32 ± 0.12 a	143 ± 11.6 a
	2	50.8 ± 0.29 aAB	26.7 ± 2.39 aA	6.08 ± 1.00 aA	31.0 ± 8.00 aA	4.83 ± 0.32 abA	6.13 ± 0.28 aB	126 ± 12.3 aAB
	5	65.9 ± 8.91 abA	36.6 ± 5.40 aA	6.74 ± 0.41 aAB	58.9 ± 34.8 aA	4.46 ± 0.27 aAB	7.15 ± 0.19 aA	180 ± 50.0 aA
	8	77.7 ± 24.1 bA	34.9 ± 16.3 aA	8.01 ± 2.05 aA	44.7 ± 14.2 aA	5.72 ± 0.81 bA	9.22 ± 1.85 bA	180 ± 59.3 aA
	12	45.7 ± 10.3 aA	30.6 ± 5.23 aA	6.67 ± 1.12 aA	45.3 ± 2.50 aA	5.26 ± 0.94 abA	10.1 ± 0.88 bA	144 ± 21.0 aA
Whole	0	56.7 ± 5.89 a	28.7 ± 3.89 ab	6.53 ± 0.34 ab	39.1 ± 1.21 a	4.47 ± 0.14 a	7.32 ± 0.12 a	143 ± 11.6 a
	2	56.2 ± 1.70 aB	25.8 ± 2.11 aA	4.96 ± 1.40 aA	54.3 ± 14.3 bB	4.64 ± 0.56 aA	7.22 ± 0.03 aC	153 ± 20.1 aB
	5	78.5 ± 4.50 bA	38.1 ± 2.65 bA	8.11 ± 0.22 bcB	58.4 ± 1.14 bA	5.64 ± 0.60 abB	9.09 ± 0.15 abA	198 ± 9.26 bA
	8	58.1 ± 12.9 aA	30.4 ± 4.22 abA	9.44 ± 1.51 cA	102 ± 3.18 cB	6.38 ± 1.69 bA	12.4 ± 3.09 bA	218 ± 26.6 bA
	12	83.3 ± 2.03 bB	51.3 ± 9.83 cB	15.6 ± 0.80 dB	91.2 ± 5.77 cB	11.39 ± 0.46 cB	16.6 ± 3.00 cB	269 ± 21.9 cB

[a] Values are the mean of three independent determinations ± standard deviation. Different lower case letters in the same carotenoid column and treatment type indicate significant differences ($P < 0.05$). Different capital letters in the same carotenoid column and day of storage indicate significant differences ($P < 0.05$).
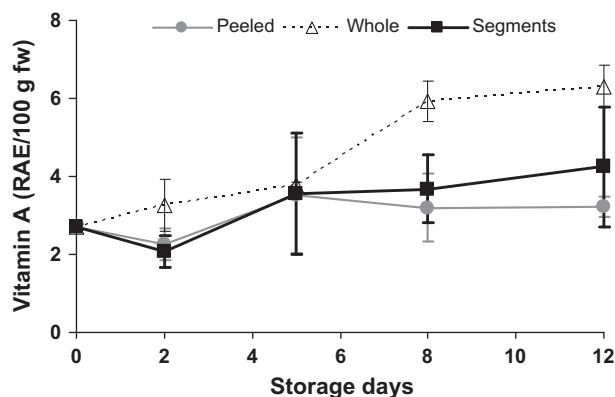

Fig. 1. Vitamin A content of minimally processed oranges during storage at 4 °C (RAE/100 g fresh weight). RAE, retinol activity equivalents.

known that maturation leads to the accumulation of carotenoids in the citrus fruit (Kato et al., 2004). After harvesting, the process of induction and accumulation of these compounds could continue during refrigerated storage, depending on the temperature applied (Carmona, Zacarías, & Rodrigo, 2007). In addition, the effect of storage temperature will depend on the maturity stage of the fruit at the moment of harvesting. In the present study, the higher carotenoid content that was observed in the whole samples, compared to the segments or peeled samples, seems to be related to a lower susceptibility of carotenoids to oxidation in the whole fruit.

3.3. Vitamin C content

The ascorbic acid and total vitamin C (ascorbic acid plus dehydroascorbic acid) contents in minimally processed oranges during refrigerated storage at 4 °C are shown in Table 3. With regard to the initial ascorbic acid and total vitamin C values (42.32 and 43.57 mg/100 g fresh weight, respectively), there was a significant decrease throughout the 12 days of cold storage for all samples (19–24% for ascorbic acid and 15–23% for total vitamin C). These results are in agreement with those reported by other authors. For instance, after 6 days at 5 °C, Gil, Aguayo, and Kader (2006) found vitamin C losses of 5% in mango, strawberry and watermelon pieces, 10% in pineapple pieces, 12% in kiwi slices and 25% in can-

Table 3
Vitamin C content of minimally processed oranges during storage at 4 °C (mg/100 g fresh weight).[a]

Sample	Storage days	L-Ascorbic acid	Total vitamin C
Segments	0	42.3 ± 1.27 c	43.6 ± 1.85 c
	2	40.4 ± 0.53 cA	42.7 ± 0.22 cB
	5	37.2 ± 0.11 bB	38.5 ± 1.35 bA
	8	36.2 ± 1.46 bA	38.3 ± 2.23 bAB
	12	32.0 ± 1.41 aA	35.2 ± 1.06 aA
Peeled	0	42.3 ± 1.27 c	43.6 ± 1.85 c
	2	37.6 ± 0.07 bcA	39.0 ± 0.20 bcAB
	5	35.8 ± 2.33 abAB	38.4 ± 2.26 abcA
	8	33.1 ± 1.30 abA	33.8 ± 0.91 abA
	12	32.0 ± 5.99 aA	33.5 ± 5.81 aA
Whole	0	42.3 ± 1.27 b	43.6 ± 1.85 b
	2	37.0 ± 3.29 abA	38.7 ± 3.46 abA
	5	32.9 ± 2.79 aA	35.7 ± 3.21 aA
	8	36.4 ± 6.93 abA	40.4 ± 3.59 abB
	12	34.0 ± 0.14 aA	36.8 ± 0.74 aA

[a] Values are the mean of three independent determinations ± standard deviation. Different lower case letters in the same compound and treatment type indicate significant differences ($P < 0.05$). Different capital letters in the same compound and day of storage indicate significant differences ($P < 0.05$).

taloupe cubes. Also, Del Caro et al. (2004) reported vitamin C losses in minimally processed citrus fruits during cold storage. Ascorbic acid is usually degraded by oxidative processes, which are stimulated in the presence of light, oxygen, heat, peroxides and enzymes, such as ascorbate oxidase or peroxidase (Plaza et al., 2006). On the other hand, at the end of refrigerated storage, there were not significant differences among the minimally processed samples studied in the present work (whole, peeled or segments). In the same way, Wright and Kader (1997) reported no significant losses in the vitamin C content in sliced strawberries compared to the intact fruit after 7 days at 5 °C.

3.4. Flavanone content

Orange is a good source of flavanone glycosides, such as narirutin and hesperidin. Table 4 shows the flavanone content, after the hydrolysis of flavanone glycosides (narirutin and hesperidin) to their corresponding aglycons (naringenin and hesperetin, respectively), in minimally processed oranges during refrigerated storage

Table 4
Flavanone content of minimally processed oranges during storage at 4 °C (mg/100 g fresh weight).[a]

Sample	Storage days	Naringenin	Hesperetin	Total flavanones
Segments	0	1.57 ± 0.07 ab	3.52 ± 0.58 a	5.09 ± 0.65 a
	2	1.69 ± 0.07 abB	4.13 ± 0.83 abA	5.82 ± 0.90 aA
	5	1.92 ± 0.41 bA	5.15 ± 0.00 cdB	7.07 ± 0.41 bB
	8	1.28 ± 0.17 aA	4.55 ± 0.10 bcA	5.83 ± 0.27 aA
	12	1.50 ± 0.44 abA	5.48 ± 0.20 dA	6.98 ± 0.64 bA
Peeled	0	1.57 ± 0.07 ab	3.52 ± 0.58 a	5.09 ± 0.65 a
	2	1.16 ± 0.03 aA	4.02 ± 0.28 abA	5.18 ± 0.31 aA
	5	1.80 ± 0.65 bA	4.86 ± 0.13 cB	6.66 ± 0.78 bAB
	8	1.39 ± 0.11 abA	4.63 ± 0.51 bcA	6.02 ± 0.62 abA
	12	1.35 ± 0.10 abA	4.82 ± 0.46 cA	6.17 ± 0.56 abA
Whole	0	1.57 ± 0.07 c	3.52 ± 0.58 a	5.09 ± 0.65 a
	2	1.11 ± 0.08 aA	3.40 ± 0.79 aA	4.51 ± 0.87 aA
	5	1.46 ± 0.27 bcA	3.77 ± 0.88 aA	5.23 ± 1.15 aA
	8	1.19 ± 0.15 abA	3.65 ± 1.21 aA	4.84 ± 1.36 aA
	12	1.60 ± 0.17 cA	6.32 ± 1.37 bA	7.92 ± 1.54 bA

[a] Values are the mean of three independent determinations ± standard deviation. Different lower case letters in the same flavanone and treatment type indicate significant differences ($P < 0.05$). Different capital letters in the same flavanone and day of storage indicate significant differences ($P < 0.05$).

Table 5
Radical-scavenging parameters of minimally processed oranges during storage at 4 °C.[a]

Sample	Storage days	EC_{50} (g fresh weight/ g DPPH·)	t_{EC50} (min)	$AE^{b} \times 10^{-3}$
Segments	0	488 ± 4.24 a	1.67 ± 0.16 a	1.23 ± 0.13 b
	2	485 ± 52.6 aA	1.92 ± 0.01 bA	1.07 ± 0.10 abA
	5	497 ± 69.4 aA	1.86 ± 0.10 bA	1.08 ± 0.11 abA
	8	499 ± 73.8 aA	1.94 ± 0.07 bA	1.03 ± 0.09 aA
	12	513 ± 43.0 aA	1.84 ± 0.10 abA	1.06 ± 0.04 abA
Peeled	0	488 ± 4.24 ab	1.67 ± 0.16 a	1.23 ± 0.13 bc
	2	414 ± 76.3 aA	1.95 ± 0.33 abA	1.24 ± 0.13 cA
	5	417 ± 68.0 aA	2.07 ± 0.01 bB	1.16 ± 0.06 abcA
	8	549 ± 21.5 bA	1.81 ± 0.18 abA	1.01 ± 0.10 aA
	12	574 ± 79.1 bA	1.69 ± 0.05 aA	1.03 ± 0.12 abA
Whole	0	488 ± 4.24 a	1.67 ± 0.16 a	1.23 ± 0.13 b
	2	496 ± 38.1 aA	1.72 ± 0.09 aA	1.17 ± 0.11 abA
	5	492 ± 40.8 aA	1.95 ± 0.06 bAB	1.04 ± 0.07 aA
	8	472 ± 17.7 aA	1.99 ± 0.01 bA	1.06 ± 0.03 abA
	12	518 ± 15.0 aA	1.84 ± 0.12 abA	1.05 ± 0.12 abA

[a] Values are the mean of three independent determinations ± standard deviation. Different lower case letters in the same parameter and treatment type indicate significant differences ($P < 0.05$). Different capital letters in the same parameter and day of storage indicate significant differences ($P < 0.05$).
[b] AE expressed as $1/[EC_{50}$ (g fresh weight/g DPPH·) $\times t_{EC50}$ (min)].

at 4 °C. The initial naringenin and hesperetin values (1.57 and 3.52 mg/100 g fresh weight, respectively) were in accordance with those found in the literature for oranges (Peterson et al., 2006).

In general, there were no significant changes in the naringenin content for the minimally processed oranges during the 12 days of cold storage at 4 °C. However, at the end of refrigerated storage, the hesperetin content showed a significant increase and, consequently the total flavanone content (37.1%, 21.2%, and 55.6% for segments, peeled and whole samples, respectively). No significant differences were found among the samples after 12 days of refrigerated storage. Several studies have reported a positive relationship between the accumulation of phenolic compounds during refrigerated storage and phenylalanine ammonia-lyase (PAL) activity (Benkeblia, 2000; Lo Piero, Puglisi, Rapisarda, & Petrone, 2005). PAL is a key enzyme in the phenolic metabolism, being involved in the phenylpropanoid biosynthesis pathway, which has been reported to protect plants against stress conditions. Klaiber et al. (2005) reported an increase on PAL activity and phenolic content in minimally processed carrots throughout refrigerated storage under aerobic conditions, but not in anaerobic ones. Moreover, increased PAL activity has been reported to occur in citrus as a response to cold stress (Lafuente, Zacarías, Martínez-Téllez, Sánchez-Ballesta, & Granell, 2003). Lo Piero et al. (2005) observed that a low temperature induced the anthocyanins accumulation in red orange vesicles after 75 days, reaching values eight times higher than those at 25 °C. In addition, they reported that the expression of PAL was strongly induced during low temperature exposure. Hence, the increase in flavanone content observed in our work appears to be related to a response to the stress conditions.

3.5. Antioxidant activity

The DPPH· stable radical scavenging activity was evaluated in minimally processed oranges during refrigerated storage at 4 °C. Values of radical-scavenging parameters are shown in Table 5. Initial EC_{50}, t_{EC50} and AE for orange extract were 487.73 g fresh weight/g DPPH·, 1.67 min and 1.23×10^{-3} 1/[(g fresh weight/g DPPH·) × min], respectively. These values were in agreement with those reported for orange juice (Kelebek, Selli, Canbas, & Cabaroglu, 2009; Sánchez-Moreno et al., 2003c). Leong and Shui (2002) tested

the antioxidant capacity of 27 fruit pulps, reporting that oranges presented medium activity.

At the end of refrigerated storage, the antioxidant activity remained stable with regard to the initial values in each sample. In addition, there were no significant changes among samples (whole, peeled, and segments). In the same way, other studies have reported that the antioxidant activity remains relatively constant during cold storage of minimally processed products (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008; Piga, Del Caro, Pinna, & Agabbio, 2003). Thus, the antioxidant activity will depend on the impact of the processing and storage conditions on the different antioxidant compounds present in oranges. In a previous study of our group, we found that vitamin C is the major contributor to antioxidant activity in commercial orange juices (Sánchez-Moreno et al., 2003b). In fact, carrying out regression analyses, the antiradical efficiency (AE) showed a statistically significant correlation with ascorbic acid ($r = 0.6441$, $P = 0.0175$) and total vitamin C ($r = 0.5821$, $P = 0.0369$). However, in addition to vitamin C, it is necessary to take into account the possible synergistic effect of other phytochemicals, the effect of which will depend on their structure, interaction mode, and concentration.

4. Conclusions

Bioactive compounds (carotenoids and flavanones) were retained in minimally processed oranges during refrigerated storage. Although some vitamin C losses were observed, the antioxidant activity remained stable. Overall, the microbiological quality and potentially health-promoting attributes of minimally processed oranges were preserved during 12 days of storage at 4 °C. Further research is needed to provide more insight in the impact of minimal processing on the orange bioactive compounds and their bioavailability.

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