Effect of High-Pressure Treatment on the Carotenoid Composition and the Radical Scavenging Activity of Persimmon Fruit Purees

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The carotenoid composition of persimmon fruit purees of two cultivars, cvs. Rojo Brillante and Sharon, grown in Spain was determined by HPLC to assess the effects of high-pressure processing on some sensory (carotenoids), nutritional (provitamin A value), and health-related (radical-scavenging capacity) parameters. Total carotenoid content was higher in untreated Rojo Brillante puree (22.11 μg g^{-1}) than in untreated Sharon puree (15.22 μg g^{-1}). Purees of both untreated cultivars showed similar carotenoid patterns after saponification with β-cryptoxanthin, β-carotene, and zeaxanthin as the main pigments. A high content of lycopene was quantified in Rojo Brillante (5.34 μg g^{-1}), whereas only traces were detected in Sharon. The provitamin A value, reported as retinol equivalents (RE), was in untreated Rojo Brillante puree (77 RE/100 g) similar to that of Sharon (75 RE/100 g). Scavenging free radical capacity, measured as antiradical efficiency (AE), showed in untreated Rojo Brillante puree a value (12.14×10^{-3}) 8.5 times higher than that in untreated Sharon (1.42×10^{-3}). Nonuniform behavior of high-pressure treatment was detected. Pressure treatments at 50 and 300 MPa/15 min/25 °C for Rojo Brillante and at 50 and 400 MPa/15 min/25 °C for Sharon increased the amount of extractable carotenoids (9−27%), which are related with the increase of vitamin A value (75−87 RE/100 g). No correlation with the increase of AE (from 1.42×10^{-3} to 16.73×10^{-3} and 19.58×10^{-3}) after some pressure treatments (150 and 300 MPa/15 min/25 °C) was found.

Keywords: *Persimmon; carotenoids; HPLC; high pressure; processing effects; radical-scavenging capacity; vitamin A value; cultivar differences*

INTRODUCTION

Persimmon fruits (*Diospyros kaki*) not only have nutritional relevance due to their high provitamin A carotenoid content (β-carotene and β-cryptoxanthin) (Daood et al., 1992; Homnava et al., 1990) but also are rich sources of dietary carotenoids (β-carotene, β-cryptoxanthin, zeaxanthin, lutein, and lycopene), which have been implicated in the reduction of degenerative human disease (Block et al., 1992; Van Poppel, 1993; Giovannucci et al., 1995; Steinmetz and Potter, 1996) due to their antioxidant and free-radical scavenging properties (Boileau et al., 1999).

Persimmon fruits show significant differences among varieties in terms of color and astringency (Ito, 1980). This fruit must be consumed completly ripe to avoid the astringent taste and, because of this, large production losses take place due to handling of overly soft products. Now, the selection of suitable cultivars and the use of ethylene, carbon dioxide, and other chemicals remove the astringency while the fruit is still unripe (Ito, 1980; Palmer-Wright and Kader, 1997). These treatments can produce undesired changes of the fruits characteristic yellow-orange color, due to the degradation of the carotenoid pattern.

Persimmon fruits are traditionally consumed in Japan and in other Asiatic countries. In the Mediterranean regions of Spain, persimmon fruit is an emergent crop that is increasing in production. The future of this crop depends on (a) the selection of high-quality cultivars that can hold up to technological treatments without color loss and (b) the development of new processing technologies that not only ensure the sensory and nutritional quality of persimmon products but also preserve or indeed increase the bioavalibility of the compounds with antioxidant capacity present in this fruit.

In this framework, application of high pressure as a new nonthermal preservation technology is being investigated as an alternative or complementary process to thermal treatment (sterilization, pasteurization, and blanching) (Farr, 1990; Mertens and Knorr, 1992; Cheftel, 1992; Knorr, 1995). High-pressure treatment results in good preservation levels, diminishing microorganism and enzyme activity, and minimally altered small molecules as volatile compounds, pigments, vitamins, and other compounds related with sensory, nutritional, and health-related qualities of the product (Knorr, 1993). There are many bibliographic references about the effect of high-pressure treatment, applied alone or combined with low temperature, on food enzyme activity [polyphenol oxidase (PPO), peroxidase (POD), lipoxygenase (LOX), and pectin methylesterase (PME)] (Seyderhelm et al., 1996; Hendrickx et al., 1998; Cano et al., 1997; Hernández and Cano, 1998) and microorganism (Smelt, 1998) and protein funtionality (Messens et al., 1997), but there is little information about the effect of high-pressure treatment on pigments and on compounds with nutritional and/or antioxidant capacity, in

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Table 1. Physicochemical Characteristics of Persimmon Fruit Cultivars before Pressurization

characteristic[a]	cv. Sharon	cv. Rojo Brillante
titratable acidity (g of citric acid/100 g of fw)	0.14 ± 0.04	0.12 ± 0.05
pH	5.31 ± 0.01	5.02 ± 0.02
soluble solids (°Brix at 20 °C)	14.50 ± 0.08	16.13 ± 0.17
moisture content (%)	82.88 ± 0.04	78.85 ± 1.93

[a] Values are average ± SD of three independent determinations.

model or in real food systems (Quaglia et al., 1996; Donsì et al., 1996; Van Loey et al., 1998; Van den Broeck et al., 1998).

The objective of this research work was to separate, identify, and quantify by HPLC the main carotenoids with radical-scavenging capacity of the saponified extracts of the flesh of two persimmon cultivars growing in Spain, cv. Sharon and cv. Rojo Brillante. This will be a useful tool to quantify sensory and nutritional losses during persimmon fruit processing in order to select the suitable variety according to losses obtained. Second, the effect of high-presssure treatment on the qualitative and quantitative carotenoid composition of both persimmon cultivars was studied, with special attention to the carotenoid compounds with radical-scavenging capacity (β-carotene, lycopene, β-cryptoxanthin, and zeaxanthin) with the aim of getting an excellent preserved product with its sensory and nutritional quality and its radical-scavenging capacity unchanged.

MATERIALS AND METHODS

Reagents and Material. Persimmon fruits, cvs. Sharon and Rojo Brillante, were harvested at commercial ripe stage in Massagrell, Valencia (Spain), and brought to Instituto del Frío, Madrid (Spain), in the next 12 h. The physicochemical characteristics of both persimmon cultivars are shown in Table 1.

The peel of ~40 fruits of each cultivar were removed and pooled, and the pulp was homogenized using a blender (Osterizer, Proctor-Silex, Inc.). The puree obtained was used for pigment analysis of untreated and high-pressure-treated samples, before and after saponification.

β-Carotene, lycopene, β-cryptoxanthin, zeaxanthin, and lutein standards were kindly provided by Hoffman-La Roche (Basel, Switzerland). HPLC grade solvents, methanol, ethyl acetate, and tetrahydrofuran (Lab Scan) were used without further purification. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma-Aldrich Química, Madrid, Spain) was used for antioxidant capacity measurements of the persimmon fruits extracts. The dye Sudan I was also obtained from Sigma-Aldrich.

High-Pressure Treatments. Three samples (100 g) of each persimmon fruit cultivar puree were vacumm packed in plactic bags (Polyskin X12) and then introduced into the pressure unit filled with pressure medium (water).

High-pressure treatments were performed in a hydrostatic pressure unit with a 2350 mL capacity, a maximun pressure of 500 MPa, and a potential maximun temperature of 95 °C (Gec Alsthom ACB 900 HP, type ACIP 665, Nantes, France). The pressures employed in the treatments selected were 50, 150, 300, and 400 MPa. Pressure was increased and released at 2.5 MPa/s. The duration of the pressure treatments was constant at 15 min, and the temperature of the immersion medium was 25 °C. After treatment, samples were immediately analyzed or frozen by inmersion in liquid nitrogen and stored at −80 °C.

Extraction, Separation, Identification, and Quantification of Pigments. All of these processes have been described in detail in previous works (Cano et al., 1996; Cano and de Ancos, 1994). Duplicates of each sample (30 g) were extracted with 100 mL of tetrahydrofuran (THF) containing butylated hydroxytoluene (BHT; 0.1 mg/g) until the extracts were colorless. One milliliter of solution of Sudan I (1 mg/mL) as internal standard was added. The combined THF extracts were concentrated on a rotatory evaporator at 35 °C under nitrogen, partitioned between ethyl ether (150 mL) and saltwater, and transferred to a separating funnel. The organic layer was washed with water (3 × 50 mL) until the ethyl ether extract was colorless. The organic layers were combined and dried over anhydrous sodium sulfate.The solvent was evaporated to dryness (T < 35 °C) and the dry residue dissolved in 2 mL of dichloromethane. Duplicate 20 μL samples for each extract were injected for the HPLC analysis of the unsaponificated extract. All steps were performed under diminished light.

Saponification. Ethereal solutions of the fruit extracts (150 mL) were reduced in the evaporator to 60 mL and were treated with methanolic potassium hydroxide (30 g/L) (4 mL) under nitrogen atmosphere at room temperature for 12 h. The solution was partioned into a saturated aqueous solution of sodium chloride and ether, and the organic layer was removed. The organic layer was washed several times with water until KOH was completly removed (pH 7.0). The solvent was evaporated to dryness and the residue dissolved in 1 mL of dichloromethane. Duplicate 20 μL samples for each extract were injected for the HPLC analysis.

Apparatus. A Hewlett-Packard model 1050 (quaternary solvent delivery) was equipped with a Hewlett-Packard 1040A UV−visible photodiode array detector, provided with a Hewlett-Packard recorder, model 9000/300.

Column. Separation was carried out with a 250 × 4.6 i.d. mm Hypersil ODS column (5 μm particle size) (Technochroma).

Chromatographic Procedure. A gradient mixture of methanol/water (75:25), eluent A, and ethyl acetate, eluent B, was used, beginning at time 0 (eluent B = 0%) until time 10 (min) with a semifinal composition of eluent B (70%). The gradient eluent composition was followed at time 10 until time 30 (min) with the final composition of eluent B (100%). The flow rate employed was 1 mL/min, and the chromatographic runs were monitored at 440 nm. At the end of the gradient, the column was reequilibrated to initial conditions by a new gradient, beginning at time 30 (min) until time 35 (min), with a final composition of eluent B (0%).

Identification. Identification of the carotenoids was carried out by HPLC through the combined use of the retention time, UV−visible absortion spectrum obtained with a photodiode array detector, and co-injection with carotenoid standards. In some cases the structure was further verified by means of chemical reaction (Eugster, 1995).

Quantification. The concentration of the carotenoids of the persimmon extracts after saponification was determined according to the procedure of Hart and Scott (1995). Calibration curves were built with a minimun of four concentration levels of each carotenoid standard, and the straight line equations and their coefficients of correlation (ranging from 0.995 to 0.999) were calculated. Correction for recovery was applied to the data. Recoveries were completed by standard (Sudan I) addition before saponification for each sample analyzed and used to correct carotenoid levels after HPLC analysis. Recovery values for the carotenoids were quite variable. Mean recoveries ranged from 82% ± 10 for β-cryptoxanthin to 87% ± 12 for β-carotene.

Free Radical Scavenging Measurement. The antiradical capacity of the sample extracts, untreated and high pressure treated, was estimated according to the procedure reported by Brand-Williams et al. (1995), which was slightly modified by Sánchez-Moreno et al. (1998).

Preparation of Sample Extracts. Untreated and high-pressure-treated samples were thawed, and the extracts were obtained by homogenizing 5 g of each sample with 20 mL of THF in an ultrahomogenizer (Omnimixer, model ES-207, Omni International, Inc., Gainsville, VA) during 3 min (8000 rpm). The extraction was carried out at 0 °C; the blender jar was cooled in an external ice bath. The extracted were filtered

under suction, and the solid materials were washed several times with THF until the resulting filtrate was colorless. The combinated THF extracts were clean up and saponified as has been previous described. After the saponification procedure, the ethyl ether layer was reduced in the evaporator to 5 mL and then made up to 50 mL with methanol.

Determination of the Scavenging Effect on DPPH Radicals. An aliquot (0.1 mL) of the sample extracts was added to 3.9 mL of DPPH$^{\bullet}$ (0.025 g L^{-1}) in methanol. Absorbances of the samples were measured using a spectrophotometer (Perkin-Elmer, model Lambda 12) at different time intervals until the reaction reached a plateau time (time at the steady time) at 515 nm against methanol without DPPH$^{\bullet}$ as the blank reference. Persimmon sample concentrations, expressed as grams of dry sample per gram of DPPH$^{\bullet}$ in the reaction medium, ranged from 0.5 to 80.

The DPPH$^{\bullet}$ concentration in the reaction medium was calculated from the following calibration curve determined by linear regresion ($r^2 = 0.999$)

$$A_{515} = 29298[\text{DPPH}^{\bullet}]_T + 4.54 \times 10^{-2}$$

where $[\text{DPPH}^{\bullet}]_T$ was expressed as g L^{-1}. The percentage of remaining DPPH$^{\bullet}$ (%DPPH$^{\bullet}_{\text{REM}}$) was calculated as follows:

$$\%\text{DPPH}^{\bullet}_{\text{REM}} = \{[\text{DPPH}^{\bullet}]_T / [\text{DPPH}^{\bullet}]_{T=0}\} \times 100$$

The antiradical efficiency (AE $= 1/\text{EC}_{50}T_{\text{EC50}}$) of the untreated and high-pressure-treated persimmon products was calculated according to the method of Sánchez-Moreno et al. (1998), where EC_{50} is the amount of sample needed to decrease by 50% the initial DPPH$^{\bullet}$ concentration and T_{EC50} is the time needed to reach the steady state at EC_{50} concentration.

Statistical Analysis. Results were processed by an analysis of variance (ANOVA), and statistical significance was determined by Student's *t* test. The computer program employed was InStat.

RESULTS AND DISCUSSION

Carotenoid Composition of Persimmon Fruit Extracts. *Carotenoid Composition of Unsaponified Extracts.* The HPLC chromatograms of the unsaponified extracts of the flesh of the two persimmon fruit cultivars studied, Rojo Brillante and Sharon, are shown in Figure 1A and Figure 2A, respectively. Three different carotenoid groups were separated by HPLC from the unsaponified extracts of both cultivars, Rojo Brillante (Figure 1A) and Sharon (Figure 2A). The pattern consisted of oxycarotenoids (xanthophylls), hydrocarbon carotenoids, and the main group of carotenol mono- and bis-fatty acid esters. The following carotenoids were identified in persimmon cv. Rojo Brillante unsaponified extract (Figure 1A) on the basis of HPLC data and UV−visible light absorption by comparison of the spectra with standards and literature values (Table 2): ~2% of the total content was xanthophylls, identified as zeaxanthin (peak 3), antheroxanthin (peak 5), and β-cryptoxanthin (peak 6); the hydrocarbon carotenoids, β-carotene (peak 11) and lycopene (peak 10), were ~36% of the total carotenoid composition; and the rest (62%) was constituted by carotenol fatty acid esters of different xanthophylls such as lutein (peaks 7 and 13), antheraxanthin (peak 8), violaxanthin (peak 12), β-cryptoxanthin (peaks 9, 14, and 15), and zeaxanthin (peaks 16−18). The HPLC chromatogram of persimmon fruit cv. Sharon (Figure 2A) showed a similar carotenoid composition before saponification, with the main qualitative difference of only traces of lycopene detected (peak 10); meanwhile, in cv. Rojo Brillante, this pigment is one of the main compounds of the extract (30%) (Figure 1A).

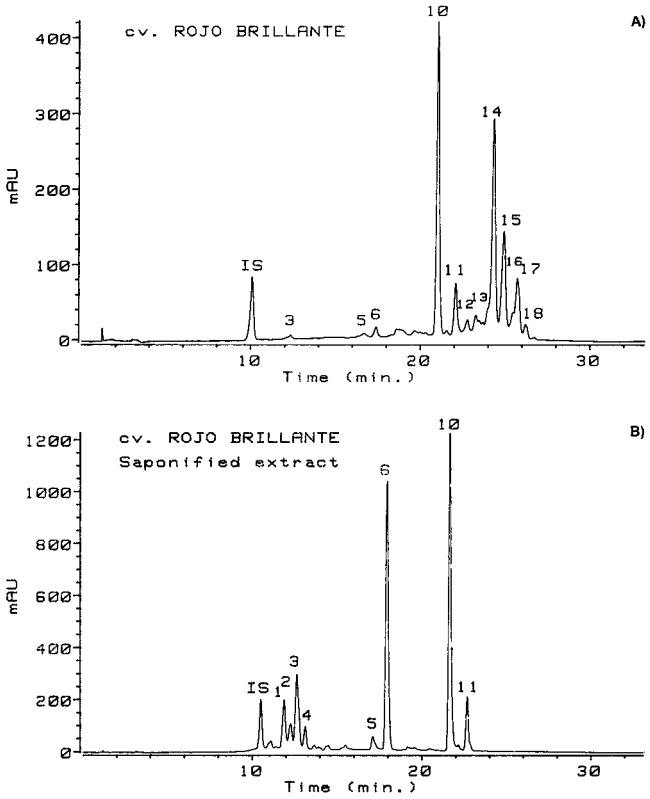

Figure 1. HPLC profiles of persimmon fruit cv. Rojo Brillante pulp extract: (A) unsaponified extract; (B) saponified extract. Peak identifications are given in Table 2.

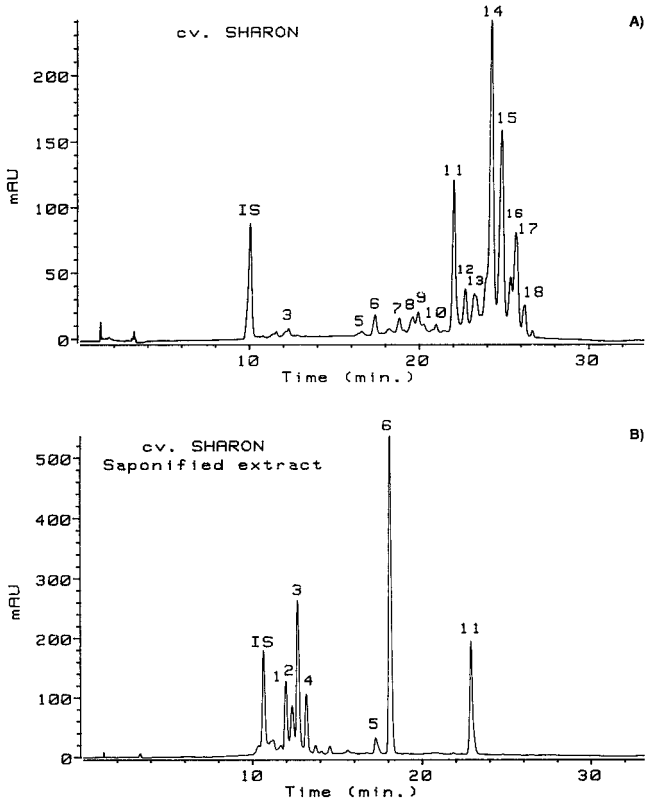

Figure 2. HPLC profiles of persimmon fruit cv. Sharon pulp extract: (A) unsaponified extract; (B) saponified extract. Peak identifications are given in Table 2.

So, cv. Sharon belongs to the category of persimmon fruit cultivar with very low lycopene content and cv. Rojo Brillante belongs to the high lycopene content

Table 2. Peak Identification of the Carotenoids of Persimmon Fruit Extracts Separated by HPLC in the Order of Elution of the C_{18} Column

peak	carotenoid	λ, nm
1	neoxanthin	416, 438, 470
2	violaxanthin	418, 442, 472
3	zeaxanthin	428, 450, 474
4	lutein	420, 444, 472
5	antheroxanthin	420, 444, 472
6	β-cryptoxanthin	428, 450, 474
7	lutein monoester	420, 444, 472
8	antheroxanthin monoester	420, 444, 472
9	β-cryptoxanthin monoester	428, 450, 474
10	lycopene	472, 502
11	β-carotene	426, 450, 472
12	violaxanthin ester	418, 442, 472
13	lutein diester	420, 444, 472
14	β-cryptoxanthin ester	428, 450, 472
15	β-cryptoxanthin ester	428, 450, 472
16	zeaxanthin diester	428, 450, 474
17	zeaxanthin diester	428, 450, 474
18	zeaxanthin diester	428, 450, 474

cultivar category (Brossard and Mackinney, 1963). Both cultivars showed β-cryptoxanthin as the main xanthophyll esterified with different fatty acids.

The carotenoid patterns of these two persimmon cultivars grown in Spain, Sharon and Rojo Brillante, were very similar to those of other more extensively studied cultivars such as the Japanese cultivars Fuju and Hachiya (Gross, 1987; Homnava, 1990) or cultivars grown in California (Philip and Chen, 1988; Palmer-Wright and Kader, 1997).

Carotenoid Composition of Saponified Extracts. Saponification of persimmon fruit extracts regenerated the parent hydroxycarotenoids. HPLC chromatograms of saponified extracts of cv. Rojo Brillante (Figure 1B) and cv. Sharon (Figure 2B) shows the disappearance of the peak assigned to the carotenol mono- and bis-fatty esters (peaks 7−9 and 12−18) and the regeneration of some xanthophylls, the majority being β-cryptoxanthin and zeaxanthin (peaks 3 and 6) and other minor hydroxycarotenoids such as violaxanthin, neoxanthin, lutein, and antheroxanthin (peaks 1, 2, 4, and 5, respectively). Peak assignment of the carotenoid compounds in saponified extracts (Table 2) was based on comparison of HPLC retention times and UV−visible absorption spectra with those of authentic samples. In some cases the structure was further verified by chemical reactions.

Both persimmon fruit cultivars analyzed in this study showed similar carotenoid patterns, with β-cryptoxanthin as the main carotenoid (5.79 μg g^{-1} in Sharon and 6.85 μg g^{-1} in Rojo Brillante) and with a great xanthophyll contribution, approximately 70 and 89% of the total carotenoid composition of Rojo Brillante and Sharon, respectively. From a comparison of the HPLC chromatograms of both cultivars (Figures 1B and 2B), the main qualitative difference was found to be the high content of lycopene (5.34 μg g^{-1}) (24%) in Rojo Brillante; in Sharon extracts only traces were detected. The quantitative distribution of carotenoid compounds in both persimmon fruit cultivars analyzed in this study after saponification is presented in Table 3 (cv. Sharon) and Table 4 (cv. Rojo Brillante). The carotenoid levels in cv. Rojo Brillante (22.11 μg g^{-1}) are significantly higher than those in cv. Sharon (15.22 μg g^{-1}).

Tables 3 and 4 showed that provitamin A carotenoids (β-carotene and β-cryptoxanthin) were predominant among other constituents of fruit pigments. Although significant differences in total carotenoid content have

Table 3. Carotenoid Content (Micrograms per Gram) of Persimmon Flesh (Cv. Sharon) Fresh and Treated with High Pressure during 15 min at 25 °C

		high-pressure treatments[a] (MPa)			
carotenoid[b]	fresh	50	150	300	400
violaxanthin	1.11 ± 0.27a	2.19 ± 0.14b	1.08 ± 0.24a	0.87 ± 0.17a	1.63 ± 0.05b
neoxanthin	1.35 ± 0.24a	1.37 ± 0.04a	0.88 ± 0.11b	0.79 ± 0.08b	1.76 ± 0.04c
zeaxanthin	3.32 ± 0.46a	3.38 ± 0.07a	2.70 ± 0.54a	3.19 ± 0.26a	3.18 ± 0.07a
lutein	1.66 ± 0.22a	2.12 ± 0.13b	1.65 ± 0.20a	1.51 ± 0.07a	1.89 ± 0.04a
antheroxanthin	0.36 ± 0.02a	0.42 ± 0.02b	0.32 ± 0.03a	0.38 ± 0.02a	0.45 ± 0.01b
β-cryptoxanthin	5.79 ± 0.18a	6.93 ± 0.33b	4.65 ± 0.48c	5.53 ± 0.31a	7.00 ± 0.07b
β-carotene	1.63 ± 0.17a	1.78 ± 0.19a	1.49 ± 0.14a	1.73 ± 0.08a	1.79 ± 0.01a
total	15.22 ± 1.56a	18.19 ± 0.92b	12.72 ± 1.74a	13.95 ± 0.99a	17.70 ± 0.29b
vitamin A value (RE[c]/100 g)	75 ± 0.1a	87 ± 0.3b	64 ± 1.2c	75 ± 2.3a	87 ± 0.2e

[a] Different letters in the same row indicate significant differences ($p \leq 0.05$). [b] Values are the mean of six determinations ± SD. [c] RE, retinol equivalents.

Table 4. Carotenoid Content (Micrograms per Gram) of Persimmon Flesh (Cv. Rojo Brillante) Fresh and Treated with High Pressure during 15 min at 25 °C

		high-pressure treatment[a] (MPa)			
carotenoid[b]	fresh	50	150	300	400
violaxanthin	2.18 ± 0.07a	1.06 ± 0.02b	1.48 ± 0.03c	1.75 ± 0.26c	2.19 ± 0.08a
neoxanthin	1.14 ± 0.08a	1.14 ± 0.14a	0.85 ± 0.07a	0.98 ± 0.18a	0.91 ± 0.02a
zeaxanthin	3.67 ± 0.16a	5.13 ± 0.27b	3.23 ± 0.31a	4.16 ± 0.69ab	3.45 ± 0.27a
lutein	1.14 ± 0.04a	1.00 ± 0.27a	1.23 ± 0.12a	1.36 ± 0.22a	1.22 ± 0.08a
antheroxanthin	0.55 ± 0.03a	0.50 ± 0.02a	0.52 ± 0.07a	0.65 ± 0.17a	0.56 ± 0.08a
β-cryptoxanthin	6.85 ± 0.41a	7.24 ± 0.39a	6.72 ± 1.18a	8.05 ± 1.38a	5.17 ± 0.69b
lycopene	5.34 ± 0.18a	5.89 ± 0.39a	5.19 ± 1.12a	7.73 ± 0.54b	5.04 ± 0.26a
β-carotene	1.24 ± 0.04a	1.17 ± 0.05a	1.14 ± 0.20a	1.63 ± 0.22a	1.08 ± 0.06a
total	22.11 ± 1.01a	23.13 ± 0.89a	20.36 ± 2.1a	26.51 ± 1.66b	19.62 ± 1.54a
vitamin A value (RE[c]/100 g)	77 ± 0.4a	76 ± 0.1a	79 ± 0.6b	83 ± 0.5c	62 ± 0.3d

[a] Different letters in the same row indicate significant differences ($p \leq 0.05$). [b] Values are the mean of six determinations ± SD. [c] RE, retinol equivalents

been quantified, the vitamin A values were similar in the two cultivars analyzed because only β-carotene and β-cryptoxanthin were the carotenoid contributors to vitamin A value, rendering 75 and 77 retinol equivalent (RE)/100 g in cv. Sharon and cv. Rojo Brillante, respectively. Considering that the female recommended daily allowance (RDA) is 800 RE, persimmons provide between 9 and 10% of the RDA in a 100 g serving. The vitamin A values quantified in the two persimmon cultivars analyzed in this study were significantly higher than that found in the literature (Homnava et al., 1990).

Effect of High-Pressure Treatment on Carotenoid Composition and Vitamin A Value. High-pressure treatments ranging from 50 to 400 MPa at 25 °C and prefixed treatment time of 15 min were assayed on the pulp of two persimmon fruit cultivars harvested in Spain, cvs. Rojo Brillante and Sharon. The effect of pressure treatments assayed on total carotenoid content and on individual pigment concentration of saponified extract of cvs. Sharon and Rojo Brillante is shown in Tables 3 and 4, respectively. An evident stability of each pigment of the two cultivars' carotenoid profiles toward pressure processing was observed. Total carotenoid content extracted of Sharon pulp high-pressure-treated at 50 MPa (18.19 $\mu g\ g^{-1}$) and at 400 MPa (17.70 $\mu g\ g^{-1}$) increased significantly, up to 19% and up to 16% of the untreated persimmon total concentration (15.22 $\mu g\ g^{-1}$) (Table 3). The increase of total pigment content was related with a significantly better extraction of some of the main pigments such as violaxanthin, lutein, antheroxanthin, β-cryptoxanthin, and β-carotene. Nonsignificant differences were detected on zeaxanthin concentration after and before pressure treatments (Table 3). Pressure treatments at 150 and 300 MPa produced a slight and nonsignificant decrease on total and individual carotenoid contents.

Table 4 shows the effect of pressure on the carotenoid composition of the pulp of Rojo Brillante. A nonuniform behavior was detected for a further increase of pressure from 50 to 400 MPa. Although a slight increase or decrease of individual and total concentrations of pigments was achieved, no significant modifications due to pressure were detected. A nonsignificant increase of total carotenoid concentration compared with the fresh product was quantified when the pulp was exposed to pressures of up to 50 MPa (9%) and up to 300 MPa (27%), due to a better extraction of the main carotenoids. For example, treatment at 300 MPa increased the extraction of lycopene, β-carotene, β-cryptoxanthin, and zeaxanthin by about 45, 3, 18. and 19% of their concentration in the fresh product, respectively (Table 4). As in cv. Sharon, the minor xanthophyll neoxanthin in Rojo Brillante was the carotenoid that decreased its concentration more (∼25% at 150 MPa) after high-pressure treatments.

In general, these results showed a high stability of the color components (carotenoids) of persimmon fruit products to high-pressure processing. Isomerization and degradation of carotenoids produced by thermal processing (Cano and de Ancos, 1994) were not detected after high-pressure treatments. In the literature, high-pressure treatments at room temperature or combined with low temperature are recommended to minimize sensory and nutritional quality losses during processing. Donsi et al. (1996) reported that high-pressure treatments ranging from 2000 to 5000 bar on orange juice had no significant effect on β-carotene content or on total carotenoid concentration. Van Loey et al. (1998) found that high-pressure treatments at room temperature or combined with mild temperature retained the initial chlorophyll content of broccoli, even after treatments of 4 h at 800 MP at 40 °C, and so minimized green color losses. Also, ascorbic acid content was retained in green peas after treatment at 9000 bar (Quaglia et al., 1996), in orange juice with treatments not lower than 3500 bar (Donsi et al., 1996), and in squeezed oranges and tomatoes treated at 8500 bar during 1 h at 50 °C (Van den Broeck et al., 1998). These results were correlated with the stability of the covalent structure of pigments and ascorbic acid to high pressure due to the compressibility of covalent bonds (Knorr, 1993).

Some high-pressure treatments assayed in this study increased the amount of extractable carotenoids with a nutritional (β-carotene and β-cryptoxanthin) or health-related value due to their radical-scavenging capacity (lycopene, β-carotene, β-cryptoxanthin, zeaxanthin, and lutein) (Tables 3 and 4). High-pressure processing could increase the amount of carotenoid available for absorption, improving the bioavailability of the carotenoids in fruit products, as happened with the lycopene in tomato products thermally treated (Gärtner et al., 1997).

β-Carotene and β-cryptoxanthin were the two contributors to vitamin A value in persimmon fruits. The better extraction of these two carotenoids after high-pressure treatments was reflected in the vitamin A value, which increased significantly from 75 to 87 RE [RE/100 g of fresh weight (fw)] in cv. Sharon after treatments at 50 and 400 MPa (Table 3) and from 77 to 83 RE (RE/100 g of fw) in cv. Rojo Brillante treated at 300 MPa (Table 4). In general, high-pressure processing slightly modified the vitamin A value, and the positive trend observed was the increase in RE (RE/100 g of fw) after pressure processing. A significant decrease was shown only after two treatments at 150 MPa in Sharon (15%) and at 400 MPa in Rojo Brillante (19%).

The nonuniform behavior detected for a further increase of pressure from 50 to 400 MPa in both persimmon fruit carotenoid contents could be explained by taking into account that the molecule of carotenoid is not free. Plant carotenoids can be bound in protein−carotenoid complexes or may be associated in the plant matrix (Boileau et al., 1999). Treatment at 50 MPa increased insignificantly the amount of carotenoid extractable in both persimmon fruits (Tables 3 and 4). Elevated hydrostatic pressure between 50 and 100 MPa slightly modified the protein structure but can affect the nuclear membrane (Smelt, 1998), producing the disprution of the chromoplasts where the carotenoids are located, improving the extraction of the pigments. In general, pressures >300 MPa cause irreversible protein denaturation at room temperature (Hendrickx et al., 1998). Treatments at 300 MPa in Rojo Brillante and at 400 MPa in Sharon improved the extraction of the individual and total carotenoid contents, perhaps because the pressure processing caused protein denaturation and released more carotenoid from the food matrix, as happened with mild thermal treatments (Dietz et al., 1988). To increase the amount of extractable carotenoids, cv. Sharon needed higher pressure treatment (400 MPa) than cv. Rojo Brillante (300 MPa). The differences between compositions of the two persimmon cultivars (Table 1) could explain this behavior. Rojo Brillante had a lower pH (5.02) than Sharon (5.31),

Table 5. Free Radical Scavenging Paramenters of Persimmon Fruit Fresh and Standards; Evolution of AE with High-Pressure Treatments

sample	EC_{50}[a] (g/g of DPPH•)	T_{EC50}[b] (min)	AE[c] × 10^{-3}
persimmon fruits[d]			
cv. Rojo Brillante			
fresh	3.07 ± 0.08	26.84 ± 0.05	12.14 ± 0.4a
50 MPa	4.41 ± 0.07	20.82 ± 1.1	10.89 ± 0.1a
150 MPa	3.13 ± 0.03	19.91 ± 0.1	16.04 ± 0.6b
300 MPa	3.57 ± 0.8	21.91 ± 0.7	12.78 ± 0.5a
400 MPa	4.87 ± 0.1	19.24 ± 0.06	10.67 ± 0.3a
cv. Sharon[e]			
fresh	39.14 ± 1.3	18.03 ± 0.1	1.42 ± 0.01a
50 MPa	54.49 ± 1.8	13.92 ± 0.1	1.32 ± 0.03a
150 MPa	2.86 ± 0.1	20.90 ± 0.6	16.73 ± 0.1b
300 MPa	2.81 ± 0.2	18.17 ± 1.5	19.58 ± 0.2b
400 MPa	51.76 ± 1.6	16.09 ± 1.6	1.20 ± 0.02b
standards			
ascorbic acid	0.076 ± 0.007	1.15 ± 0.08	11441.22 ± 111
DL-α-tocopherol	0.20 ± 0.011	9.52 ± 0.71	525.21 ± 31.2
BHA	0.088 ± 0.005	113.35 ± 7.8	100.25 ± 10.8
BHT	0.092 ± 0.008	122.12 ± 8.6	89.00 ± 4.5

[a] EC_{50} is the amount of sample needed to decrease by 50% the initial DPPH• concentration. [b] T_{EC50} is the time needed to reach the steady state at EC_{50} concentration. [c] Different letters in the same column of persimmon fruit treatments indicate significant differences ($p \leq 0.05$). [d] Persimmon extract concentration expressed as grams of dry sample per gram of DPPH• in the reaction medium.

and, as is well-known, a more acidic environment is more greatly affected by pressure. The literature showed similar conclusions about the correlation of the composition of the products and the effect of pressure treatments on plant food enzymes (Seyderhelm et al., 1996; Cano et al., 1997) or on quality parameters such as ascorbic acid (Van den Broeck, 1998).

Free Radical Scavenging Capacity of Persimmon Fruit As Affected by High Pressure. The free radical scavenging activity of the saponified extracts of the two persimmon fruit cultivars analyzed in this study was compared with that of two well-known natural antioxidants, ascorbic acid and α-tocopherol, and with that of two synthetic antioxidants, buylated hydroxyanisole (BHA) and BHT (Table 5). The AE is a new parameter to measure the free radical scavenging of samples, and it combines not only the widely used parameter EC_{50} (amount of antioxidant necessary to decrease by 50% the initial DPPH•) but also the time of the reaction with the parameter T_{EC50}, defined as the time needed to reach a steady state at the concentration corresponding to EC_{50} (Sánchez-Moreno et al., 1998). The results given in Table 5 showed the kinetic behavior of Rojo Brillante and Sharon fruits. On the basis of the T_{EC50} values (Table 5) of the two cultivars, ranging from 13.92 to 26.84 min, persimmon fruit had an "intermediate" kinetic behavior, according to the classification of Sánchez-Moreno et al. (1998). The data given in Table 5 show that Rojo Brillante's AE value (AE = 12.14) was 8.5 times higher than that for Sharon (AE = 1.42). Therefore, Rojo Brillante had a greater ability to scavenge free radicals than Sharon. These differences may be explained by a high content of lycopene in Rojo Brillante, whereas in Sharon only traces were detected, although the persimmon flesh studied could have other compounds with antioxidant capacity such as vitamin C and polyphenols. AE values of persimmon fruit cv. Rojo Brillante (AE = 12.14) were comparable with the AE values of different red wines (Larrauri et al., 1999), a recently important subject of many studies due to their antioxidant properties.

In general, pressures assayed on Rojo Brillante fruit produced slighlty insignificant changes on AE. Only treatments at 150 MPa significantly increased the AE value from 12.14 to 16.04 (Table 5). More significant was the effect of pressure on AE values of Sharon fruits. AE values significantly increased after treatments at 150 (from 1.42 to 16.73) and 300 MPa (from 1.42 to 19.58), reaching the AE values of Rojo Brillante. The nonuniform behavior of high pressure on the AE of persimmon cannot be related with the increase of the amount of extractable carotenoids after treatments. Nevertheless, other important bioactive compounds such as ascorbic acid and polyphenols are important components of persimmon fruit. Although ascorbic acid content slightly changed after pressure treatments at room temperature (Van den Broeck et al., 1998), the synergistic effect between the bioactive compounds may be improved upon pressure. Also, the high content of polyphenols in persimmon fruits may be related with changes in free radical scavenging capacity found in this study. Further research on the radical scavenging capacity of pressurized persimmon products and the relationship with their polyphenol content is needed.

Conclusions. The carotenoid profile of both persimmons, Rojo Brillante and Sharon, was mainly composed by β-cryptoxanthin, zeaxanthin, β-carotene, lycopene, and carotenoids. The high content of β-carotene and β-cryptoxanthin gave to these fruits an important provitamin A value, providing approximately 10% of the RDA in 100 g of pulp consumed. High lycopene concentration in Rojo Brillante conferred to this fruit an AE value similar to that found in red wine. Processing persimmon fruits by high pressure could be an efficient method to preserve these products (enzyme and microorganism inactivation) that would not induce important pigment compound changes and that would retain or indeed improve their vitamin A values and their radical-scavenging capacities.

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