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Phytochemical composition and antioxidant activity of peach as affected by pasteurization and storage duration

Ana Oliveira [a], Manuela Pintado [a], Domingos P.F. Almeida [b,c,*]

[a] CBQF, Esc. Sup. Biotecnologia, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
[b] Faculdade de Ciências, Universidade do Porto, Rua Campo Alegre 687, 4169-007 Porto, Portugal
[c] Frulact, S.A., Rua do Outeiro, 589, 4475-150 Gemunde, Maia, Portugal

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A B S T R A C T

Fruit are very perishable and are often preserved as heat-processed foods. Clingstone peach [*Prunus persica* (L.) Batsch 'Catherine'] fruit were heat-treated at 90 °C for 5 min and stored under aseptic conditions at room temperature (*ca.* 22 °C) for 90 days. Significant reductions in total carotenoids were observed immediately after pasteurization but total antioxidant activity and the concentration of total phenolics were unaffected. Pasteurization induced significant reductions in the concentration of protocatechuic acid (from 10.2 to 5.8 μg/g fw), zexanthin and β-cryptoxanthin. Significant reduction in antioxidant activity, expressed as ascorbic acid equivalents, from 0.52 to 0.25 mg/g fw, was observed during storage of pasteurized peach for 90 days. Total phenolics, expressed as gallic acid equivalents, decreased during storage from 0.57 to 0.28 mg/g fw and total carotenoids decreased from 4.0 to 1.3 μg/g fw. Procyanidin B1 increased from 15.8 to 26.8 μg/g fw and chlorogenic acid and neochlorogenic acid increased 35 and 43%, respectively. (−)-Epicatechin decreased during storage from 13.1 to 4.0 μg/g fw and quercetin-3-glucoside from 7.3 to 4.4 μg/g fw. All carotenoids decrease significantly with the exception of zeaxanthin, which increased during storage. Storage duration strongly affected the concentration of phenolics and carotenoids in pasteurized peach.

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1. Introduction

Fruit are an essential part of a healthy diet and a vehicle for several unique health-promoting phytochemicals (Block, Patterson, & Subar, 1992; Kalt, Forney, Martin, & Prior, 1999; Nicoli, Anese, & Parpinel, 1999). Fresh fruit are very perishable, therefore requiring preservation techniques to prevent undesirable changes and, eventually, total loss due to spoilage. Peaches are often thermally processed by pasteurization, to assure the absence of vegetative forms of spoilage microorganisms or human pathogens, or by sterilization to assure destruction of microbial spores (Asami, Hong, Barrett, & Mitchell, 2003).

Phenolics and carotenoids are natural components of peaches that possess beneficial properties for human health. Phenolics play a role in plant defence against insect and mammal herbivory and oxidative damage (Chang, Frankel & Barrett, 2000; Duval, Shetty, & Thomas, 2000). Peach carotenoids act as pigments and are also involved in the protection of plant cells against oxidative stress. Considering the relevant role of phenolic and carotenoid phytochemicals in human health, it has become increasingly important to investigate the impact of postharvest and processing conditions on their levels in foods (Asami et al., 2003).

Thermal processing is widely used in the food industry due to its proven efficacy in preventing enzymatic changes and microbial spoilage. Heat treatments, however, may cause undesirable biochemical changes that affect the sensory and nutritional quality of the final product (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007). Thermal decomposition is the most likely cause for losses of bioactive compounds. These degradative processes depend on the chemical structure of the phytochemicals, *e.g.*, molecules with unsaturated covalent bonds are more prone to degradation (Rawson, Patras, Tiwari, Noci, Brunton & Koutchma., 2011). Therefore, it is anticipated that phytochemicals in a fruit matrix will be differentially affected by heat treatments.

Identification of markers sensitive to the processing conditions can provide a basis for the development of an audit system for the preservation of health-promoting phytochemicals during

* Corresponding author. Faculdade de Ciências, Universidade do Porto, Rua Campo Alegre 687, 4169-007 Porto, Portugal. Tel.: +351 220 402 489; fax: +351 220 402 490.

E-mail address: dalmeida@fc.up.pt (D.P.F. Almeida).

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processing. This study was conducted to investigate the effect of pasteurization and subsequent storage duration on peach phenolics and carotenoids in an attempt to identify relevant markers for process evaluation aimed at the preservation of functional and nutritional properties.

2. Materials and methods

2.1. Chemicals

Methanol, 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), sodium carbonate, hexane, ammonium acetate, acetonitrile were purchased from Sigma–Aldrich (St. Louis, MO, USA), Folin–Ciocalteu's reagent, potassium persulfate, potassium hydroxide, and dichloromethane from Merck (Damstadt, Germany), and sodium chloride from Panreac (Barcelona, Spain). Standards of ascorbic acid, gallic acid, procyanidin B1, protocatechuic acid, (+)-catechin, (−)-epicatechin, chlorogenic acid, dihydroxibenzoic acid, and β-carotene were obtained from Sigma–Aldrich (St. Louis, MO, USA), whereas zeaxanthin, lutein and β-cryptoxanthin were purchased from Extrasynthese (Lyon, France).

2.2. Pasteurization treatment and storage conditions

Clingstone peach [*Prunus persica* (L.) Batsch 'Catherine'] were harvested at commercial maturity in Cova da Beira, Portugal, shipped under refrigerated conditions to the laboratory and processed within 12 h after harvest. Fruit were peeled, the pit removed, and the flesh cut by hand with a sharp knife into cubes with *ca.* 10 mm sides.

Fruit pieces (*ca.* 100 g) were placed in 250 mL glass flasks, covered with aluminium foil, and heated in a water-bath at 90 °C. The temperature at the centre of the cubes, monitored with a HD 8802 thermometer (Delta OHM, Padova, Italy), reached 84.5 °C ± 2.2 °C (mean ± SD, $n = 40$) after 15 min and remained that temperature for 5 min thereafter. The flasks were then capped and cooled to room temperature in a water bath during 30 min. Flasks with pasteurized fruit pieces were stored in the dark for 90 days at room temperature (*ca.* 22 °C). Sampling of individual flasks was carried out at 18, 36, 54, 72 and 90 days of storage. This procedure was performed in triplicate.

2.3. Extract preparation

Hydrophilic extractions were performed by homogenizing 5 g of fruit in 50 mL of methanol in water at the concentration of 800 mL/L. Homogenization (IKA Ultra-turrax T18, Wilmington, USA) was performed at 24,000 rpm for 30 s. The slurry was then centrifuged at 4000× g at 4 °C for 10 min, the supernatant filtered through a 0.45-μm cellulose acetate filter (Orange Scientific, Braine-l'Alleud, Belgium), the volume of the filtrate recorded and used for the assessment of total activities and quantification of individual compounds. A 10-mL aliquot of the extract was evaporated to dryness in a RVC 2-18 speed-vacuum evaporator (Christ, Osterode am Harz, Germany) at 30 °C and the residue dissolved in 3 mL of methanol. The extract was then filtered through a 0.45-μm cellulose acetate filter (Orange Scientific, Braine-l'Alleud, Belgium) and analysed by HPLC-DAD.

Carotenoids were extracted as described by Wright and Kader (1997). Briefly, 5 g of peach tissue were suspended in 10 mL of cold ethanol and homogenized at 24,000 rpm for 3 min using an IKA T18 Ultra-turrax (Wilmington, USA). Hexane (8 mL) was added to the homogenate and the resulting mixture was homogenized for an additional 2 min before the slurry was centrifuged for 10 min at 4000× g. The hexane layer containing the carotenoids was transferred to a polypropylene tube. A solution of saturated sodium chloride (5 mL) and an additional 8 mL of hexane were added and the resulting mixture and homogenized for 1 min. The mixture was centrifuged as described above, and the hexane layer recovered for analyses. All extracts were performed in triplicate samples.

2.4. Determination of antioxidant activity

The ABTS radical scavenging activity was measured in the methanolic extracts using the method described by Gião et al. (2007). Ascorbic acid was used as a standard to prepare a calibration curve in the range of 0.02–0.50 mg/mL.

2.5. Analysis of phenolic compounds

The concentration of total phenolic compounds was determined colorimetrically in the methanolic extracts by the Folin–Ciocalteu method (Singleton & Rossi, 1965). Quantification was done at 750 nm (UV mini 1240, Shimadzu, Tokyo, Japan) with gallic acid as standard in the range 0.015–1.00 mg/mL.

2.6. Analysis of total carotenoids

Saponification of the extract was carried out as described (Kimura, Rodriguez-Amaya, & Godoy, 1990). Carotenoids were quantified after saponification by measuring the absorbance at 454 nm with a UV mini 1240 spectrophotometer (Shimadzu, Tokyo, Japan). β-Carotene was used to prepare calibration curve in the range of 0.063–4.0 μg/mL.

2.7. Identification and quantification of phenolics and carotenoids

Qualitative and quantitative profiles of phenolics and carotenoids were determined by HPLC-DAD (Waters Series 600, Mildford MA, USA). Separation was performed in a reverse phase Symmetry® C18 column (250 × 4.6 mm i.d., 5 μm particle size and 125 Å pore size) with a guard column containing the same stationary phase (Symmetry® C18). Chromatographic separation of phenolic compounds was carried out with a solvent A – formic acid, water and methanol (92.5:5:2.5) – and solvent B – methanol and water (94:6) – under the following conditions: linear gradient starting at 0–10% solvent B in 10 min at 0.5 mL/min, 10–30% in 40 min at 0.65 mL/min, 30–50% in 20 min at 0.75 mL/min and from 50 to 0% in 10 min at 1 mL/min. Injection volume was 20 μL. Detection was achieved by a diode array detector (Waters, Massachussets, EUA) at wavelengths ranging from 200 to 600 nm in 2 nm intervals. Absorbance was measured at 280 nm (flavan-3-ols) and 320 nm (cinnamic acids).

Retention times and spectra of compounds were analysed by comparison with pure standards and quantification performed by the calibration curves of procyanidin B1, protocatechuic acid, (+)-catechin, (−)-epicatechin, chlorogenic acid and 2,5-dihydroxibenzoic acid and expressed as micrograms per gram of fresh biomass.

Carotenoids were eluted using acetonitrile, methanol, dichloromethane, hexane and ammonium acetate (55:22:11.5:11.5:0.02) under isocratic conditions at 1.0 mL/min flow rate during 20 min, at 25 °C. Injection volume was 40 μL and the detector was set at 454 nm. β-Carotene, zeaxanthin, lutein, β-cryptoxanthin were quantified using a calibration curve built with pure standards and expressed as micrograms per gram of fresh biomass.

Three independent analyses were performed in each of the triplicate extracts obtained for each treatment.

2.8. Statistical analysis

All measurements were performed in three independent replicates and the results reported as mean and standard deviation. The results were statistically analysed using the Student's *t*-test using the statistical package GraphPad Prism, version 5.00 for Windows.

3. Results and discussion

3.1. Effect of pasteurization

Pasteurization of fresh peach significantly affected the concentration of carotenoids but antioxidant activity and the concentration of total phenolics were not significantly altered by the heat treatment (Table 1). Decreases in the antioxidant activity of unpeeled peach puree pasteurized at 100 °C for 30 min have been reported (Talcott, Howard, & Brenes, 2000), but the 20% reduction of antioxidant activity after pasteurization reported herein for peach cubes was not statistically significant. The content of total phenolics in the methanolic extracts was similar in fresh and pasteurized peach pieces (Table 1). In contrast, a 20% reduction in total phenolics after pasteurization of peach samples at 110 °C was observed by Asami et al. (2003).

Fresh peach contained 11.6 μg/g of total carotenoids, expressed as β-carotene, a level that decreased by 65% after pasteurization (Table 1). Carotenoids exist in fruit in the form of complexes with proteins, protected by the cellular structure (Rodriguez-Amaya, 1999). Heat processing causes the denaturation of proteins and rupture of cell walls exposing the carotenoids to isomerization and epoxidation reactions (Rodriguez-Amaya, 1999, 2001, p. 64). Decreases in total carotenoids have been reported in heat-treated fruit, such as canned peach (Lessin, Catigani, & Schwartz, 1997), microwaved orange juice (Fratianni, Cinquanta, & Panfili, 2010) and pasteurized orange juice (Gama & Sylos, 2007).

Phenolic compounds reported in peach (Bengoechea et al., 1997; Chang, Tan, Frankel, & Barrett, 2000; Gil, Tomás-Barberán, Hess-Pierce, & Kader, 2002; Lavelli, Pompei, & Casadei, 2009; Tomás-Barberán et al., 2001) were identified by comparison of their absorption spectra with standards. The effect of pasteurization on individual phenolics (Table 2) and carotenoids (Table 3) was analysed. Quercetin-3-rutinoside (rutin), (+)-catechin, (−)-epicatechin, and procyanidin B1 were present in the fresh peach samples but their concentration was not significantly affected by the pasteurization (Table 2). The main hydroxycinnamic acids present in fresh peach were chlorogenic acid and neochlorogenic acid, consistent with previous characterizations (Bengoechea et al., 1997; Chang et al., 2000; Lavelli et al., 2009). Pasteurization of fresh peaches had no significant effect on these hydroxycinnamic acid derivatives (Table 2). Protocatechuic acid and 2,5-dihydroxybenzoic acid were the hydroxybenzoic acids identified. A significant reduction of protocatechuic acid levels was induced by the heat treatment (Table 2).

Table 1
Total antioxidant capacity, total phenolic content and total carotenoids of extracts obtained from fresh and pasteurized peach. Values are mean ± SD ($n = 3$).

Sample	Antioxidant activity (mg/g fw)	Total phenolics (mg/g fw)	Total carotenoids (μg/g fw)
Fresh peach	0.6 ± 0.1^{a}	0.7 ± 0.1^{a}	11.6 ± 1.6^{a}
Pasteurized peach	0.5 ± 0.02^{a}	0.6 ± 0.03^{a}	4.0 ± 0.5^{b}

Different letters within the same column indicate significant differences between means ($P < 0.05$).

Table 2
Phenolic compounds present in methanolic extracts obtained from fresh and pasteurized peach samples. Values are mean ± SD ($n = 3$).

Identification	Phenolics concentration (μg/g)	
	Fresh peach	Pasteurized peach
Procyanidin B1	14.7 ± 2.1^{a}	15.8 ± 1.6^{a}
Protocatechuic acid	10.2 ± 1.5^{a}	5.8 ± 0.2^{b}
Neochlorogenic acid	25.0 ± 5.1^{a}	20.5 ± 0.6^{a}
(+)-Catechin	42.3 ± 5.2^{a}	36.2 ± 0.9^{a}
(−)-Epicatechin	9.2 ± 0.7^{a}	13.1 ± 2.1^{a}
Chlorogenic acid	29.3 ± 3.3^{a}	31.5 ± 4.5^{a}
2,5-Dihydroxybenzoic acid	98.5 ± 2.0^{a}	77.4 ± 6.7^{a}
Quercetin-3-rutinoside	10.2 ± 2.9^{a}	7.4 ± 0.5^{a}

Different letters within the same row indicate significant differences between means ($P < 0.05$).

Zeaxanthin, lutein, β-cryptoxanthin, and β-carotene were the carotenoids identified in fresh peach. Pasteurization of fresh peach induced a significant decrease in the concentration of zeaxanthin and β-cryptoxanthin, but levels of lutein and β-carotene were unaffected by the heat treatment (Table 3). Lutein was not affect by pasteurization in a mixture of orange and carrot juice (Maiani et al., 2009) or in tomato purees (Sanchez-Moreno, Plaza, de Ancos, & Cano, 2006). The observed decreases in the levels of zeaxanthin and β-cryptoxanthin are possibly due to isomerization. The formation of *cis*-isomers of β-cryptoxanthin at temperatures of 60 °C–90 °C have been shown in red cashew apple fruits (Zepka & Mercadante, 2009) and at temperatures of 80–95 °C in yellow tomarillo (Mertz, Brat, Caris-Veyrat, & Gunata, 2010). The occurrence of oxidation reactions during the heat treatment cannot be excluded (Rodriguez-Amaya, 1999, 2001, p. 64), but its contribution is likely more relevant during storage.

3.2. Effect of storage duration

The stability of phenolics, carotenoids, and antioxidant activity was evaluated during storage of pasteurized peach. Antioxidant capacity of peaches immediately after pasteurization, expressed in ascorbic acid equivalents, was 0.50 mg/g fw and decreased by *ca.* 50% during the 90-day storage period (Fig. 1). Total phenolic content of pasteurized peach, expressed in gallic acid equivalents, decreased from 0.57 to 0.28 mg/g fw during the initial 18 days in storage and remained relatively constant thereafter (Fig. 1). A reduction in total phenolics was also observed in a pasteurized peach puree stored at 40 °C during 4 weeks (Talcott et al., 2000) and in canned peaches stored at room temperature for 3 months (Hong, Barrett, & Mitchell, 2004).

Total carotenoids content decreased from 4.0 to 1.3 μg/g fw during the 90-day storage period (Fig. 2). Carotenoid degradation during storage at moderate temperatures is largely determined by oxidation reactions leading to the formation of low molecular weight epoxicarotenoids and apocarotenoids (Rodriguez-Amaya, 1999, 2001, p. 64).

Table 3
Carotenoids present in extracts obtained from fresh and pasteurized peach samples. Values are mean ± SD ($n = 3$). ND, not detected.

Identification	Carotenoids concentration (μg/g)	
	Fresh peach	Pasteurized peach
Zeaxanthin	0.8 ± 0.1^{a}	0.1 ± 0.03^{b}
Lutein	0.2 ± 0.00^{a}	0.2 ± 0.04^{a}
β-Cryptoxanthin	0.2 ± 0.04^{a}	ND
β-Carotene	0.1 ± 0.02^{a}	0.1 ± 0.02^{a}

Different letters within the same row indicate significant differences between means ($P < 0.05$).

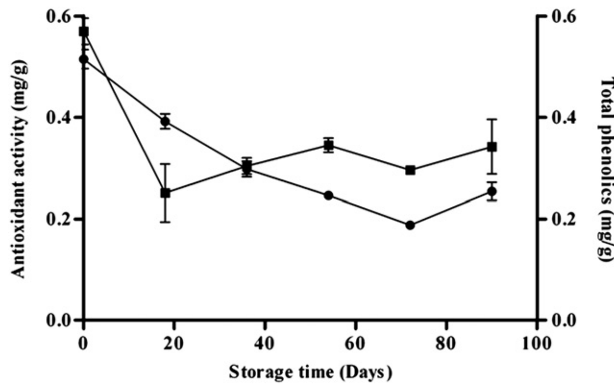
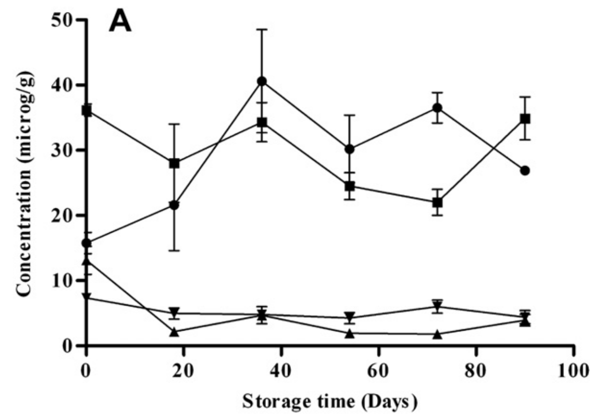


Fig. 1. Total antioxidant activity (●) and total phenolic content (■) in samples of pasteurized peach stored at 22 °C for 90 days. Values are mean ± SD ($n = 3$).

The antioxidant activity of phenolic compounds depends on their chemical structure, namely on the number and arrangement of the hydroxylated groups (Cao, Sofic, & Prior, 1997; Rice-Evans, Miller, & Paganga, 1996; Sang et al., 2002). In this class of phytochemicals, hydroxylation is directly related to antioxidant activity (Srisvastava, Akon, Fischer, & Krewer, 2007).

Changes during storage were also observed for individual phenolics (Fig. 3) and carotenoids (Fig. 4). The concentration of quercetin-3-rutinoside decreased rapidly during the first 18 days in storage, from 7.4 to 5.0 μg/g, and remained relatively unchanged during the remaining storage period (Fig. 3A). The reduction of quercetin-3-rutinoside levels strongly contributes to the decrease in antioxidant activity. The contribution of the 3-hydroxyl group in flavonoids is very relevant for their antioxidant activity (Heijnen, Haenen, van Acker, van der Vigh, & Bast, 2001); blocking the 3-hydroxyl group in the B ring (i.e., rutin) significantly decreases the antioxidant activity of flavonoids (Heijnen et al., 2001; López, Martínez, Del Valle, Ferrit, & Luque, 2003; Salah et al., 1995; Villaño, Fernández-Pachón, Troncoso, & García-Parrilla, 2005). Although the decrease in quercetin-3-rutinoside during storage was not significant (Table 2) the quantitative changes may account for some of the variation in antioxidant activity. (−)-Epicatechin exhibited significant reduction, from 13.1 to 2.2 μg/g fw, during the first 18 days in storage and little variation thereafter (Fig. 3A). Thermal processing induces a decrease in (−)-epicatechin subunits as report for processed Chinese quince fruit (Hamauzu, Kume, Yasui, & Fujta, 2007). The procyanidin B1 increased significantly from 15.7 to 40.6 μg/g fw during the first 36 days of storage and then decrease to 26.9 μg/g fw by the end of the 90-day storage period (Fig. 3A). Similarly, flavan-3-ols are affected by pasteurization in grape juice. In this matrix, increased levels of total procyanidins result from depolymerization of procyanidins with a high molecular weight into dimers and trimers (Fuleki & Ricardo-da-Silva, 2003). However, reductions in the concentration of procyanidins have been reported during storage of canned peach, possibly



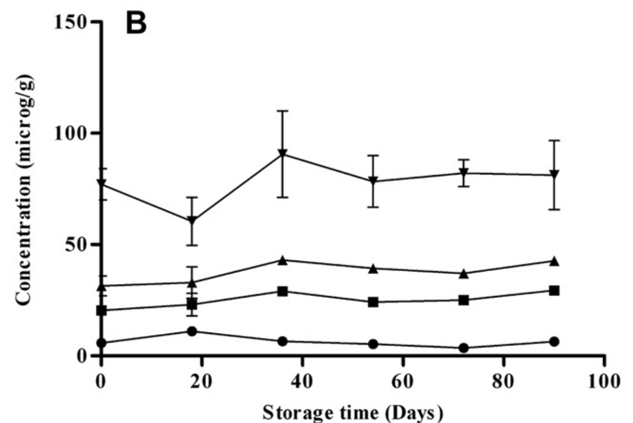


Fig. 3. Concentration of individual flavonoids (A; ● procyanidin B1; ■ (+)-catechin; ▲ (−)-epicatechin; ▼ rutin) and phenolic acids (B; ■ neochlorogenic acid; ▲ chlorogenic acid; ▼ 2,5-dihidroxybenzoic acid; ● protocatechuic acid) in samples of pasteurized peach stored at 22 °C for 90 days. Values are mean ± SD ($n = 3$).

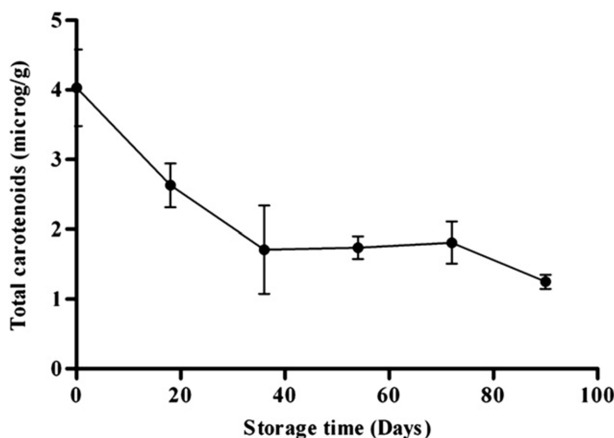


Fig. 2. Total carotenoids in samples of pasteurized peach stored at 22 °C for 90 days. Values are mean ± SD ($n = 3$).

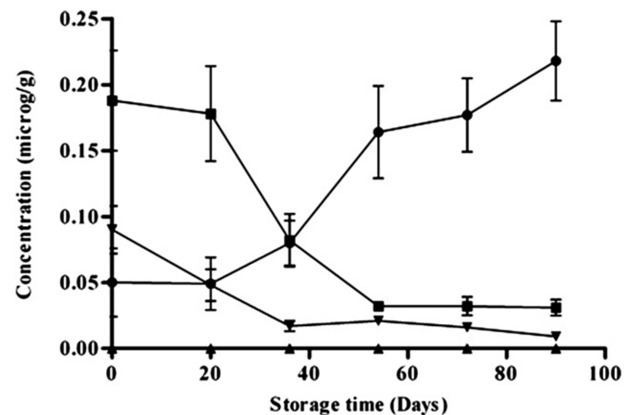


Fig. 4. Concentration of individual carotenoids (● zeaxanthin; ■ lutein; ▲ β-cryptoxanthin; ▼ β-carotene) in samples of pasteurized peach stored at 22 °C for 90 days. Values are mean ± SD ($n = 3$).

due to the migration from the fruit matrix into the syrup (Hong et al., 2004).

The levels of chlorogenic acid and neochlorogenic acid increased during the first 36 days of storage by 35% and 43%, respectively (Fig. 3B) and remained unchanged thereafter. The heat-inactivation of polyphenoloxidase (PPO) reduces the oxidative conversion of these phenolic acids into quinones, preserving the levels of chlorogenic acid (Bakshi & Masoodi, 2010). The reasons for the increased levels of chlorogenic acid are not clear. Chlorogenic acid is a water-soluble compound located in the cell vacuole (Skrede, Wrolstad, & Durst, 2000). Easier extraction may be due to a gradual equilibration of vacuolar contents of pasteurized peach cubes and the extracellular matrix during storage. Levels of protocatechuic acid and 2,5-dihydroxybenzoic acid increased slightly, but not significantly (Fig. 3B).

The rate of decrease in the levels of phenolic acids and flavonoids in cubes of heat-treated peach stored at room temperature was, by decreasing order, (−)-epicatechin > rutin > (+)-catechin. For the phenolics whose levels increased during storage, the rate of variation was procyanidin B1 > neochlorogenic acid > chlorogenic acid > protocatechuic acid > 2,5-dihydroxibenzoic acid.

The overall reduction in antioxidant activity during storage of pasteurized peach cubes can be largely explained by the variations in the different classes of phenolic compounds. Flavan-3-ols, the phenolic compounds with more hydroxyl groups, have a higher specific activity against the ABTS radical (Apak et al., 2007; Lien, Ren, Bui, & Wang, 1999; Salah et al., 1995). Rutin, a flavonoid hydroxilated in the position 3, has also a high specific antioxidant activity, as measured by the ABTS method (Lien et al., 1999; Salah et al., 1995). Therefore, the decrease in total antioxidant activity during storage (Fig. 1) is likely related to the decrease in the levels of (+)-catechin, (−)-epicatechin, and rutin (Fig. 3A). Chlorogenic acid do not reduce the ABTS radial (Villaño et al., 2005) and protocatechuic acid and 2,5-dihidroxybenzoic acid (Nenadis, Wang, Tsimidou & Zhang, 2004; Villaño et al., 2005) have a moderate antioxidant activity against the same radical. Therefore, the variations in the levels of these compounds (Fig. 3B) had little contribution to the changes in the overall antioxidant activity.

Heat treatment eliminated β-cryptoxanthin from processed peach (Table 3) and this xanthophyll remained undetected throughout the storage period (Fig. 4). β-Carotene and lutein decreased 90 and 83%, respectively, during the 90-day storage period (Fig. 4). Oxidation and isomerization reactions are likely responsible for the decreased levels of these two carotenoids (Rodriguez-Amaya, 1999). In contrast, the concentration of zeaxanthin increased during storage from 0.05 to 0.2 μg/g fw (Fig. 4). A similar trend was also observed in sweet corn after a heat treatment (Updike & Schwartz, 2003). Potato zeaxanthin proved to be very heat-stable (Maiani et al., 2009).

Gradual dissociation during storage of zeaxanthin from the native protein complex stimulated by previous heat treatment may explain the observed increase (Sánchez-Moreno, Plaza, Ancos, & Cano, 2003). The rate of reduction in the levels of carotenoids, by decreasing order, was β-cryptoxanthin > β-carotene > lutein, with increases in the levels of zeaxanthin during storage. Individual carotenoids were significantly correlated with the antioxidant activity determined by ABTS. Antioxidant activity was positively correlated with lutein ($r^2 = 0.87$), β-carotene ($r^2 = 0.88$) but negatively correlated with zeaxanthin ($r^2 = 0.66$).

4. Conclusions

Pasteurization of fresh peaches significantly reduced the concentration of total carotenoids, completely eliminating β-cryptoxanthin. Subsequent changes in carotenoids occurred during storage with increased levels of zeaxanthin and reduction in the concentration of lutein and β-carotene. Peach phenolic compounds were less affected by the heat treatment, but significant decreases in protocatechuic acid occurred immediately after pasteurization. During storage, the levels of all phenolics changed significantly with the exception of protocatechuic acid and 2,5-dihydroxibenzoic acid levels. Heat treatment differentially affected individual carotenoids and phenolic compounds. Carotenoids are good markers for the nutritional and functional quality of processed peach since they were very sensitive to pasteurization and changed during storage.

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