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Enhancement in the oxidative stability of green peas by *Ilex paraguariensis* addition in a blanching process before their refrigerated and frozen storage

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ABSTRACT

Green peas have a short shelf-life because their unsaturated fatty acids are exposed to oxidative damage induced by pro-oxidant enzymes leading to organoleptic changes and nutritional quality losses. Blanching (BL) is a crucial treatment in the successful production of high quality frozen vegetables to inactivate the pro-oxidant enzymes to prevent possible food deterioration reactions. *Ilex paraguariensis* extract (IP) has evidenced antioxidant effect and it was ascribed to their phenolic constituents. The main aim of the present work was to evaluate the effect of BL combined with IP on the oxidative stability of green peas along refrigerated and frozen storage. Fresh peas were exposed to different treatments for 2 min and compared to untreated samples: BL in water at 97 °C; dipping in IP at 25 °C (IPD), BL combined with IP at 97 °C (BL/IP). Afterwards, samples were stored at 4 and -18 °C for 60 days. Lipoxygenase activity and oxidative stability (taking malondialdehyde and hexanal as oxidation markers) have been monitored in stored peas as indicators of the assayed treatments effectiveness. BL/IP extract was the most effective treatment to inhibit oxidation and it can be a new alternative in order to enhance oxidative stability of peas.

1. Introduction

As green legumes are good sources of nutrients, they are strongly recommended for a healthy diet. The current lifestyle increases the consumption of foods stored in refrigerated or frozen conditions to ensure their ready availability at home. Frozen ready meals are the largest sector of food industry in terms of volume and value and their marketing is focused on convenience as these products require short preparation time (Canet, Alvarez, Luna, & Fernández, 2004). Green peas (*Pisum sativum* L.) belong to the family of cool season legume crops. They are rich in proteins, carbohydrates, and water-soluble fibers (Bhattacharya & Malleshi, 2012). In addition, they contain starch with a low glycemic index and high contents of ascorbic acid, β-carotene, thiamine, riboflavin and unsaturated fatty acids as oleic, linoleic (LA) and linolenic acids (Tharanathan & Mahadevamma, 2003). Fresh vegetables have a short shelf-life because these unsaturated fatty acids are exposed to oxidative damage induced by pro-oxidant enzymes such as lipoxygenase (LOX) (Szymanowska, Jakubczyk, Baraniak, & Kur, 2009). LOX is a non-heme iron dioxygenase which catalyses the oxidation reaction of unsaturated fatty acids containing a *cis*, *cis*-1,4-pentadiene into conjugated hydroperoxides as primary products. Then, these unstable compounds are cleaved by hydroperoxide lyase (HPL). HPL mediated lysis of hydroperoxides yields hexanal and (3Z)-hexenal, (3Z,6Z)-nonadienal, (3Z)-nonenal and malondialdehyde (MDA) as secondary oxidation products. These oxidative processes lead to organoleptic changes and losses of food nutritional quality. The presence of LOX in peas has previously been reported (Rodríguez-Concepción & Beltrán, 1995; Gardner, Sherrier, Kardailsky, & Brewin, 1996; Wisniewski, Gardner, & Brewin, 1999); however, these studies focused on the enzyme genetic or the molecular properties of its isoforms; by contrast, there is scarce information about variations in oxidation product profile during green pea storage.

To reduce oxidative damage of foods, preservation technologies are necessary to combine shelf-life extension with the maintenance of sensorial and nutritional features (Jabbar et al., 2014). In this sense, BL is a crucial treatment in the successful production of high quality of frozen vegetables and its main aim is to inactivate the pro-oxidant enzymes such as LOX and peroxidase to prevent possible deterioration reactions off-flavor and undesirable changes in color. During this process, vegetables are immersed in hot water (70–100 °C) for several minutes; the blanched samples are drained and cooled before being sent to the next processing operation.

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The selection of suitable BL conditions is an important factor for food industry because a non-appropriate choice may have an adverse effect on physical and nutritional properties of the foods (Wang et al., 2017). In addition, water-soluble nutrients, such as vitamins, flavors, minerals, carbohydrates, sugars, and proteins can leach out from the plant tissue into the BL water (Kinalski & Noreña, 2014). To mitigate those drawbacks, novel technologies have been developed and reported, e.g., BL by microwave irradiation, by high-humidity hot air exposure, and by infrared irradiation, among others (Chandrasekaran, Ramanathan, & Basak, 2013). On the other hand, it has been informed that the BL with 0.2% ammonium bicarbonate retains quality parameters of green beans stored for 6 months at −18 °C (Samah, 2016). Other chemicals, such as sodium sulfite and sodium metabisulfite, are also often added to the BL water to preserve product color and to inactivate microbial activity (Kinalski & Noreña, 2014).

On the other hand, it has been informed that the addition of preservatives to foods would increase their stability (Aziz & Karboune, 2016). In this sense, the addition of *Ilex paraguariensis* extract (IP) has evidenced antioxidant effect in some food products, such as chicken (Racanicci, Danielsen, & Skibsted, 2008) and sausages (Beal, Faion, Cichoski, Cansian, Valduga, & De Oliviera, 2011). IP is a native plant from South America, widely consumed as tea-like beverage and nowadays, is globally popular. The antioxidant properties of IP extract are attributed to their phenolic constituents, and their levels are greater than those of green tea and similar to those found in red wine (Valerga, Reta, & Lanari, 2012). Moreover, it has also been demonstrated that it can reduce the oxidative stress levels of melon plants exposed to thermal stress during the early production of this crop (Yonny et al., 2016). However, the use of IP in BL processes of vegetables has not been reported yet.

The main aim of the present work was to evaluate the effect of the combined BL and IP treatments on the oxidative stability of green peas along their refrigerated and frozen storage. We propose herein that IP aqueous extract can retard oxidative degradation during the storage of peas.

2. Materials and methods

2.1. Reagents and solvents

Malondialdehyde *bis*(diethylacetal) or 1,1,3,3-tetraethoxypropane (TEP), hexanal, 1,1-diphenyl-2-picrilhidrazyl radical (DPPH$^{\bullet}$), and chlorogenic and caffeic acids were purchased from Sigma (St. Louis, SA). Thiobarbituric acid (TBA) was obtained from Merck (Darmstast, Germany). Trichloroacetic acid (TCA) was purchased from Biopack (Buenos Aires, Argentina). Acetophenone was purchased from Anedra (Buenos Aires, Argentina). All aqueous solutions were prepared with Milli-Q water.

2.2. Preparation of IP extract

IP ground dried leaves were bought at a local supermarket. The extract was prepared by decoction of IP minced leaves in boiling water for 40 min. The concentration was 20 g L^{-1}; being this procedure similar to the preparation of the popular beverage known as cooked-mate. Afterwards, the extract was allowed to reach room temperature and filtered through a colander (Anbinder, Deladino, Navarro, Amalvy, & Martino, 2011).

Antiradical activity (ARA) against DPPH$^{\bullet}$ of the IP extract was expressed as vitamin C equivalent antioxidant capacity (VCEAC) and was 2.26 ± 0.04 mg ascorbic acid equivalent g^{-1} (Kim, Lee, & Lee, 2002).

2.3. Determination of phenolic compounds in IP extract

HPLC analyses were carried out with a Lab Alliance chromatographic system, equipped with two pumps and a C_{18} column (250 × 4.6 mm ID, 5 μm). Chromatographic separations were carried out using as mobile phase a water, acetonitrile (ACN) and formic acid (FA) mixture (94.7: 5: 0.3) (A) and an ACN-FA solution (99.7: 0.3) (B) with a flow rate of 1.0 mL min^{-1}. The gradient program was: 0–20 min pure A; 20–30 min A/B 90/10; 30–40 min A/B 70/30; 40–55 min A/B 40/60; 55–57 min A/B 20/80; 57–59 min pure A. Identification and quantification were carried out by comparison of retention times and diode array (DAD) UV–Vis spectra with those from analytical grade commercial standards. Determinations were performed by triplicate.

2.4. Samples and treatments

Samples of 60 g of fresh peas were washed, drained and divided into four groups: control without treatment; BL in water for 2 min at 97 °C (BL); dipping in IP extract at 25 °C for 2 min (IPD); and BL with the mentioned IP extract at 97 °C for 2 min (BL/IP). For blanched peas, 50 g samples were soaked in 5 L of extract or water according to the recommended guidelines for blanching foods (Chioffi & Mead, 1991).

Then, samples were packaged in individual sealed bags and stored in fridge (4 °C) and freezer (−18 °C). The analyses were performed at 0, 3, 7, 30 and 60 days.

2.5. Determination of LOX activity in peas

LOX activity was evaluated in the treated peas stored under different conditions as suggested by Gökmen, Bahçeci, and Acar (2002). Enzyme extraction was carried out by homogenizing 10 g of fresh peas (washed and drained) with 50 mL of water at 4 °C in a tissue homogenizer at 17,000 rpm for 2 min. The slurry was filtered and centrifuged at 15,000 × g for 30 min. The supernatant containing LOX was used as the crude enzyme extract. The substrate solution, 2.5 mmol L^{-1} linoleic acid, was prepared in Tween-20 adding 0.067 mol L^{-1} sodium phosphate buffer (pH 6.0). The oxidation reaction started when the enzyme aliquot was added to the substrate micelle system. The formation of conjugated dienes was monitored at 234 nm in a 0.2 cm path-length cuvette. One unit of LOX activity was defined as an increase in absorbance at 234 nm of 0.001 min^{-1} g^{-1} of sample under assay conditions.

2.5.1. Inhibition of pea LOX activity by phenolic compounds in a model system

The effect of IP extract and of their main constituents as pure compounds was determined on pea LOX activity, in triplicate, following the same procedure as described in 2.4 Section. Before its addition to the linoleic acid-Tween 20 micelles, the enzyme was incubated for 10 min with 20 g L^{-1} IP aqueous extract, chlorogenic and caffeic acids, or the mix of them in the levels quantified in 2.3 Section; the control being the system in absence of phenolic compounds. Percentage enzyme inhibition was calculated by the following equation:

$$\%\ LOX\text{inh} = \frac{A_1 - A_2}{A_1} \times 100$$

being A_1 the LOX activity of control and A_2 the LOX activity of sample.

2.6. Oxidative stability in stored peas: determination of thiobarbituric reactive species and hexanal levels

Lipid oxidation products levels were evaluated in pea samples taking into account the different storage conditions assayed. Among the secondary oxidation products, MDA and hexanal are recognized markers of an advanced oxidative status (Janero, 1990).

The methodology used to determine the lipid oxidation advance is the thiobarbituric reactive species (TBARS) assay according to the method of Noreen and Ashraf (2009) with minor modifications. Pea sample was homogenized at 17,000 rpm for 3 min, using 1g L^{-1} TCA solution *w/v*. Subsequently, the homogenate was centrifuged at 15,000 × g at 4 °C for 15 min. A supernatant aliquot of 1.0 mL was

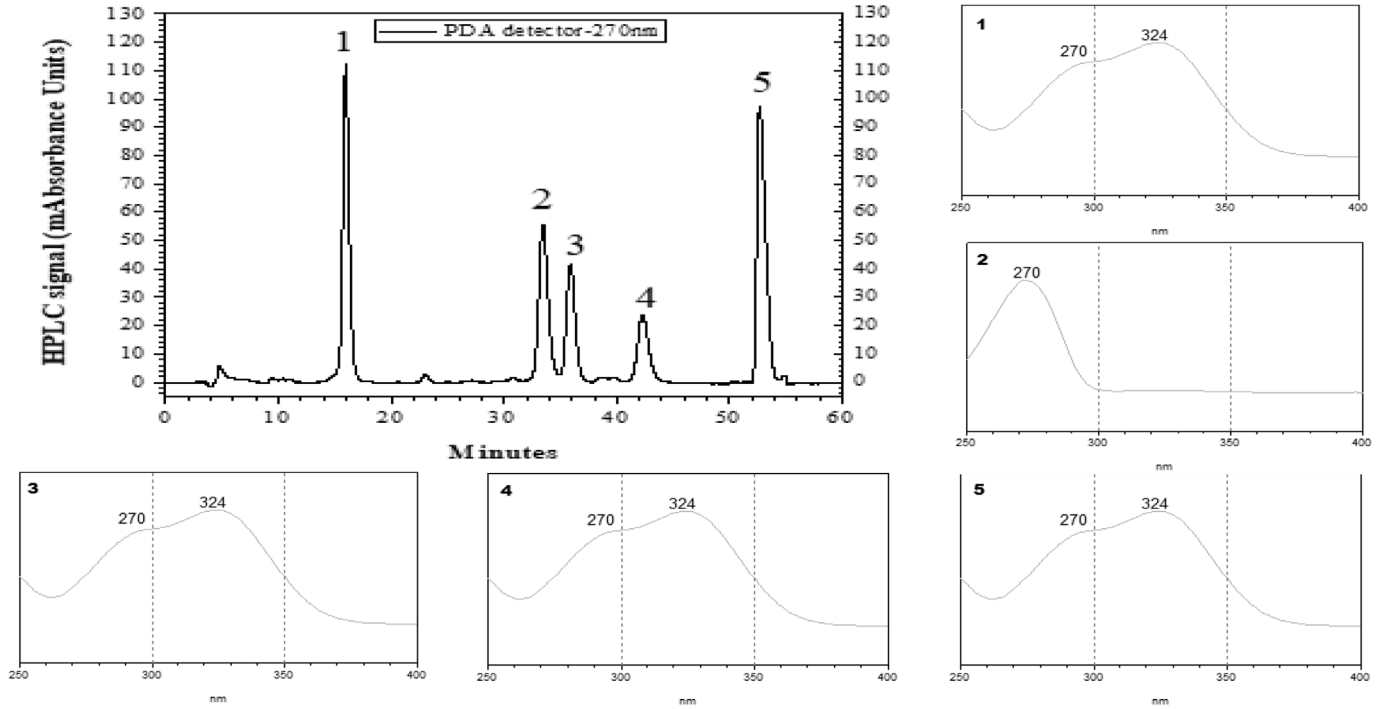

Fig. 1. Phenolic profile of IP extract analyzed by HPLC-DAD. Peak 1) chlorogenic acid (*trans*-5-O-caffeoylquinic acid). Peak 2: caffeic acid. Peaks 3, 4 and 5: 3,4–3,5 and 4,5-di-caffeoylquinic acids, respectively.

Table 1
Reduction of fresh pea LOX activity by antioxidant addition in LA oxidation model system.

Treatment	Enzyme residual activity (ULOX g^{-1} min^{-1})	Inhibition percentage (%)
Control	10.14 ± 0.38	Considered as 0%
IP	4.06 ± 0.14	59.96 ± 1.95
Chlorogenic acid	4.62 ± 0.13	54.44 ± 1.81
Caffeic acid	5.54 ± 0.13	45.36 ± 1.81
Chlorogenic and caffeic acids	5.00 ± 0.12	50.69 ± 1.67

taken and mixed with 4.0 mL of 5 g L^{-1}*w/v* TBA in 200 g L^{-1}*w/v* TCA. The reaction mixture was incubated at 95 °C for 30 min. Then, it was cooled in an ice bath for 10 min and centrifuged at 10,000 × g at 4 °C for 10 min. Finally, the absorbance was determined at 532 nm. TEP dilutions were prepared in 1 g L^{-1}*w/v* TCA and incubated at 40 °C for 30 min to obtain MDA for quantification purposes by external calibration ($y = -0.018 + 0.16\,x$; $R^2 = 0.9998$). MDA concentration was expressed in g kg^{-1} of peas.

To determine hexanal, the method proposed by Elisia and Kitts (2011) was used with minor modifications. Pea samples were placed in vials, the internal standard (acetophenone) was added. Vials were sealed and incubated at 50 °C for 15 min. Then, the solid phase micro-extraction (SPME) fiber was inserted into the vial headspace (HS) for 15 min allowing that volatile compounds are absorbed into the polymer fiber (polydimethylsiloxane, 100 μM). Finally, they were desorbed and quantified by placing the fiber in the gas chromatograph detection flame ionization KONIK 3000 C equipped with a capillary Zebron ZB-FFAP column with polar phase (15 m × 0.32 mm × 0.25 μm) and using N_2 as the mobile phase at a flow of 35.3 mL min^{-1}. Injector and detector temperatures were set at 250 °C, and the column temperature was kept 4 min at 80 °C, increased at a rate of 8 °C. min^{-1} up to 140 °C and kept for 5 min, increased at a rate of 15 °C min^{-1} up to 230 °C, and kept for 1 min.

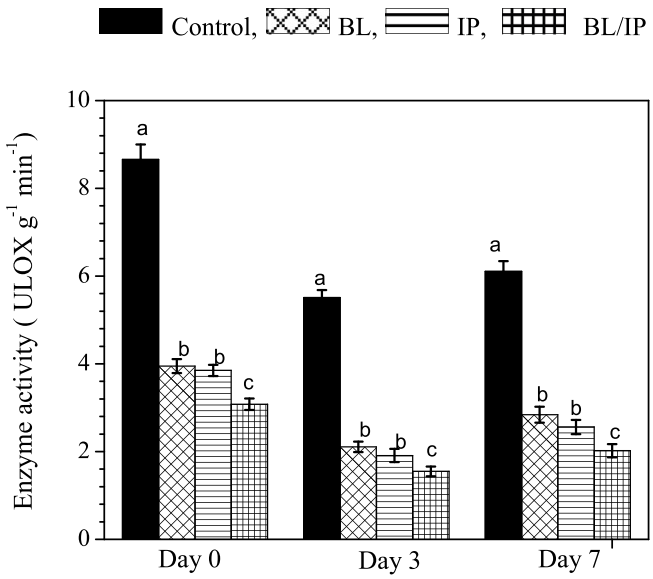

Fig. 2. LOX residual activity in fresh peas after their treatments and storage at 4 °C.

2.7. Determination of the antiradical activity against DPPH• in stored peas

Portions of 2.0 g of peas were homogenized using methanol as extraction solvent and afterwards, ultrasound irradiated for 30 min. Finally, the extract was centrifuged at 10,000 rpm at 4 °C for 15 min.

The antiradical activity (ARA) against DPPH• was measured spectrophotometrically and calculated as suggested in Chaillou and Nazareno (2006) report and the results are expressed as VCEAC in g ascorbic acid equivalent kg^{-1}.

2.8. Statistical analysis

The results were expressed as mean ± standard deviation; and they were statistically evaluated using analysis of variance (ANOVA)

Table 2
LOX residual activity of fresh peas after their treatments and storage at −18 °C.

Treatment	Storage time (days)				
	0	3	7	30	60
	Enzyme activity (ULOX g^{-1} min^{-1})				
Control	(8.66 ± 0.34)[a]	(6.74 ± 0.23)[a]	(7.03 ± 0.34)[a]	(7.13 ± 0.40)[a]	(7.04 ± 0.30)[a]
BL	(3.95 ± 0.16)[b]	(2.37 ± 0.14) [b]	(3.04 ± 0.23)[b]	(2.86 ± 0.17)[b]	(2.96 ± 0.22)[b]
IP	(3.85 ± 0.13)[b]	(2.19 ± 0.13)[b,c]	(2.72 ± 0.16)[b]	(2.67 ± 0.17)[b]	(2.52 ± 0.18)[c]
BL/IP	(3.38 ± 0.13)[c]	(1.95 ± 0.11)[c]	(2.21 ± 0.15)[c]	(2.17 ± 0.14)[c]	(2.20 ± 0.17)[c]
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

The values are expressed are means ± standard deviations, n = 3 for each treatment. Different letters in column represent statistically different mean values (P < 0.05).

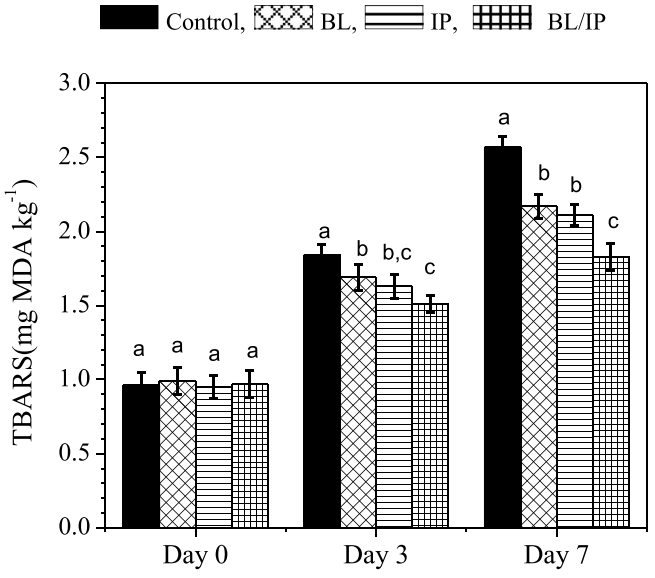

Fig. 3. Oxidative stability of fresh peas after their treatments and storage at 4 °C.

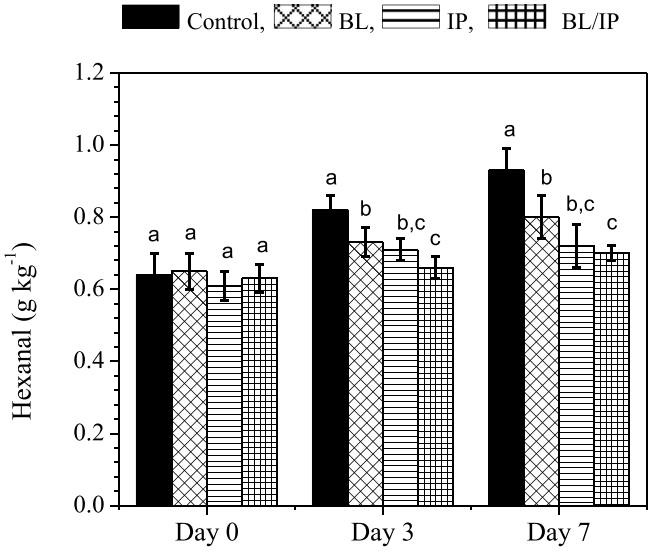

Fig. 4. Hexanal levels of fresh peas after their treatments and storage at 4 °C.

INFOSTAT statistical package, version 2012, INFOSTAT Group, Faculty of Agricultural Sciences, National University of Cordoba, Argentina. When the effects were found to be significant ($p < 0.05$), Fischer LSD-test was used to compare means.

3. Results and discussion

3.1. Phenolic compounds in IP extract

The identification and quantification of the major constituents of IP extract was carried out by HPLC-DAD (Fig. 1). Chlorogenic and caffeic acids were the major phenolic compounds present in the IP extract as Fig. 1 shows. Other compounds were also observed and may correspond to dicaffeoylquinic acid isomers as suggested by Gonzáles de Mejía, Song, Heck, and Ramirez-Mares (2010) and Gebara et al. (2017), who informed that these isomers correspond to the 3,4–3,5 and 4,5-di-caffeoylquinic acids in IP aqueous extract under the same experimental conditions. Thus, the content of chlorogenic acid and the isomers (expressed as chlorogenic acid) in our IP extract is 4421.2 μg mL^{-1}, and the content of caffeic acid and 243.9 μg mL^{-1}. The IP extract composition has been previously characterized by several reports (Filip, L, ó, pez, Giberti, Coussio, & Ferraro, 2001; Gonzáles de Mejía et al., 2010; Valerga et al., 2012), being caffeoyl derivatives such as chlorogenic and caffeic acids as the major compounds; although, it is strongly dependent on its industrial processing, geographical provenance, among others factors.

3.2. Enzyme activity

3.2.1. LOX activity in inhibition model system

The residual activity of LOX was determined after addition of IP extract, chlorogenic and caffeic acids, and their mixture is shown in Table 1. This result proved for the first time, IP extract had an inhibitory effect on pea LOX and it can be attributed to their phenolic compounds.

The ability of chlorogenic and caffeic acids to inhibit soybean LOX activity has been reported by Chaillou and Nazareno (2006). In addition, it has been demonstrated that the caffeic acid inhibits LOX in pea seeds var. Telephone (Szymanowska et al., 2009). In the present work, the inhibitory effect on pea LOX of chlorogenic acid and IP extract containing both phenolics has been demonstrated.

It has been proven that the inhibition degree depends on the number and position of phenolic groups because these play a fundamental role as inhibitors in oxidation processes. In this sense, both compounds mentioned above present the catechol group in their structure, which is one of the structural groups required for exhibiting high antioxidant activity (Benavente-García, Castillo, Marin, Ortuño, & Del Río, 1997).

These results support the possibility of using IP aqueous extract combined with BL method on fresh peas as a new alternative in order to prevent their oxidative degradation.

3.2.2. LOX activity in stored peas

LOX activity in stored peas has been monitored as an indicator of the effectiveness of assayed treatments. Fresh peas were exposed to three different treatments and compared to control (BL, IPD, BL/IP and

Table 3
Oxidative stability of fresh peas after their treatments and storage at −18 °C.

Treatment	Storage time (days)				
	0	3	7	30	60
	TBARS (mg MDA kg^{-1})				
Control	(0.95 ± 0.07)[a]	(2.16 ± 0.09)[a]	(3.14 ± 0.09)[a]	(3.13 ± 0.07)[a]	(3.18 ± 0.08)[a]
BL	(0.98 ± 0.08)[a]	(1.96 ± 0.09)[b]	(2.85 ± 0.07)[b]	(2.90 ± 0.08)[b]	(2.84 ± 0.05)[b]
IP	(0.94 ± 0.07)[a]	(1.80 ± 0.08)[b,c]	(2.76 ± 0.06)[b,c]	(2.73 ± 0.06)[c]	(2.75 ± 0.06)[b,c]
BL/IP	(0.96 ± 0.07)[a]	(1.70 ± 0.09)[c]	(2.65 ± 0.08)[c]	(2.62 ± 0.07)[c]	(2.66 ± 0.05)[c]
P value	0.6740	0.0010	0.0003	0.0001	< 0.0001
	Hexanal (g kg^{-1})				
Control	(0.63 ± 0.05)[a]	(0.89 ± 0.06)[a]	(1.65 ± 0.08)[a]	(1.65 ± 0.09)[a]	(1.59 ± 0.06)[a]
BL	(0.64 ± 0.06)[a]	(0.79 ± 0.03)[b]	(1.52 ± 0.03)[b]	(1.46 ± 0.03)[b]	(1.50 ± 0.08)[b]
IP	(0.60 ± 0.04)[a]	(0.76 ± 0.03)[b,c]	(1.44 ± 0.05)[b]	(1.36 ± 0.07)[b]	(1.39 ± 0.09)[b,c]
BL/IP	(0.62 ± 0.04)[a]	(0.70 ± 0.03)[c]	(1.28 ± 0.09)[c]	(1.27 ± 0.06)[c]	(1.30 ± 0.05)[c]
P value	0.7727	0.0025	0.0010	0.0012	0.0155

The values are expressed are means ± standard deviations, n = 3 for each treatment. Different letters in column represent statistically different mean values (P < 0.05).

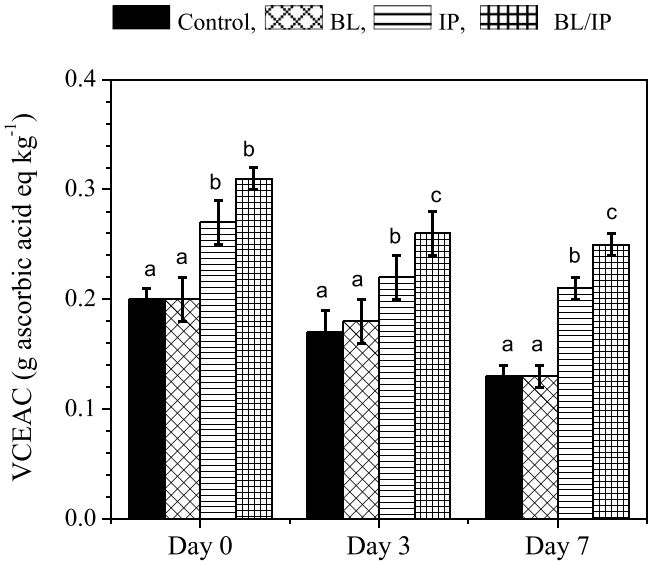

Fig. 5. Antiradical Activity of fresh peas after their treatments and storage at 4 °C.

C). Afterwards, pea LOX was extracted and its activity was evaluated in treated peas stored at 4 (Fig. 2) and −18 °C (Table 2) for 7 and 60 days, respectively. LOX residual activities immediately after BL, IPD and BL/IP treatments were 45.6, 44.4 and 35.6%, respectively and were significantly different compared to C (Fig. 2). After food preservation at 4 and -18 °C, results showed significant statistical differences along storage time (Supplementary material). Bahçeci, Serpen, Gökmen, and Acar (2005) reported that 90% of LOX activity was lost in green peas after water BL. On the other hand, Güne and Bayindirli (1993) obtained approximately 30% remaining or residual activity after water BL in green beans.

Enzyme activity decreased after 3 days storage at both temperatures for all treatments. Then, after 7 days, the LOX activity levels slightly increased both at 4 and -18 °C, being the lowest residual activity for the BL/IP treatment and the highest for the untreated peas.

3.3. Oxidation product levels in peas after storage

The oxidative stability of peas was monitored as TBARS and hexanal levels as advanced lipid oxidation products.

Oxidation products were analyzed in pea samples after storage at 4 °C; TBARS and hexanal levels are shown in Figs. 3 and 4, respectively. Measurements were considered until 7 days because after that, pea microbiological spoilage was observed.

Food oxidative stability was monitored up to 60 days in treated peas stored at −18 °C. TBARS and hexanal levels are shown in Table 3. The highest levels of TBARS and hexanal were registered after 7 days of storage; afterwards, significant changes in their concentrations were not observed.

These results could be explained taking into account the activity of the oxidant enzymes present in peas. As shown Section 3.2, LOX activity progressively increased after inhibition due to the treatments, up to 7 days and then it remained without marked changes during storage. Hornostaj and Robinson (2000) registered in their experiments that the maximum activity of HPL is found between the 3 and 7 days and then, this activity decreased slowly. In this way, it could be said that both enzymes involved in the oxidative process influenced the generation of secondary oxidation products.

Table 4
Antiradical Activity of fresh peas after their treatments and storage at −18 °C.

Treatment	Storage time (days)				
	0	3	7	30	60
	VCEAC (g ascorbic acid equivalent kg^{-1})				
Control	(0.21 ± 0.01) [a]	(0.17 ± 0.01) [a]	(0.16 ± 0.01)[a]	(0.15 ± 0.01) [a]	(0.15 ± 0.02) [a]
BL	(0.20 ± 0.02) [a]	(0.19 ± 0.01) [a]	(0.16 ± 0.02)[a]	(0.17 ± 0.02) [a]	(0.17 ± 0.02) [a]
IP	(0.26 ± 0.02) [b]	(0.23 ± 0.02) [b]	(0.23 ± 0.01)[b]	(0.22 ± 0.02) [b]	(0.22 ± 0.01) [b]
BL/IP	(0.32 ± 0.01) [c]	(0.29 ± 0.03) [c]	(0.29 ± 0.03)[c]	(0.27 ± 0.01) [c]	(0.27 ± 0.01) [c]
P value	0.7727	0.0025	0.0010	0.0012	0.0155

The values are expressed are means ± standard deviations, n = 3 for each treatment. Different letters in column represent statistically different mean values (P < 0.05).

After 7 days, the levels of the secondary oxidation products were higher in peas stored at −18 °C than the ones stored at 4 °C. This result could be explained considering that the peas stored in freezer may have damaged the cellular structure due to the presence of microcrystals of water (Reno, Torres Prado, & Villela de Resende, 2011). This situation allowed a major contact between different cellular material, oxidant enzymes and lipids, and therefore, oxidation reaction is induced. In this sense, Hornostaj and Robinson (2000) found an approximate 80-fold increase of hexanal formation in frozen shoot/roots sections of peas compared to fresh sections.

Concerning sample treatments, under both storage conditions, statistical differences in the TBARS and hexanal levels were observed and their levels in treated peas were lower than those of the control samples. The BL/IP was the best treatment. Results obtained in this work evidenced the additional protective effect of IP bioactive substances to prevent the oxidation advance of blanched peas under short-term storage. This may be explained considering that IP is rich in antioxidants compounds which are good pea LOX inhibitors.

3.4. Antiradical activity of stored peas

Variations in the ARA of the peas after treatment and stored at 4 and -18 °C were shown in Fig. 5 and Table 4, respectively. The disappearance of DPPH$^{\bullet}$ radical was monitored after the addition of an aliquot of the pea methanolic extracts. The results were calculated using the equation $y = -1.58 + 3.95x$ ($R^2 = 0.9996$) obtained from external calibration with ascorbic acid.

Table 4 shows higher ARA capacity in IP treated peas than that found in C and BL peas. This can be explained as the polyphenols present in the extract are able to diffuse into the peas. Similar results were obtained by Rodriguez-Arzuaga et al. (2016) in apple pieces treated with IP extract.

The ARA decreased as a function of the sample storage time for all the treatments.

Significative statistical differences were found among the mean values of the sample treatments within the same storage temperature. The BL/IP treatment presented the highest ARA followed by the IPD at 25 °C. This result is consistent with the lowest TBARS and hexanal formation, thus, the bioactive substances present in IP extract are effective to retard the oxidative degradation process in the stored peas.

After 7 days, ARA decreased a 35.0% from the initial value in the untreated peas stored at 4 °C (Fig. 5). This value is approximately the half of the value found by Babatola, Ojo, and Lawal (2008) after storing pea samples at 8 °C. The ARA decrease was also 35.0% after water BL treatment in respect to the initial value. The last value is in agreement with Hunter and Fletcher (2002), who observed a 30.0% ARA decrease after BL and storage of peas. ARA decreased 22.0 and 19.0% when peas were treated with IPD and BL/IP, respectively. The mean ARA values at −18 °C (Table 4) indicate that this activity decreased to 24.0% from the initial value to control peas and 20.0% in the case of BL treatment. These values are in agreement with those expressed by Puupponen-Pimia et al. (2003), who found that the ARA decrease 30 and 20% in frozen peas without and with BL; ARA decreased 11 and 9% after 60 days in IPD and BL/IP samples.

A higher retention of ARA was found for frozen peas than refrigerated peas. This result is coincident to Ninfali and Bacchiocca (2003) report.

4. Conclusion

This work proposes an alternative BL method to prevent the oxidative deterioration in fresh peas, taking into account that the oxidation induced by LOX can be modulated by the action of antioxidants.

The most effective treatment to inhibit pea LOX activity was the combination BL/IP extract, being capable to decrease the formation of oxidation products (MDA and hexanal), and to increase the intrinsic protective food ARA during the storage time. The second one was IPD at 25 °C. To the best of our knowledge, this is the first time that BL is carried out using an IP extract to enhance the oxidative stability of peas stored under refrigerated and frozen conditions. Thus, this combined treatment of peas before their storage constitute an innovative strategy to improve the food oxidative stability; although beyond the scope of this study, changes in organoleptic traits in IP treated peas deserve further studies. The evaluation of possible variations in sensorial properties as well as consumer acceptance of IP treated peas are new research aspects to be addressed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2018.01.063.

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