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Effect of thermal blanching and of high pressure treatments on sweet green and red bell pepper fruits (*Capsicum annuum* L.)

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Abstract

The effect of pressure treatments of 100 and 200 MPa (10 and 20 min) and of thermal blanching at 70 °C, 80 °C and 98 °C (1 and 2.5 min), on sweet green and red bell peppers was compared. Pressure treated peppers showed a lower reduction on soluble protein and ascorbic acid contents. Red peppers presented even an increased content of ascorbic acid (15–20%), compared to the untreated peppers. Peroxidase and pectin methylesterase (whose activity was only quantifiable in green peppers) showed a higher stability to pressure treatments, particularly the latter enzyme, while polyphenol oxidase was inactivated to the same final level by the thermal blanching and pressure treatments. Pressure treatments resulted in comparable (in green pepper) to higher (in red pepper) microbial loads compared to blanching. Pressure treated green and red peppers presented similar to better firmness before and after tunnel freezing at −30 °C, compared to thermally blanched peppers, particularly those blanched at 98 °C. The results indicated that pressure treatments of 100 and 200 MPa can be used to produce frozen peppers with similar to better nutritional (soluble protein and ascorbic acid) and texture (firmness) characteristics, comparable activity of polyphenol oxidase and higher activity of pectin methylesterase, while pressure treated peppers show a higher level of peroxidase activity. It would be interesting to use higher pressures in future studies, as an attempt to cause a higher reduction on microbial load and on enzymatic activity.

Keywords: Bell pepper; *Capsicum annuum*; Pressure; Blanching; Freezing; Vitamin C; Texture; Enzymes

1. Introduction

Native from the Americas, sweet bell pepper is a Solanaceous fruit belonging to the *Capsicum annuum* L. species, whose consumption is growing in popularity, mainly due to its occurrence in a wide variety of colours (ranging from green, yellow, orange, red, and purple), shapes, and sizes and its characteristic flavour (Lucier & Lin, 2001). Bell peppers are used to produce dehydrated products (such as paprika), pickled peppers, and sliced or diced frozen peppers to be used in pizzas or to be eaten raw as salads. The demand for sliced and diced frozen raw peppers has been increasing considerably in the last years, due to consumers' willingness to eat raw, minimally processed vegetable products, as part of healthier food habits.

Prior to freezing, foods of vegetable origin are submitted to thermal blanching to reduce the microbial load and inactivate deleterious enzymes (Cano, 1996), since many of the quality changes that occur during distribution and storage of these foods, are due to detrimental reactions catalyzed by enzymes, such as peroxidase (POD), polyphenol oxidase (PPO), and pectin methylesterase (PME) (Bahçeci, Serpen, Gökmen, & Acar, 2004; Cano, 1996). However, heating causes also losses of sensorial (texture, taste, flavour, and colour) and nutritional quality attributes, such as reduction of ascorbic acid content (Howard, Smith, Wagner, Villalon, & Burns, 1994; Rao, Lee, Katz, & Cooley, 1981).

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For these reasons, it is desirable to keep blanching treatment conditions at a level strictly sufficient to cause inactivation of the deleterious enzymes, to minimize quality losses. This is particularly relevant for frozen sweet bell peppers intended to be stored frozen and eaten raw after thawing, since thermal blanching can cause considerable deleterious effects on texture, which is particularly important for this product due to its characteristic texture properties, namely firmness and crispness. This limitation has been a driving force for food processors to seek for other processing technologies that can substitute the conventional thermal blanching and, at the same time, cause less damaging effects on bell pepper texture.

In the last 15–20 years, high pressure processing has been increasingly explored and used to process foods, to destroy microorganisms and inactivate enzymes, with minimal deleterious effects on quality (Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998; Knorr, 1993; Torres & Velazquez, 2005), with a considerable number of high pressure pasteurized food products already commercially available worldwide.

The aim of this work was to study the effect of pressure treatments and conventional thermal blanching on sweet bell pepper fruits. In order to compare the effect of both treatments on quality of the processed peppers, the following parameters were quantified: soluble protein and ascorbic acid contents, activity of three enzymes (PPO, PME, and POD), texture (firmness), and microbial load (total aerobic mesophiles, enterobacteraceae, total and faecal coliforms, and *Escherichia coli*). The latter two quality factors are the two main indicators used to evaluate the quality of thermally blanched bell peppers fruits at the industrial level. Physicochemical characterization of the peppers used was also carried out. The effect of freezing, carried out at an industrial plant, using the usual processing conditions to produce commercial frozen peppers, on firmness, was also evaluated for both thermally blanched and high pressure treated peppers. Sweet bell peppers in two different colour maturation stages (green and red) were used in this study.

2. Materials and methods

2.1. Materials

Green and red bell peppers (*C. annuum* L.) were supplied by a local company. The fruits were harvested, brought to the company and immediately taken to the laboratory, where they were stored at 4 ± 1 °C, until further use. All chemicals used in this study were of analytical grade.

2.2. Physicochemical analysis

2.2.1. Dry matter and ash content

One gram of homogenised pepper fruit was dried until constant weight, first at 70 °C (about 3 h) and, subsequently, at 105 °C (about 16 h) to quantify the dry matter (adapted from AOAC, 1990). Afterwards, the dried fruit pepper residue was burnt in a muffle at 525 °C for 16 h and the residue weighted to determine the ash content (adapted from AOAC, 1990).

2.2.2. pH, titratable acidity, and soluble solids

Pepper juice was extracted from a 10 g sample with an Ultra-Turrax (T25, IKA-Labortechnik), followed by centrifugation (10,000*g*, 10 min), at 4 °C. The supernatant was recovered for pH, titratable acidity, and soluble solids measurements. The pH was measured at 20 °C. Titratable acidity was determined by titration with 0.1 N NaOH until pH 8.1 was reached and reported as g citric acid/100 g fresh weight. Soluble solids content was determined at 20 °C with a refractometer and reported as °Brix. All assays for the physicochemical analysis were performed in triplicate.

2.3. Protein content

The protein content (g protein/100 g fresh weight) was quantified using the Folin–Lowry method (Lowry, Rosenbrough, Farr, & Randall, 1951) and bovine serum albumin (BSA) as standard. Preparation of the protein extract for quantification of the protein content is presented in Section 2.5.1.

2.4. Ascorbic acid content

Five grams of pepper fruit were homogenised with 50 ml of 4% (w/v) solution of metaphosphoric acid for 15 min. The mixture was filtered and diluted to 100 ml and divided into several aliquots. The aliquots were then frozen in liquid nitrogen and stored at −20 °C until quantification of ascorbic acid (AsA) by HPLC, based on the method described by Daood, Biacs, Dakar, and Hajdu (1994). All these operations were carried out with protection from light, using aluminium foil, to avoid oxidation of AsA. An aliquot of AsA extract was thawed and filtered through a 0.45 μm millipore filter prior to injection onto the chromatographic column. The HPLC apparatus used consisted of an L-6200A pump, with a 20 μl injection loop and a L-4250 UV/Vis detector, with a D-2500 Chromato-integrator. The column was a LiChrosorb 100 RP-18 column (250×4.6 mm), with particle size of 5 μm (Merck). The mobile phase was constituted by 0.1 M phosphate and methanol (97:3), containing 0.75 mM ammonium tetrabutylhydroxide at pH 2.75, and a flow rate of 1.0 ml min^{-1} was used. The detection was performed at 254 nm for AsA, which was first identified and further quantified by comparing retention time, absorption spectra and peak areas with those of AsA standard (Sigma).

2.5. Enzymatic activity

2.5.1. Crude extract preparation

Activity of soluble and ionically bound forms of polyphenol oxidase (PPO), pectin methylesterase (PME), and

peroxidise (POD) were quantified separately, since these two forms can show different behaviour during maturation, as found for POD (Silva, Lourenço, & Neves, 1990) and different properties like stability to temperature and pressure. Fifty grams of thermal and pressure treated and unprocessed pepper samples were thawed at 4 °C, homogenised with 75 ml of 0.2 M sodium phosphate buffer (pH 6.5), a relatively low ionic strength buffer, and 4% (w/w) polyvinylpyrrolidone (PVP) in a Warring blender, followed by agitation during 1 h at 4 °C. The homogenate was filtered through several layers of cheesecloth and then centrifuged (10,000*g*, 20 min) at 4 °C. The supernatant, further designated enzymes soluble fraction (SF) extract, was collected and the precipitate was re-suspended in 30 ml of 0.2 M sodium phosphate buffer (pH 6.5), followed by agitation for 30 min, and extraction performed again, alike, to ensure that all enzymes from SF were extracted. The resulting pellet was mixed with 50 ml of 0.2 M sodium phosphate buffer (pH 6.5) with 1 M NaCl for 2 h, a high ionic strength buffer, in order to obtain the enzymes ionically bound fraction (IF) extract. Extraction was carried out twice as described for the SF and with the same purpose. All the extracts were divided into aliquots, frozen in liquid nitrogen, and stored frozen until quantification of the enzymatic activities. No activity was found for the enzymes studied in the second extraction of SF and IF, revealing that first extraction was complete for both fractions. Protein content was quantified using the same extracts used for quantification of enzymatic activities and no protein was found for the second extraction of SF and IF, revealing that the first extraction was enough to fully extract the soluble protein. Total enzymatic activity for each enzyme and total protein content, was calculated as the sum of the values obtained for SF and IF fractions. The standard deviation, S_T, for the total amount of enzymatic activity for each enzyme and protein content, was calculated by $S_T = \sqrt{S_{SF}^2 + S_{IF}^2}$ (Miller & Miller, 2000), where S_{SF} and S_{IF} are, respectively, the standard deviation obtained for the SF and IF. All procedures to obtain the enzymatic extracts were carried out at 4 °C and the enzymatic activities and protein content were determined in triplicate.

2.5.2. Polyphenol oxidase

A spectrophotometric assay using cathecol as substrate at pH 6.5, used to quantify PPO activity, as described by Weemaes et al. (1997), using a 6405 UV/Vis JenWay spectrophotometer. The reaction mixture consisted of 1000 μl of substrate at 25 °C and 40 μl of enzyme extract. PPO activity was determined from the slope of the linear portion of the curve, relating absorbance at 411 nm with time, and was expressed as ΔAbs_{411} min^{-1}/100 g fresh weight.

2.5.3. Pectin methylesterase

Activity determination was carried out according to the method described by Hagerman and Austin (1986). In order to achieve a constant starting pH for the reaction, all the solutions (pectin, indicator dye and water) and the enzymatic extracts were adjusted to pH 7.5 with NaOH solutions of adequate concentration, to facilitate fine pH adjustment. Four milliliter of citrus pectin solution (0.5%, w/v) were mixed with 300 μl of bromothymol blue (0.01%, w/v), and distilled water and enzyme extract, up to 6 ml. Change of absorbency was recorded at 620 nm, at 25 °C and PME activity was expressed as ΔAbs_{620} min^{-1}/100 g fresh weight. The initial absorbency remained constant until the enzyme was added, indicating that no reaction occurred in the absence of the enzyme.

2.5.4. Peroxidase

Activity was determined based on the method described by Worthington (1978). The substrate solution was composed by hydrogen peroxide (0.975 mM), phenol (83.1 mM) and 4-aminoantipyrine (1.19 mM) in 0.1 M sodium phosphate buffer (pH 7.0). To 1.450 ml of daily prepared substrate solution, incubated at 25 °C, 50 μl of enzymatic extract were added and the increase in absorbency recorded at 510 nm. The slope of the linear portion of the curve relating absorbance at 510 nm with time was used to calculate the enzyme activity, expressed in ΔAbs_{510} min^{-1}/100 g fresh weight.

2.6. Texture measurements

The texture of the peppers was measured using a texture analyser (TA-HDplus, Stable Micro System) with a 7 mm diameter hole, and the following parameters: 5 kg force load cell, 2 mm diameter aluminium cylinder probe, and 2.0 mm s^{-1} test speed. The property "firmness" (hardness), the maximum force applied to puncture the pepper tissue, was measured as an indicator of texture, which is very similar to the one performed by mastication that takes part during eating. The measurements were done on both sides of the pepper tissue that is, from the skin and the flesh sides. Rupture of the skin from the flesh side required a lower force when compared with the same action from the skin side. An average value of firmness from five puncture measurements was calculated for each experimental condition. Texture analyses were carried out within 1 h after the thermal and pressure treatments have been applied, and the samples were kept at 4 °C during this period. All the measurements were conducted at room temperature, preceded by equilibration of the samples to room temperature. The results are presented as relative firmness (%), calculated from the ratio between the firmness of each sample and the control.

2.7. Microbial analysis

For the microbiological study, the pepper samples were washed with a chlorine solution (150 ppm), before being submitted to thermal blanching and pressure treatments (control samples were taken after the washing step). Quantification of total aerobic mesophiles, enterobacteraceae,

total and faecal coliforms, and *E. coli*, are the most important microorganisms used to assess microbial conformity of fresh and minimally processed ready-for-use foods of vegetable origin (French legislation is an example – Arrêté du 22 mars, 1993). These same microorganisms were quantified in this work. An amount of 10 g of pepper sample was weighted under aseptic conditions, 90 ml of sterile 0.1% peptone water added, and the mixture was homogenised in sterile bags using a Stomacher (Model 400, Laboratory blender) for 2.5 min. All decimal dilutions were prepared from this homogenate. Counts of total aerobic mesophiles were obtained by incubation onto standard plate count agar (PCA) at 30 °C for 72 h. Quantification of enterobacteraceae was done by incubation on violet red bile glucose (VRBG) agar at 37 °C for 48 h. Coliforms were quantified by incubation on violet red bile lactose (VRBL) agar at 30 °C and 44 °C, for total and faecal coliforms, respectively, for 48 h. *E. coli* was quantified using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG), as indicator of β-glucuronidase activity, and incubation at 44 °C for 24 h. All media used were from Oxoid, Hampshire, UK.

2.8. Thermal blanching and pressure treatments

Temperature, pressure, and time duration of the thermal blanching and pressure treatments studied are shown in Table 1. Temperature and time duration of the thermal blanching treatments were the same as those typically used for the industrial production of frozen peppers: for very ripened peppers or varieties with lower firmness, the temperature of 70 °C is applied, while for ripened peppers, with medium to high firmness, temperatures of 80 °C and 98 °C are, respectively, applied. Time duration of the treatments (1 or 2.5 min) is used, additionally, to better adjust, at each temperature, the applied treatment to the precise firmness stage of the peppers, with 1 min being used for lower firmness peppers and 2.5 min for higher firmness peppers. The lowest blanching temperature (70 °C) is less frequently used, particularly to produce frozen peppers intended to be eaten raw. Nevertheless, this blanching temperature was studied to evaluate the effect of a lower, less damaging blanching temperature on bell pepper quality.

Table 1
Temperature, pressure and time conditions used for thermal blanching and pressure treatments of green and red peppers

Sample code	Operating conditions
C	Control, unprocessed sample
BI.1	Blanching at 70 °C for 1 min
BI.2	Blanching at 70 °C for 2.5 min
BII.1	Blanching at 80 °C for 1 min
BII.2	Blanching at 80 °C for 2.5 min
BIII.1	Blanching at 98 °C for 1 min
BIII.2	Blanching at 98 °C for 2.5 min
PI.1	Pressurization at 100 MPa for 10 min
PI.2	Pressurization at 100 MPa for 20 min
PII.1	Pressurization at 200 MPa for 10 min
PII.2	Pressurization at 200 MPa for 20 min

For the blanching treatments, the pepper samples were cut in slices of 15 × 75 mm and placed in a plastic bag that was heat sealed. The packaged samples were immersed in a thermostated water bath (Grant, Y28), pre-set at the adequate temperature, for 1 or 2.5 min, cooled immediately in a water bath at 4 °C for 5 min, and then equilibrated at room temperature for the texture measurements. For the pressure treatments, the pepper samples were packed in a second plastic bag, which was heat sealed under vacuum. The samples were then pressurized using an Autoclave Engineers (Erie, PA, USA) isostatic press (Model IP3-23-30), with a cylindrical pressure chamber (i.d. 76 mm, height 610 mm), containing a pressure medium consisting of water with 2% hydraulic fluid (Hydrolubric 142; E.F. Houghton and Co., Valley Forge, PA, USA). The pressure was build up at room temperature (18–20 °C), up to 100 or 200 MPa, which took ca. 45 and 60 s, respectively, maintained during 10 or 20 min, followed by decompression (ca. 45 s). Temperature increment during pressurization was very small, due to the low range of pressures used (100 and 200 MPa) and the slow pressurization rate used. The maximum temperature reached during pressurization was 22–24 °C for 100 MPa and 24–26 °C for 200 MPa and declined during the pressurization holding time to 18–20 °C Since the temperature increment was very small, control samples were kept at 18–20 °C. This procedure allowed studying the full (pressure and temperature increment) effect of the pressure treatments applied, compared to the untreated samples and the blanched samples.

For determination of soluble protein and ascorbic acid contents, enzymatic activities, and microbiological analyses, thermally blanched and pressure treated peppers were frozen in liquid nitrogen and stored frozen until used.

2.9. Effect of freezing on firmness

To carry out the freezing experiments, thermally blanched and pressure treated peppers were kept at 4 °C, transported to the company and frozen in a tunnel freezer at −30 °C, with an air-blast freezing system, following the same procedure used to freeze commercial peppers. The frozen samples were stored at −20 °C and prior to the texture measurements, the samples were thawed and equilibrated at room temperature.

2.10. Data analysis

ANOVA and bilateral Tukey's test were carried out to determine significant differences ($P < 0.05$).

3. Results and discussion

3.1. Physicochemical analysis

3.1.1. Dry matter and ash content

Moisture content and ash content (Table 2) are within the ranges (92.8–94.5% and 1.33–1.38%, respectively)

Table 2
Physicochemical parameters (±standard deviation, $n = 3$) of green and red bell peppers

Physicochemical parameters	Green	Red
Moisture (%)[a]	94.5 ± 0.4	92.8 ± 0.2
Ash content (%)[a]	1.38 ± 0.01	1.33 ± 0.02
pH	4.65 ± 0.01	4.81 ± 0.01
Titratable acidity (g/L of citric acid)	10.0 ± 0.5	26.1 ± 0.9
Soluble solids (°Brix at 20 °C)	5.27 ± 0.01	6.01 ± 0.01

[a] % on fresh weight basis.

reported in the literature for pepper fruits (Guil-Guerrero, Martínez-Guirado, Rebolloso-Fuentes, & Carrique-Pérez, 2006).

3.1.2. pH, titratable acidity, and soluble solids

The pH (Table 2) was lower than the values found by Estrada, Bernal, Diaz, Pomar, and Merino (2000) (pH 5.6) and Castro, Van Loey, Saraiva, Smout, and Hendrickx (2005) (pH 5.0–6.4), but in agreement with the values reported by Biles, Wall, Waugh, and Palmer (1993) (pH 4.6–5.4). Titratable acidity increased ($P < 0.05$) about 2.5-fold from green to red peppers (Table 2), results that are similar to those reported by Luning et al. (1994). This increase in titrable acidity could be caused by an increment of the concentration of protonated, lower acidity constants, organic acids (Luning et al., 1994). The amount of soluble solids (Table 2) found in this work was within the range reported by Guil-Guerrero et al. (2006) and significantly ($P < 0.05$) higher (about 14%) for red pepper.

3.2. Protein content

Total and SF protein contents were not significantly different ($P > 0.05$) for unprocessed green and red peppers, while protein content of IF was higher ($P < 0.05$) for unprocessed red peppers (Fig. 1). The protein content of the IF constituted about 1% of the total amount of protein of green peppers and almost 10% for red peppers. Blanching treatments reduced ($P < 0.05$) the total soluble protein content by about 15–60% of green peppers (except for treatment BI.2) and 15–35% in red peppers, an effect that increased progressively with the increment of the blanching temperature/time. Pressure treated green peppers showed a decrease

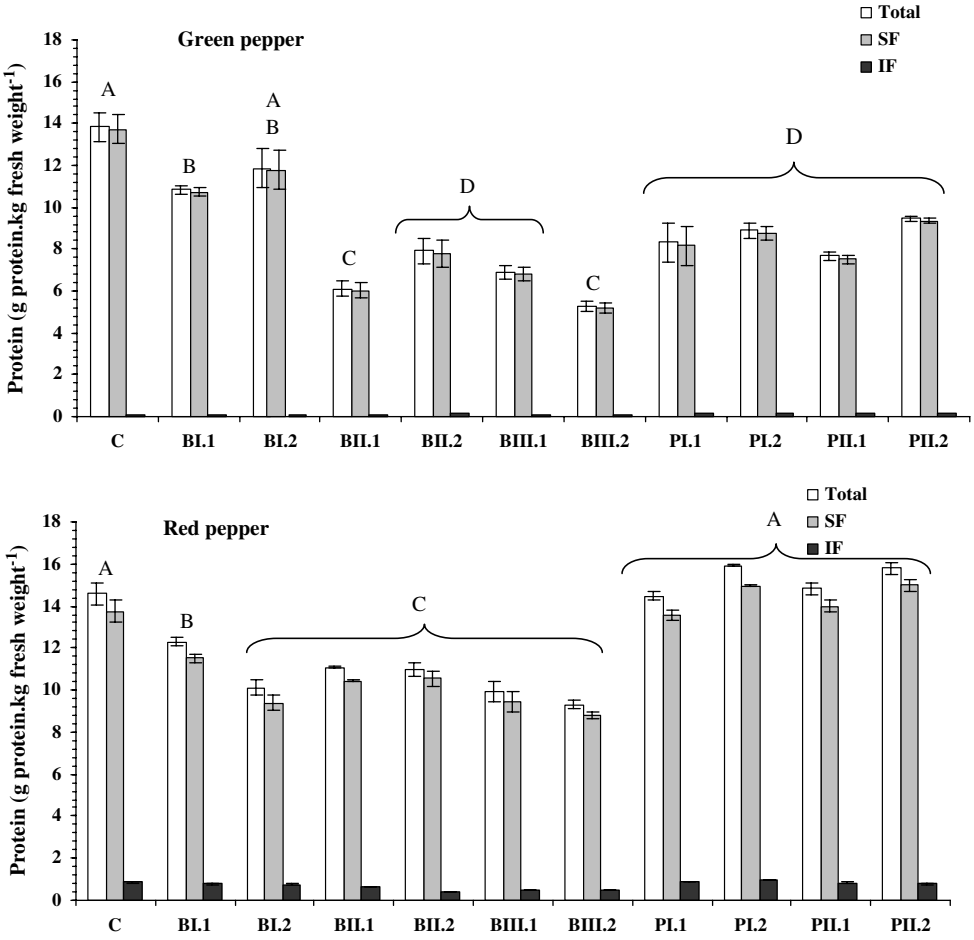

Fig. 1. Protein content (g protein/kg fresh weight) of the soluble fraction (SF), ionically bound fraction (IF), and total content (SF + IF), measured for unprocessed, thermally blanched, and pressure treated green and red peppers. The bars represent the standard deviation ($n = 3$). The meaning of the abbreviations used in the abscissas axis is shown in Table 1. Different letters indicate cases of major effects for total protein content.

($P < 0.05$) of total soluble protein content of about 32–45%, compared to untreated peppers. Green peppers treated at 100 and 200 MPa for 20 min, had a significant ($P < 0.05$) lower reduction in total protein content, than green peppers blanched at 80 °C for 1 min and 98 °C for 2 min, and higher ($P < 0.05$) than that obtained with blanching at 70 °C. Pressure treated red peppers showed no variation ($P > 0.05$) of the total protein content compared with the control sample, results that are clearly better than those observed for all the blanching treatments. Globally, the protein content of red peppers was less affected by both blanching and pressure treatments, indicating an effect of the colour maturation stage.

3.3. Ascorbic acid content

AsA content for both green (88.5 ± 1.5 mg/100 g fresh weight) and red peppers (107.4 ± 2.3 mg/100 g fresh weight) are within the ranges found in other studies, for green (12–180 mg/100 g fresh weight – Yahia, Contreras-Padilla, & Gonzalez-Aguilar, 2001) and red peppers (75–277 mg/100 g fresh weight – Howard et al., 1994). These results confirmed peppers as a good source of AsA, with 100 g exceeding the recommended daily allowance (RDA) of 60 mg (Carr & Frei, 1999) by, respectively, 48% and 79%, for green and red peppers.

For unprocessed peppers, AsA content was significantly higher ($P < 0.05$) for red peppers (Fig. 2), a result that is in accordance with studies by other authors (Guil-Guerrero et al., 2006). AsA content decreased progressively as blanching conditions were more severe (higher temperature and longer treatment time duration), to about 45% and 30% of the initial value, for green and red peppers, respectively. Jalapeño pepper cultivar blanched prior to pasteurization lost 75% of its AsA (Howard et al., 1994), while Matthews and Hall (1978) reported a 40% loss of AsA during water blanching of green peppers. Differences in ascorbic acid retention may be attributed to differences in genetics, maturity stage, brine composition, blanching method, and treatment time and temperature (Lee & Howard, 1999), that can cause different degrees of inactivation of ascorbic acid oxidase and removal of residual oxygen from vegetable tissue (Selman, 1994). For example, retention values for AsA obtained from water blanched peas could be improved if samples were packaged without water (Quaglia, Gravina, Paperi, & Paoletti, 1996).

Pressurized green bell peppers showed a decrease of about 15–20% of AsA content (Fig. 2), while red peppers showed an increase of about 10–20% ($P < 0.05$). Orange juice showed no substantial modification in the composition of AsA when pressurized at 500 MPa (García, Butz, Bognàr, & Tausher, 2001) and peas pressurized at 900 MPa showed higher retention of AsA (82%), compared to water blanching (12%) (Quaglia et al., 1996). The reason for the augmentation of AsA in pressurized red peppers is unknown, but may be related to an increased extractability, that result from the pressurization process (Sancho et al., 1999) and to a higher stability to pressure compared to temperature, of AsA and/or other components, e.g. oxidation inhibitors, which can inhibit the oxidation of ascorbic acid (Lee, Howard, & Villalon, 1995). Globally, pressurized green bell peppers showed a similar to higher retention of AsA when compared to the blanching treatments of 80 °C and 98 °C, respectively, while pressurized red peppers showed a higher content of around 50–100%, when compared to the same blanching treatments. AsA was clearly better retained in pressurized red peppers than in green peppers, indicating an effect of the colour maturation stage, as already observed for the soluble protein content.

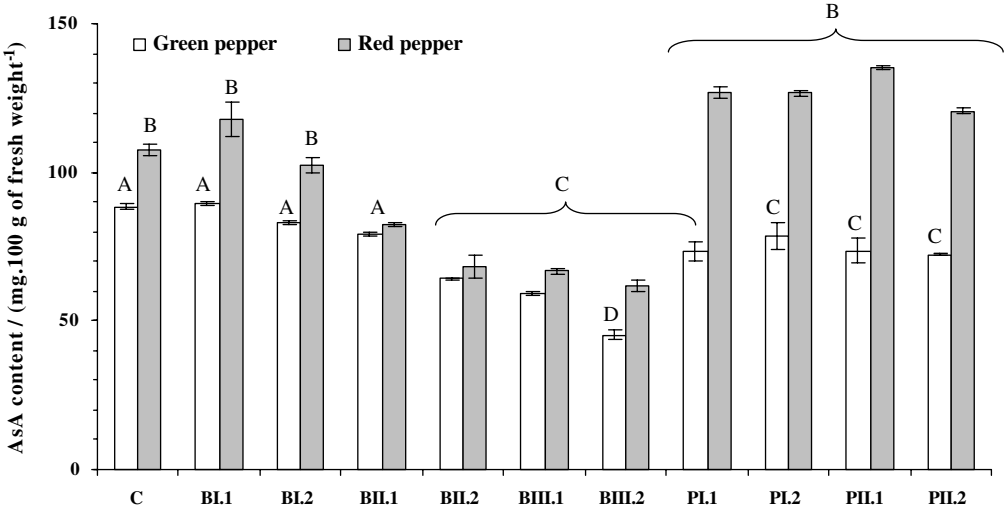

Fig. 2. Effect of thermal blanching and pressure treatments, on ascorbic acid (AsA) content (mg of AsA/100 g fresh weight) of green and red peppers. The bars represent the standard deviation ($n = 3$). The meaning of the abbreviations used in the abscissas axis is indicated in Table 1. Different letters indicate cases of major effects.

3.4. Enzymatic activity

3.4.1. Polyphenol oxidase

PPO activity (Fig. 3) of unprocessed green peppers was found to be about 50% higher than that of unprocessed red peppers due to a lower activity of SF extract of red peppers (activity of the SF extract represented 85% and 70% of total activity for green and red peppers, respectively). PPO activity in eggplant was also found to be mostly in the SF (Concellón, Añon, & Chaves, 2004).

PPO was found to be more stable to both thermal blanching and pressure treatments in red peppers with only the blanching treatments at 98 °C causing significant ($P < 0.05$) reduction of activity (Fig. 3). For green peppers, all treatments decreased PPO activity, with the thermal blanching treatments causing, generally, a progressive decrease on activity (from 25% to 75%), with increasing severity of the treatments, while all pressure treatments caused a decrease of activity of about 50%. While PPO is considered a moderately heat-stable enzyme, its baroresistance varies greatly depending on the enzyme source (Weemaes et al., 1997). For example, mushroom, potato, and avocado PPO are very pressure stable, since treatments at 800–900 MPa are needed to reduced enzyme activity at room temperature (Gomes & Ledward, 1996; Weemaes et al., 1997), while apricot and strawberry PPO were reported to be inactivated by pressures exceeding 100 and 400 MPa, respectively (Amati, Castellari, Matricardi, Arfelli, & Carpi, 1996; Jolibert, Tonello, Sageghi, & Raymond, 1994). Globally, green and red pressure treated peppers showed a PPO activity level similar or lower to that of thermally blanched peppers, except for the most severe blanching treatment (BIII.2). PPO of red peppers is

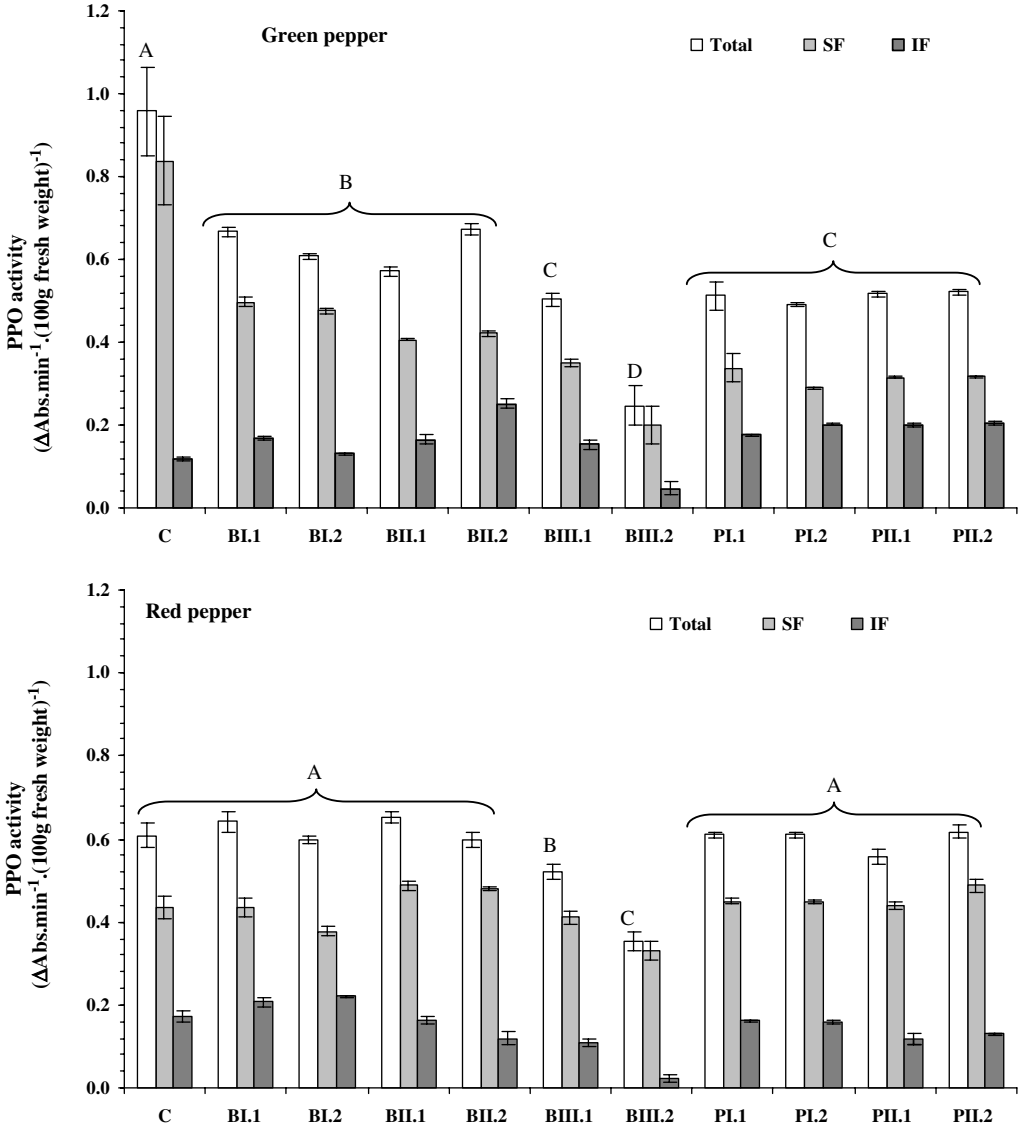

Fig. 3. Effect of thermal blanching and pressure treatments on polyphenol oxidase activity of green and red peppers (SF – activity of the soluble fraction; IF – activity of the ionically bound fraction; Total – activity of SF + IF). The bars represent the standard deviation ($n = 3$). The meaning of the abbreviations used in the abscissas axis is indicated in Table 1. Different letters indicate cases of major effects for total activity.

more stable to pressure and temperature than PPO of green peppers, results that indicate also an effect of the colour maturation stage.

3.4.2. Pectin methylesterase

PME activity was only detected in green peppers, even when increased amounts of enzymatic extract were used and longer reaction times were studied (absence of PME activity in red peppers indicates again an effect of the colour maturation stage). Absence of PME activity in red peppers can be due to absence of the enzyme, or to the occurrence of a well known PME inhibitor, a glycoprotein that usually appears or increases its amount with ripening (Giovane et al., 2004). Both Jen and Robinson (1984), and Sethu, Prabha, and Tharanathan (1996) reported a decrease in PME activity in *C. annuum* fruits during ripening.

In green peppers PME activity was, generally, equally distributed between SF and IF extracts (Fig. 4). PME activity of green peppers declined progressively, as blanching temperature and time increased, until reaching almost absence of activity for treatment BIII.2. Castro et al. (2005) concluded that green pepper PME was completely inactivated after heating at 80 °C for 5 min, both in crude extract and in Tris buffer at pH 5.6, results that are in agreement with those obtained in this work.

Pressure treated green peppers showed a slight increase in activity, which was due to the increment of PME activity of both SF and IF. Castro et al. (2005) also found that PME activity of green peppers pieces increased, after the pepper pieces have been pressure treated up to 500 MPa at room temperature. Likewise, Shook, Shelhammer, and Schwartz (2001) observed a significant increment of PME activity, when diced tomatoes were pressurized at 400 MPa and 45 °C compared to non-pressurized samples. Augmentation of PME activity of tomato (*Lycopersicon esculentum*) cell cultures, caused by pressure treatments up to 150 MPa, was ascribed by Dörnenburg and Knorr (1998) to a more effective extraction of the enzyme, due to damage of plant cell wall/membrane and changes in cell wall association state of the enzyme. The same phenomena might be responsible for the increment of PME activity found in this work for pressure treated green peppers.

3.4.3. Peroxidase

POD activity was found to be mainly present in SF, for both green and red peppers, and no significant ($P > 0.05$) variation was found between unprocessed green and red peppers (Fig. 5). In tomato (Thomas, Jen, & Morr, 1981) and strawberry (Civello, Martínez, Chaves, & Añon, 1995), POD activity decreased with ripening. In other fruits, POD activity has been found to increase with ripening (Silva et al., 1990; Thomas et al., 1981). All the blanching treatments reduced significantly ($P < 0.05$), by more than 85%, POD activity with exception of treatment BI.1 for red peppers (where a reduction of 70% was verified), indicating that pepper POD has a low stability to temperature. In general, POD is well known as a heat-stable enzyme, and for this reason is used to evaluate the adequacy of fruits and vegetables thermal blanching (Barrett & Theerakulkait, 1995). For instance, Bahçeci et al. (2004) found that a blanching treatment at 90 °C for 3 min was necessary to inactivate 90% of the activity of green bean POD. Using absence of POD activity as an indi-

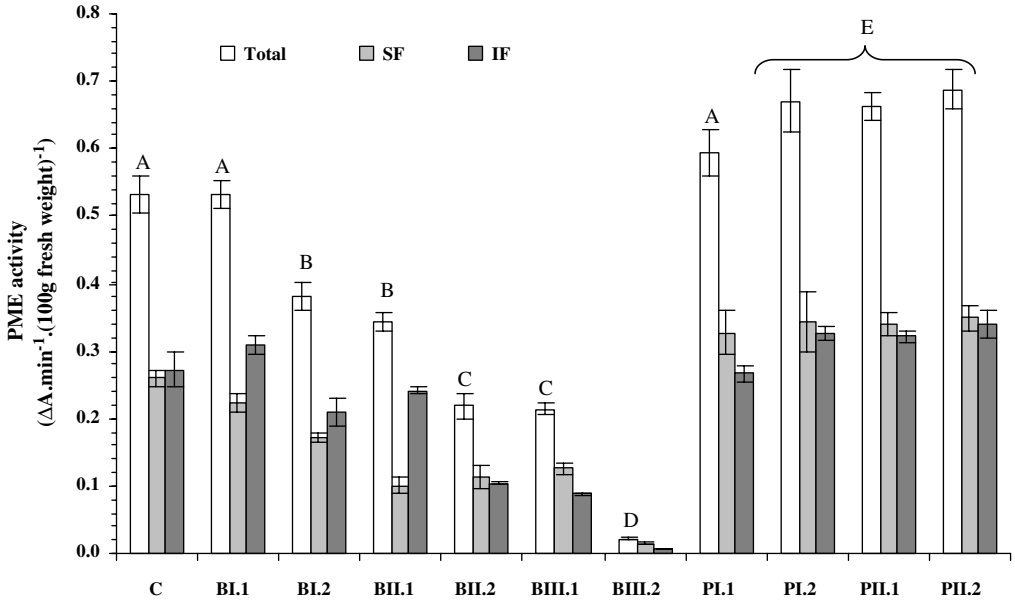

Fig. 4. Effect of thermal blanching and pressure treatments on pectin methylesterase activity of green peppers (SF – activity of the soluble fraction; IF – activity of the ionically bound fraction; Total – activity of SF + IF). The bars represent the standard deviation ($n = 3$). The meaning of the abbreviations used in the abscissas axis is indicated in Table 1. Different letters indicate cases of major effects for total activity.

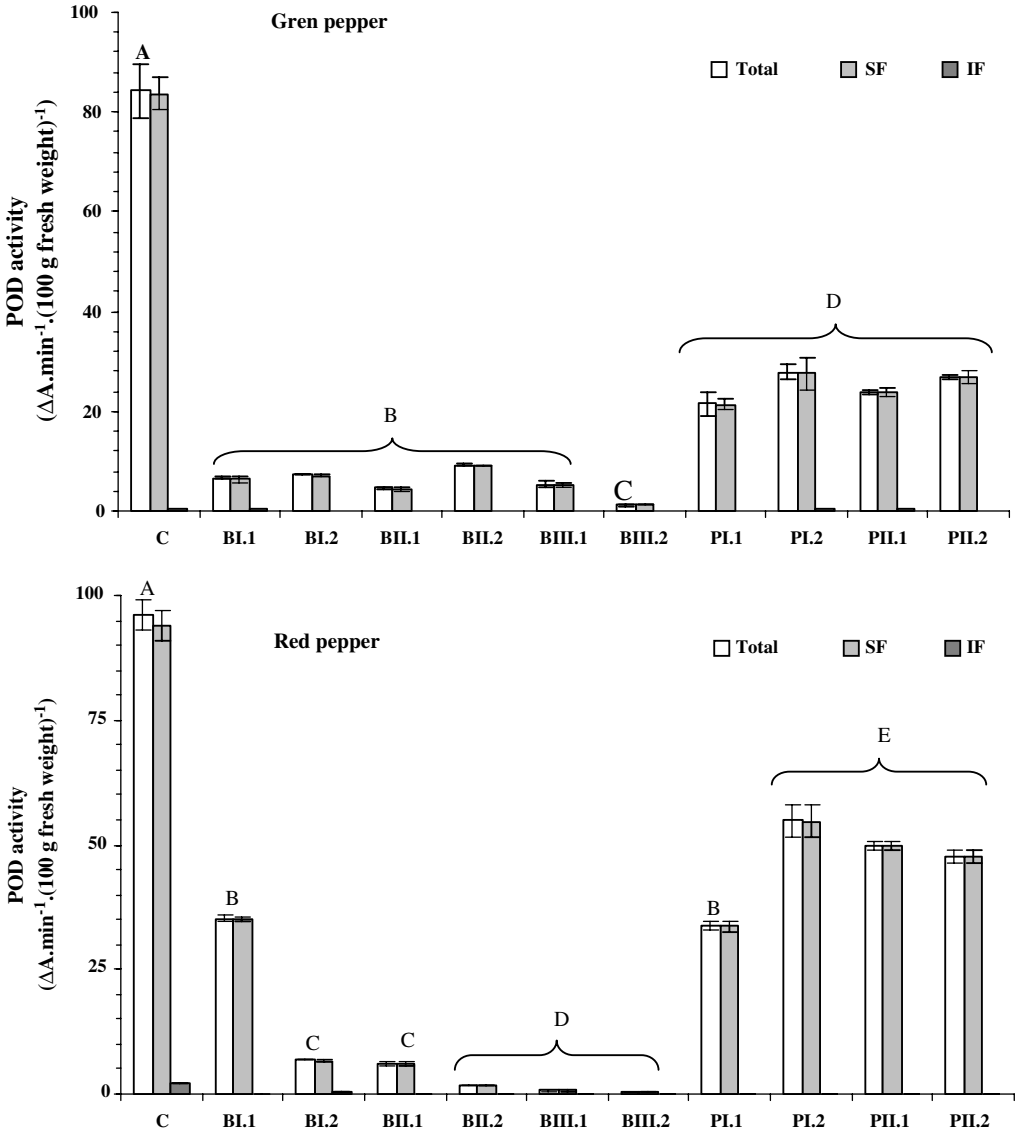

Fig. 5. Effect of thermal blanching and pressure treatments on peroxidase activity of green and red peppers (SF – activity of the soluble fraction; IF – activity of the ionically bound fraction; Total – activity of SF + IF). The bars represent the standard deviation ($n = 3$). The meaning of the abbreviations used in the abscissas axis is indicated in Table 1. Different letters indicate cases of major effects for total activity.

cator of adequate thermal blanching is inadequate for peppers, since pepper POD shows a lower stability to temperature compared to PPO and PME.

Pressure treated peppers show a reduction of POD activity of about 70% and 40%, for green and red peppers, respectively. Results found in the literature indicate that POD can show a wide spectrum in what concerns stability to pressure. Pressure treated green beans at 900 MPa for 10 min at room temperature, showed an 88% inactivation of POD and combination with temperature treatments enhanced the inactivating effect at 600 MPa (Quaglia et al., 1996). Green bean POD showed 75% residual activity after being pressure treated at 500 MPa for 60 s at room temperature (Krebbers, Matser, Koets, & Van den Berg, 2002). Less intense pressure conditions (300 and 400 MPa) could inactivate POD from strawberry puree and orange juice at room temperature (Cano, Hernandez, & De Ancos, 1997). In tomato puree, an increase in POD activity was reported for pressure treatments below 350 MPa at room temperature, while a significant inactivation was obtained above 350 MPa (Hernández & Cano, 1998). Pepper POD shows a low stability to pressure, with POD from red peppers presenting a higher stability to pressure than POD from green peppers. Overall, thermal blanching treatments caused higher inactivation of POD activity, than the pressure treatments.

3.5. Microbial analysis

Thermal blanching caused a reduction of total aerobic mesophiles of about 1 and 2 decimal reductions (1D and 2D reductions), for green and red peppers, respectively,

and about 1D reduction for faecal coliforms, for green and red peppers, except for red peppers and blanching treatments BI.1 and BII.1 (Fig. 6). For red peppers, thermal blanching also caused about 2D reduction of enterobacteraceae, with the exception of treatments BI.1 and BII.1. Blanched red peppers showed a progressive reduction of total coliforms, with the severity of the blanching treatment, up to a 2D reduction, while in green peppers, blanching caused less than 1D reduction. Generally, pressure treatments caused a lower reduction on microbial counts, compared to thermal blanching treatments, particularly for red peppers, where almost no reduction on microbial counts was verified. This is expectable, since the pressure levels used (100 and 200 MPa) are much lower than those used (500–600 MPa) to pasteurize food products (Torres & Velazquez, 2005).

Most of the microorganisms present in fresh-cut fruits and vegetables are derived from soil, air, and water contamination. The presence of coliforms in fresh-cut produce should not be surprising since many coliforms are associated with the soil. Their presence at levels of 10^2–10^4 colony forming units per gram (CFU g^{-1}) in produce is not rare, when enumeration is done on media selective for enterobacteriaceae (Nguyen-The & Carlin, 1994). Microorganisms, such as *Listeria monocytogenes*, *Salmonella*, *E. coli* O157:H7 and other pathogens of concern were also found in washed fresh-cut vegetables, even after cutting and packaging (Alzamora, Tapia, & López-Malo, 2000). Heard (2002) reported that aerobic mesophilic counts can range from 10^3 to 10^8 CFU g^{-1}, in fresh vegetable produce. French legislation (Arrêté du 22 mars, 1993) imposes maximum acceptable values for total mesophilic counts, faecal coliforms, and *E. coli*, for minimally processed ready-for-use fresh vegetables and vegetable salads, or raw foods of vegetable nature ready-for-use of, respectively, 5×10^6, 10^3, and 10^3 (all values presented as CFU g^{-1}). For this type of products, a maximum acceptable value of 10^4 for total coliforms and enterobacteriaceae is also common to be requested by buyers to the processing suppliers. As can be seen in Fig. 6, all thermally blanched and pressurized samples showed a microbial content for total mesophilic counts, enterobacteriaceae and total and faecal coliforms, below the maximum acceptable values, indicating conformity of the processed peppers for consumption. *E. coli* counts are not reported in Fig. 6, but they were less than 10 CFU g^{-1} in all samples, which is also below the maximum accepted values (Arrêté du 22 mars, 1993). The results depicted in Fig. 6 also point out for the importance of the microbial quality of the peppers, prior to the thermal blanching and pressure treatments, and so, of the washing step with chlorine solution and of good handling and manufacturing procedures, for the microbial quality of the final product, since the treatments caused at maximum 2D reductions of the microbial load of the raw peppers.

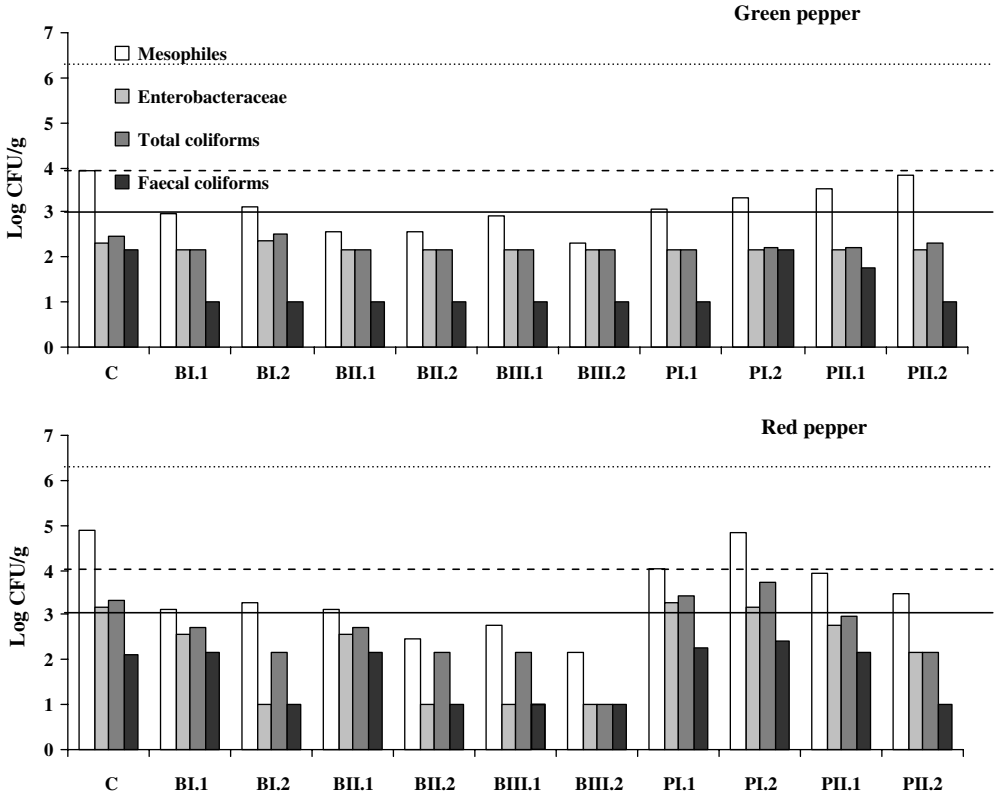

Fig. 6. Effect of thermal blanching and pressure treatments on the microbial counts of total aerobic mesophiles, enterobacteraceae, and total and faecal coliforms of green and red peppers. Horizontal lines indicate maximum allowable CFU g^{-1}, according to French legislation (Arrêté du 22 mars, 1993), for total aerobic mesophiles (···), and total (---) and faecal coliforms (—). The meaning of the abbreviations used in the abscissas axis is indicated in Table 1.

3.6. Texture

Firmness (kg force) measured from the skin side (green, 1.12 ± 0.13; red, 1.03 ± 0.06) was about threefold higher ($P < 0.05$) than firmness measured from the flesh side (green, 0.446 ± 0.043; red, 0.414 ± 0.074).

Generally, the results (Figs. 7 and 8) showed a trend for firmness of red peppers to be more sensitive to both the thermal blanching and the pressure treatments, than firmness of green peppers, especially, when texture was measured from the skin side. These results might be related to the absence of PME activity in red peppers, previously mentioned. For green peppers, PME activity can cause de-methylation of pectin molecules in the middle lamella (Alvarez, Canet, & Tortosa, 2001). The de-esterified pectins are consequently less susceptible to β-eliminative degradation and, therefore, more heat resistant and less soluble, which is generally thought to increase the cell–cell adhesion (Ng & Waldron, 1997), and can crosslink with calcium ions, forming calcium pectates that contribute to increase firmness (Lee & Howard, 1999). This hypothesis is supported by observation of the results shown in Figs. 4, 7, and 8 for green peppers: decrement of firmness caused by the blanching treatments follows a pattern similar to the decrease of PME activity caused by the same treatments, while for pressure treatments that caused no reduction of PME activity, no decrease of firmness was observed. In fact, PME activity due to pressure activation (treatments higher than 100 MPa) has been reported in the literature as causing beneficial effects on firmness (Sila, Smout, Vu, & Hendrickx, 2004). Globally, pressurized peppers showed similar to better values for firmness, compared to thermally blanched peppers.

3.7. Effect of freezing on firmness

A decrease of about 27% and 33% on firmness, measured from the flesh side, was observed for frozen, unprocessed green and red peppers, respectively, after thawing, while firmness quantified from the skin side was not

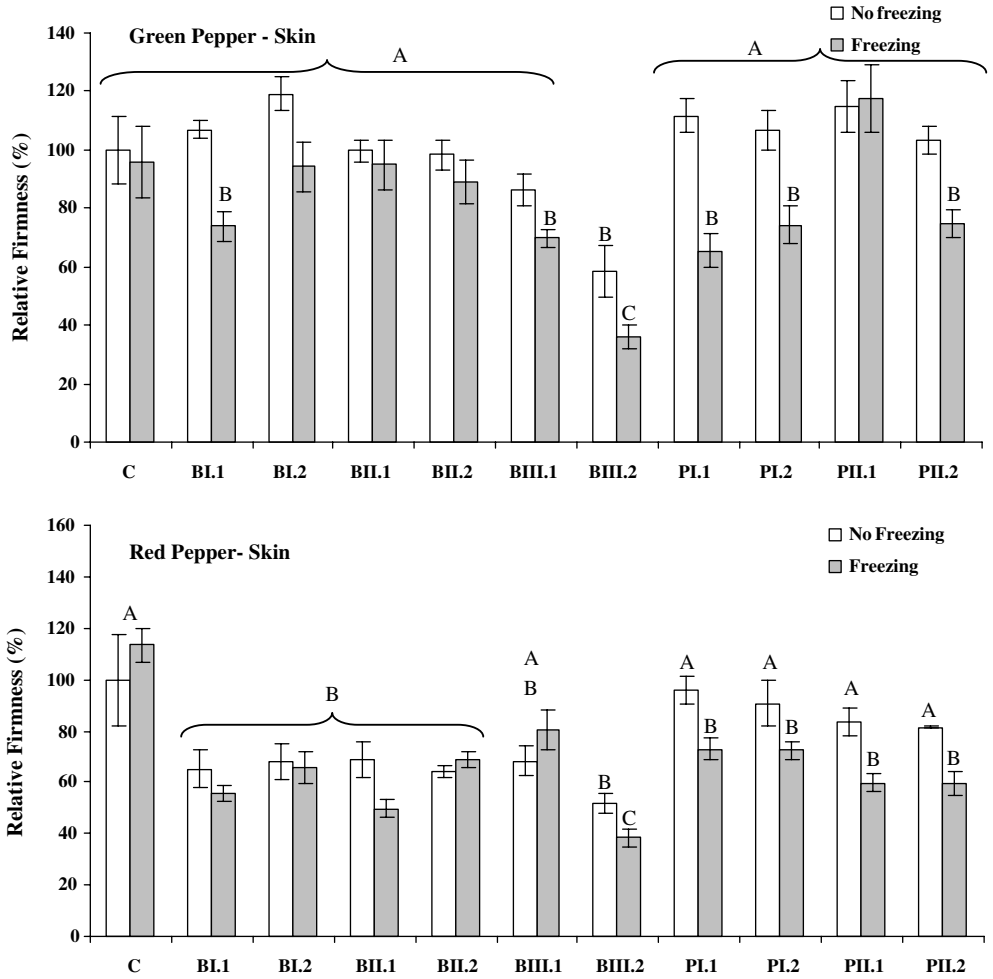

Fig. 7. Effect of thermal blanching and pressure treatments and freezing on the firmness, measured from the skin side, of green and red peppers. The bars represent the standard deviation ($n = 5$). The meaning of the abbreviations used in the abscissas axis is indicated in Table 1. Different letters indicate cases of major effects.

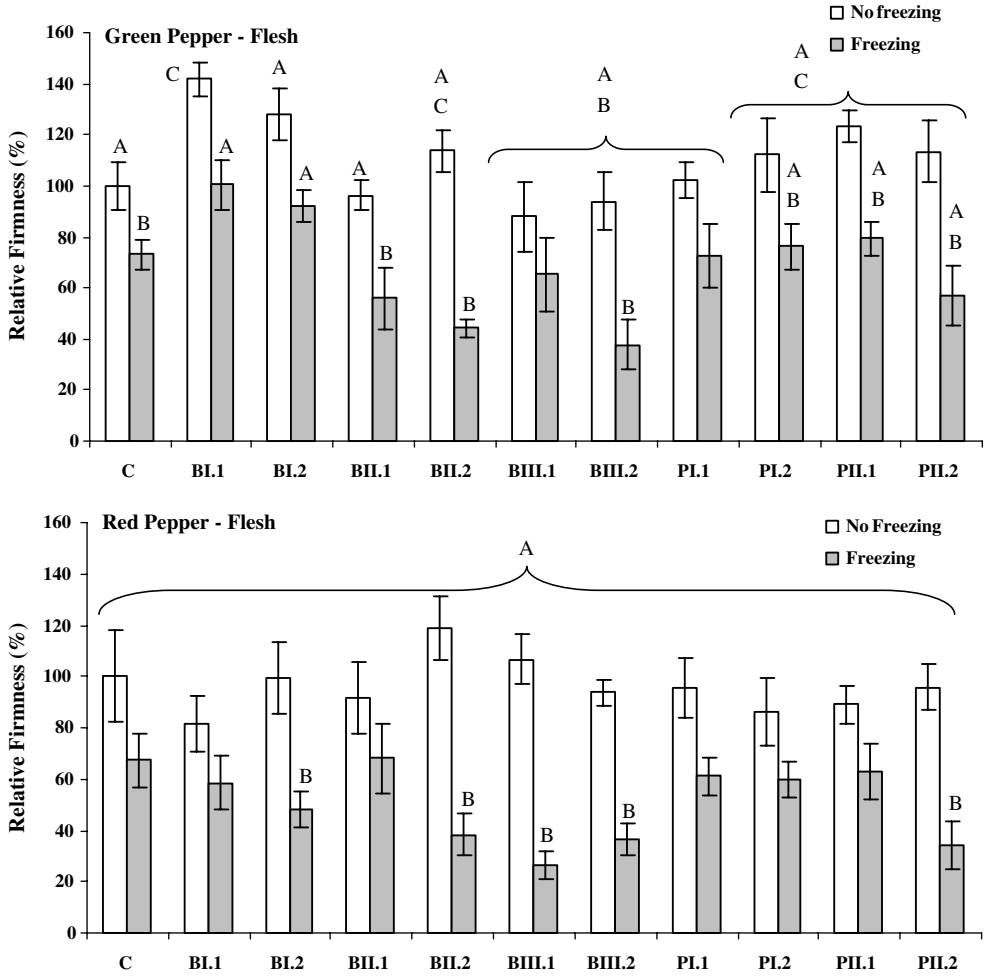

Fig. 8. Effect of thermal blanching and pressure treatments and freezing on the firmness, measured from the flesh side, of green and red peppers. The bars represent the standard deviation ($n = 5$). The meaning of the abbreviations used in the abscissas axis is indicated in Table 1. Different letters indicate cases of major effects.

affected (Figs. 7 and 8). Moreover, freezing also caused a higher decrease on firmness, for the thermally blanched peppers, when it was measured from the flesh side, indicating, possibly, the occurrence of more detrimental effects on pepper flesh cells, caused by the treatments. Globally, thawed peppers that were pressure treated, showed a similar to better texture, compared to the thermally blanched peppers.

4. Conclusions

Pressure treatments applied to green and red peppers (100 and 200 MPa for 10 and 20 min) caused a lower reduction on soluble protein and ascorbic acid contents, than thermal blanching, particularly for red peppers, that showed even an increase in the amount of ascorbic acid content, compared to the unprocessed peppers.

Both green and red pressure treated peppers showed a level of residual polyphenol oxidase activity similar of that of thermally blanched peppers. Pectin methylesterase activity was only detected in green peppers and its activity declined progressively, as blanching temperature and time increased, while the pressure treatments caused a slight increase of its activity. Peroxidase was more stable to the pressure treatments, than to the blanching treatments, particularly for red peppers, and showed a lower stability to the thermal blanching treatments than polyphenol oxidase and pectin methylesterase. Thermal blanching and pressure treatments caused at maximum 1–2 decimal reductions on microbial load, pointing out the importance of the washing step with chlorine solution for the microbial quality of the final product.

Firmness was equally to better retained in pressure treated peppers compared to thermally blanched peppers, before and after freezing, while red peppers showed higher sensitivity to lose firmness, compared to red peppers. Firmness was more affected by freezing when it was measured from the flesh side. Globally, the pressure treated peppers present similar to better levels of the quality parameters studied, pointing to the possible use of pressure treatments as an alternative to the conventional thermal blanching of sweet bell peppers. Pressure treatments at higher pressures

should be tested in further studies, to evaluate if they can cause higher reductions in microbial loads and enzymatic activity, and still yield peppers with better nutritional and texture characteristics, compared to blanched peppers.

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