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High hydrostatic pressure treatment as an alternative to pasteurization to maintain bioactive compound content and texture in red sweet pepper

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ABSTRACT

Red sweet peppers (*Capsicum annuum)* are an excellent source of essential nutrients and bioactive compounds. High hydrostatic pressures (HHP) not only increase shelf-life but also maintain nutritional and organoleptic properties better in a number of food products. The aim of this work was to measure the effect of HHP and a thermal treatment, pasteurization (PA) in a water bath at 70 °C for 10 min, on some bioactive compounds (fibre, carotenoids and antioxidant activity) and on the texture (TPA; firmness and shear force) of red Lamuyo-type sweet peppers, in order to discover the relationship between treatment (HHP and PA), tissue microstructure and bioactive compound extractability. The results show that HHP at 500 MPa and PA treatments had less impact on the microstructure, bioactive compound content (fibre and antioxidant activity) and texture of red sweet peppers, than when low pressures were used. Consequently, new functional foods could be developed using red sweet pepper tissues treated with high pressures (500 MPa) and/or PA.

Industrial relevance: Today's consumers demand foods that are rich in bioactive compounds with beneficial health effects and safer, more natural, minimally-processed food products. Red sweet peppers (*Capsicum annuum)* are an excellent source of essential nutrients and bioactive compounds such as carotenoids and fibre. High hydrostatic pressure (HHP) processing is considered one of the most economically viable of the non-thermal technologies that helps to preserve red sweet peppers with high nutritional and quality parameters. Therefore, it would be interesting to study the microstructure of HHP-treated red sweet pepper tissues in order to discover whether this treatment promotes the extractability of bioactive compounds, and to compare the results with those obtained by pasteurizing the red sweet pepper. Thus, these enhanced red sweet peppers could be used as ingredients in the formulation of new functional foods.

1. Introduction

Because food and health are closely related, consumers nowadays increasingly prefer and choose foods that not only provide the essential nutrients for life but also contain substances, such as bioactive compounds, which may have healthy effects in the long term (Drago, López, & Sainz, 2006). For instance, traditional foods such as some fruit and vegetables are now considered to contain important bioactive components that are beneficial to health (Santiago-Silva, Labanca, & Gloria, 2011).

Sweet peppers belong to the species *Capsicum annuum.* They are an excellent source of essential nutrients such as carbohydrates, vitamins and minerals (Faustino, Barroca, & Guiné, 2007). In recent years, sweet peppers have attracted the attention of researchers owing to their high content of some bioactive compounds, such as fibre, phenols, flavonoids and carotenoids, which possess antioxidant and anti-inflammatory activity (Duma & Alsina, 2012). Beneficial properties are attributed to sweet peppers and their consumption appears to improve scar formation, prevent atherosclerosis and haemorrhages, stop blood cholesterol levels rising and improve stamina (Faustino et al., 2007). Sweet peppers are an important part of the daily human diet; they can be eaten fresh; however, they are usually preserved for further consumption (Gázquez, 2007).

Bioactive compounds are extra-nutritional constituents which can be found in small quantities in a variety of foods (Kris-Etherton et al., 2002). They are easily degraded by oxygen, light, temperature and pH but have protective effects in diets, as has been proved in many studies (Araya, Clavijo, & Herrera, 2006; Ferrari, Maresca, & Ciccarone, 2010). They can lower the risk of cardiovascular diseases, strokes and cancer (Kris-Etherton et al., 2002). Furthermore, they appear to lessen the effects of diabetes, promote bowel movement and reduce the serum cholesterol level (Belitz, Grosch, & Schieberle, 2008). Bioactive compounds include, for example, carotenoids, phenols, dietary fibre and other phytochemicals. Carotenoids are important for colour and for other biological functions, such as antioxidant activity, provitamin A activity or enhancement of the immune system (Fernández-García et al., 2012). Dietary fibre can produce a sensation of fullness and therefore help in diets. Moreover it can reduce the risk of stomach cancer (Belitz et al., 2008). The insoluble fibre fraction seems to be linked to regulating the

intestinal tract, while the soluble fibre is related to lowering blood cholesterol levels and to intestinal absorption (Ramulu & Udayasekhara, 2003).

It has been shown (Boileau, Moore, & Erdman, 1999) that when natural products are consumed, the assimilation of some bioactive compounds, such as carotenoids, is relatively low for the quantities ingested. Bioavailability is the fraction of a compound that is absorbed during the complete digestion process. The bioavailability of bioactive compounds like fibre, phenols and carotenoids seems to depend not only on factors related to the food matrix but also on the nutritional level and genetic profile of each individual (Maiani et al., 2009). The term "bioaccessibility" defines the fraction of nutrients that are liberated from the food matrix in the gastrointestinal tract. Some preservation treatments (osmotic dehydration, modified atmospheres, frying, microwave, freezing, and pasteurization) cause microstructural modifications in the treated foods (Guardeño, Sanz, Fiszman, Quiles, & Hernando, 2011; Hernández-Carrión et al., 2011; Llorca et al., 2003; Quiles et al., 2004; Soliva-Fortuny, Lluch, Quiles, Grigelmo-Miguel, & Martín-Belloso, 2003) and could influence the fraction liberated from the food matrix, and therefore also the fraction that is absorbed during digestion. Microstructural characterization of these foods is fundamental and would help to elucidate whether particular methods of treating the food might influence the ability to extract these compounds from the food matrix.

The demand for safe foods that possess sensory freshness characteristics and biological properties that go beyond the strictly nutritional have led researchers and manufacturers to develop new processing and conservation technologies. Of these new technologies, high hydrostatic pressure (HHP) is one of the most economically viable of what are known as non-thermal treatments (Devlieghere, Vermeiren, & Debevere, 2004; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). The effects of HHP on the nutritional and bioactive compounds and the microstructure of the food have been studied in some foods. Hernández-Carrión, Vázquez-Gutiérrez, Hernando, and Quiles (2014) studied the impact of HHP on the structure and extractability of some bioactive compounds present in persimmons and concluded that this treatment favoured the structural compaction and extractability of carotenoids but appeared not to influence the fibre content. Vázquez-Gutiérrez et al. (2013) studied the changes in the structure and antioxidant properties of HHP-treated onions and found that the treatment caused structural changes and enhanced the extractability of phenols and other compounds with antioxidant effects. On studying the impact of HHP on the structure, soluble compound diffusion and texture properties of persimmons, Vázquez-Gutiérrez, Hernández-Carrión, Quiles, Hernando, and Pérez-Munuera (2012) concluded that HHP treatments favoured the extractability of tannins and other soluble compounds and their diffusion into the intercellular spaces and diminished the firmness and cohesiveness of the samples. It would be interesting to study the effect of HHP on the tissues of other plant products, such as sweet peppers, that are rich in bioactive compounds.

The aim of this study was to detect the effects of HHP and a traditional thermal pasteurization treatment (PA) on the bioactive compound content (fibre, carotenoids and antioxidant activity) and texture of red Lamuyo-type sweet peppers in order to ascertain the relationship between type of treatment (HHP or PA), tissue microstructure and bioactive compound extractability. This would make it possible to select the pepper tissue with the highest bioactive compound content in order to develop ingredients of interest for formulating functional foods.

2. Materials and Methods

2.1. Plant material and sample preparation

The plant material used was red Lamuyo-type sweet peppers at commercial maturity stage. The red peppers, acquired from a local market in September 2013, were washed, cut into pieces measuring about 15 mm along each side and heat-sealed in 200 x 200 mm plastic bags (Doypack type, Amcor, Spain). Each bag contained approximately 100 g of sweet red pepper. One batch was not subjected to any treatment (Control). The second, third, fourth and fifth batch were treated by HHP at different pressures (100, 200, 300, and 500 MPa). The last batch was pasteurized (PA) in a water bath at 70 ºC for 10 min (come-up time to temperature = 30 min). The bags were then stored at 4 °C until they were analysed. The microstructure, colour and texture properties were analysed within 24 h of treatment.

2.2. High hydrostatic pressure (HHP) treatments

Bags with approximately 100 g of red sweet pepper were placed inside a hydrostatic pressure unit with a 135-L capacity (Hyperbaric type 135, Burgos, Spain), using water as the pressure medium. Different HHP treatments were studied, coded T1 (100 MPa), T2 (200 MPa), T3 (300 MPa), and T4 (500 MPa) during 15 min at 25 ºC.

2.3. Microstructure analysis

2.3.1. Light Microscopy (LM)

For the LM, the samples (2 mm^3) were fixed with a 25 g L^{-1} glutaraldehyde solution (0.025 M phosphate buffer, pH 6.8, 4 °C, 24 h), post-fixed with a 20 g L^{-1} OsO4 solution (1.5 h), dehydrated using a graded ethanol series (300, 500 and 700 g kg^{-1}), contrasted in 20 g L^{-1} uranyl acetate, dehydrated with ethanol (960 and 1000 g kg^{-1}) and embedded in epoxy resin (Durcupan; Sigma-Aldrich, St. Louis, MO, USA) at 65.5 ºC for 72 h. The samples were cut using a Reichert Jung ultramicrotome (Leica Mycrosystems, Wetzlar, Germany). Semi-thin sections (1.5 μm) were stained with toluidine blue and examined under a Nikon Eclipse 80i light microscope (Nikon, Tokyo, Japan).

2.3.2. Transmission Electron Microscopy (TEM)

The same protocol of fixation, dehydration and infiltration was followed as for LM. Ultramicrotomy was carried out in the same equipment, but in this case 0.05-μm-thick sections were obtained. These ultra-thin sections were stained with 40 g L^{-1} lead citrate and 20 g L^{-1} uranyl acetate and observed with a Philips EM 400 (Philips, Eindhoven, Holland) transmission electronic microscope at 80 kV.

2.3.3. Image Analysis

The image analysis was carried out using ImageJ software (Rasband, W.S., ImageJ v. 1.43 s, National Institute of Health, Bethesda, Maryland, USA). The cell area was measured from the LM images and the cell wall thickness from the TEM images. The area and thickness were assessed from at least six randomly-acquired LM and TEM images, respectively. The cells and cell walls were labeled manually and their area (μm^2) and thickness (μm) in each image were measured.

2.4. Physicochemical analysis

2.4.1. Sweet Pepper Purée Preparation

A 120-g portion of red sweet pepper cut into small pieces was homogenised in a food processor (Thermomix TM31, Wuppertal, Germany) using two different stirring speeds: 6500 rpm for 1 min followed by 10200 rpm for 30 s. The red sweet pepper purée was then stored in hermetically sealed glass jars at -80 °C in a deep freezer (Dairei Europe, Denmark) until its analysis, when it was thawed at room temperature before measuring the carotenoid content and antioxidant activity. The purée was prepared in triplicate.

2.4.2. Total, Insoluble and Soluble Dietary Fibre

The total dietary fibre (TDF) and insoluble dietary fibre (IDF) were determined according to AOAC official method 991.43 (AOAC, 1992) using the Fibertec E system (model TM1023, Foss Analytical AB, Höganäs, Sweden). For this purpose, 1 g of freeze-dried sample was

used (72 h, -45 ºC, 1.3 10^{-3} mPa, Lioalfa-6 freeze-drier, Telstar, Terrassa, Spain). Duplicate samples underwent sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglycosidase to remove the starch and protein. For TDF, the enzyme digestate was treated with ethanol to precipitate the soluble dietary fibre before filtering and the TDF residue was washed with ethanol, dried and weighed. For IDF, the enzyme digestate was filtered and the residue (IDF) was washed with warm water, dried and weighed. The TDF and IDF residue values were corrected for protein, ash, and blank. The soluble dietary fibre (SDF) was determined by the difference between TDF and IDF. The results were expressed as g/100 g of dry weight. Three different digestions were made for each treatment.

2.4.3. pH

The pH was measured in duplicate from the three separate sweet pepper purées, using a Basic 20+ pH-meter (Crison, Barcelona, Spain).

2.4.4. Carotenoid Content

The total carotenoid content was measured by the method described by Hornero-Méndez and Mínguez-Mosquera (2001), with modifications. The sweet pepper purée (5 g) was extracted with 25 mL of cool acetone using a homogeniser (IKA T25 Basic Ultraturrax) and vacuum-filtered until no more colour was extracted. The extract was added gradually to 50 mL of ethyl ether in a decanting funnel. With each addition of extract, enough NaCl solution (100 g L^{-1}) was added to separate the phases and transfer the pigments to the ether phase, then the aqueous phase was removed. This process was carried out in several steps to ensure maximum elimination of the aqueous phase. The ether phase was treated several times with anhydrous Na_2SO_4 (20 g L^{-1}) to remove residual water and was evaporated to dryness in a rotary evaporator (model RII; Buchi Labortechnik, Flawil, Switzerland) at a temperature below 35 ºC. Finally, the pigments were collected with acetone to a volume of 200 mL and the absorbance was measured at 450 nm using a spectrophotometer (model Helios Zeta UV Visible; Thermo Fisher Scientific Inc., Cambridge, UK). The calibration curve was constructed with different concentrations of β carotene (Sigma Aldrich, Madrid, Spain) in acetone (Panreac, Barcelona, Spain). The results were expressed as mg β-carotene/100 g of dry weight. Three separate carotenoid extractions were made for each treatment and the measurements were performed in triplicate.

2.4.5. Colour Measurements

The measurements were carried out with a Chroma meter CR-400 (Konica Minolta Sensing Americas, Inc. USA). The results were expressed in accordance with the CIELAB system with reference to illuminant C and a visual angle of 2°. The colorimeter was calibrated with a white standard pattern (Y = 92.9; x = 0.3137; y = 0.3198). The parameters determined were lightness, L* (L* = 0 [black] and L* = 100 [white]), a* (-a* = greenness and + a* = redness), and b* (-b* = blueness and + b* = yellowness). Total colour difference (ΔE*) was calculated as follows (Francis & Clydesdale, 1975).

$$\Delta E^* = \left[(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b)^2\right]^{1/2} \quad (1)$$

The values used to determine whether the total colour difference was apreciable by the human eye were the following (Bodart, de Peñaranda, Deneyer, & Flamant, 2008):

$\Delta E^* < 1$ colour differences are not obvious for the human eye.
$1 < \Delta E^* < 3$ colour differences are not appreciate by the human eye.
$\Delta E^* > 3$ colour differences are obvious for the human eye.

Measurements were performed in triplicate.

2.4.6. Antioxidant Activity

The antioxidant activity was measured by a ferric reducing antioxidant power assay (FRAP). The sweet pepper purée (5 g) was homogenised in an Ultraturrax with 25 mL of 960 g kg^{-1} ethanol. The homogenate was centrifuged (27716 *g*, 20 min, 4 ºC) and filtered. The supernatant was kept. More supernatant was extracted from the pellet with 25 mL of 960 g kg^{-1} ethanol and added to the first supernatant. The total supernatant was brought up to 100 mL with 960 g kg^{-1} ethanol. Distilled water (30 μL), sample (30 μL), and FRAP reagent (900 μL) were placed in each cuvette. The cuvettes were incubated for 30 min in a water bath at 37 ºC and the absorbance was measured at 595 nm. The calibration curve was obtained using different concentrations of Trolox in 960 g kg^{-1} ethanol. The results were expressed as μmol Trolox/g of sample. Three separate extractions were made for each treatment and the measurements were performed in triplicate.

2.4.7. Texture Properties

The texture properties were measured at room temperature with a TA.XTplus Texture Analyser (Stable Micro Systems, UK). Flesh firmness, shear force, and texture profile analysis (TPA) parameters were studied in the epicarp and endocarp of red sweet pepper samples. The flesh firmness was expressed as the load in newtons (N) required to break the flesh of the red sweet pepper pieces with a 2-mm diameter flat-tipped cylindrical probe at a test speed of 1 mm s^{-1}. The shear force was measured as the load in Newtons (N) needed to cut the sweet red pepper pieces (15-mm wide) with a knife blade at a test speed of 1 mm s^{-1}. A texture profile analysis was performed to measure the hardness, cohesiveness, springiness, chewiness and gumminess. The red sweet pepper pieces (15-mm wide) were axially compressed in two consecutive cycles at a test speed of 1 mm s^{-1} with 15% compression with a 50-mm diameter flat plunger. For each treatment values were an average of the measurements from 12 pieces of red sweet pepper.

2.5. Statistical analysis

The data were subjected to variance analysis (ANOVA), using the least significant difference (LSD) test with a 95% ($p < 0.05$) confidence interval to compare the test averages (Statgraphics Plus 5.1, Manugistics, Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Microstructure

The parenchyma of the red Lamuyo-type sweet peppers was composed of turgid cells, mostly round or semi-round in appearance (Fig. 1A), with mean areas of 10113 ± 2311 μm^2. The cell walls, with a mean thickness of 0.82 ± 0.34 μm, turned an even blue colour when stained with toluidine blue (Fig. 1B) and had a well-defined appearance in the TEM images (Fig. 1D), showing their high degree of integrity.

In most of the cells the plasmalemma remained close to the cell wall (Fig. 1D, E and F). The cell interior could be seen to be occupied by an enormous vacuole (Fig. 1C), surrounded by the tonoplast, which remained close to the plasmalemma in most areas (Fig. 1D). In general, the cell membranes showed a high level of structural integrity. Plasmodesmata could be observed, connecting the protoplasms of adjacent cells. The middle lamella could be seen to be intensely coloured, continuous and intact, keeping the cell walls united with those of the adjacent cells (Fig. 1D and F). The intercellular spaces were mostly triangular and packed with solutes. A high carotenoid pigment content, accumulated inside the chromoplasts, could also be seen in the interior of the cells (Fig. 1A and B). These organelles were distributed throughout the symplast, specifically between the plasmalemma and the tonoplast (Fig. 1B and C). A clearly-defined chromoplast membrane could be

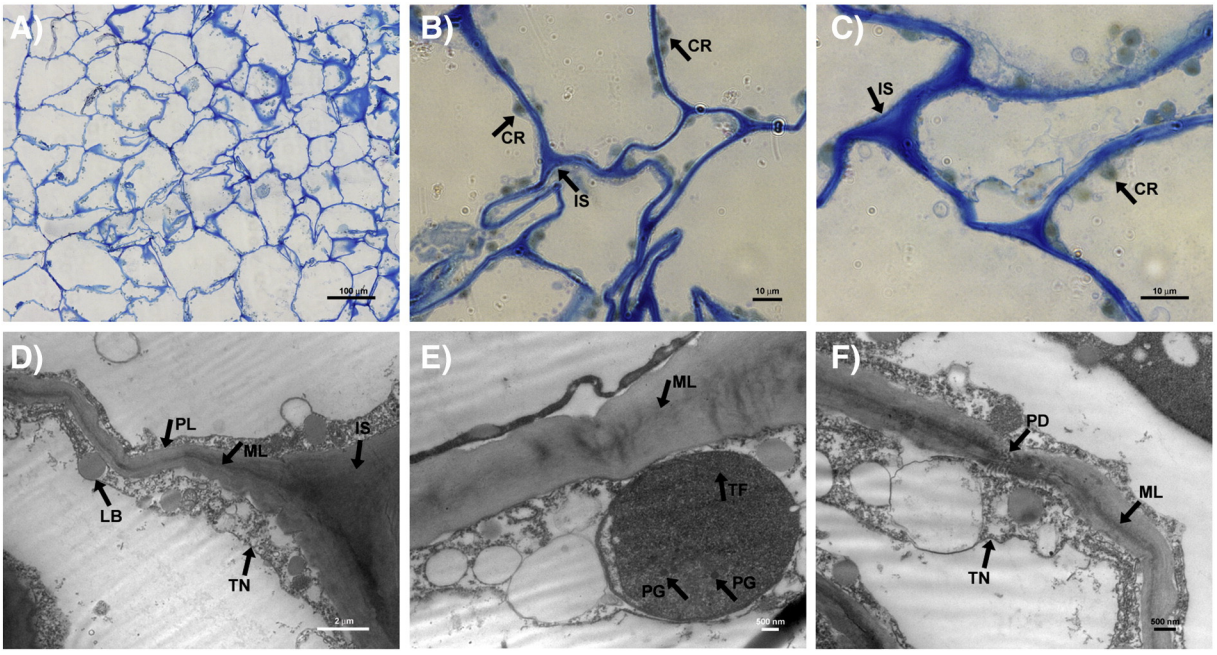

Fig. 1. Light microscopy (A, B, C) and transmission electron microscopy (D, E, F) micrographs of untreated red Lamuyo sweet pepper. CR, chromoplast; IS, intercellular space; LB, lipid body; ML, middle lamella; PD, plasmodesmata; PG, plastoglobuli; PL, plasmalemma; TF, tubular formations; TN, tonoplast. Magnification: 10x (A), 60x (B), 100x (C), 1500x (D), 2000x (E), and 2500x (F).

seen and, within it, plastoglobuli and the carotenoid pigments clustered into tubular structures (Fig. 1E).

When the red pepper was subjected to gentle HHP treatment at pressures of 100 MPa (treatment T1, Figs. 2 and 3), its tissues broke down completely (Fig. 2A) when compared to the untreated pepper (Fig. 1A). In the pepper with the HHP T1 treatment, the parenchymal cells (mean area 7246 ± 1647 μm^2) were found to be deformed and longer in shape than those of the untreated pepper. The cell walls, which were thicker (1.57 ± 0.45 μm) than in the untreated pepper, were observed to be very lightly blue-stained or even not stained at all (Fig. 2A, D and G), confirming their advanced degree of breakdown and loss of fibril bundling (Fig. 3A and D). Areas where the cell walls had degraded completely could be seen throughout the parenchymal tissue (Figs. 2D and 3D). However, these areas were occupied by clumps of matter – probably the remains of the middle lamella and cell material, possibly lignified – organised like bridges connecting the other walls to each other, maintaining the continuity and the boundaries of the cells. No middle lamella was observed in any of the walls of the pepper subjected to HHP T1 (Fig. 3A and D), so this treatment can be said to lead to a high level of dissolution of the middle lamella. The cell walls of the neighbouring cells were completely separated from each other (Fig. 2A and D), greatly increasing the proportion of apoplast. In this way, the triangular intercellular spaces typical of the untreated pepper (Fig. 1A) gave way to the appearance of large intercellular spaces (Fig. 2A). HHP treatments with gentle pressure caused cell membrane rupture (Fig. 3A) and withdrawal of the cell's content into its interior (Fig. 2A and D). In the pepper subjected to HHP T1, the chromoplasts appeared degraded and their membranes ruptured (Fig. 2D and G).

The HHP T4 treatment (Fig. 2B, E and H) led to visible structural changes in the parenchyma compared with the untreated pepper (Fig. 1A), but less tissue breakdown than with the HHP T1 treatment (Fig. 2A). Generally speaking, the gentle pressure applied in treatments T1 (Fig. 2A, D and G) and T2 (data not shown) caused greater structural breakdown than the high pressures of treatments T4 (Fig. 2B, E and H) and T3 (data not shown). The red sweet pepper parenchymal cells subjected to HHP T4, with a mean area of 9662 ± 2773 μm^2, were found to be round in shape (Fig. 2B) like those of the untreated pepper (Fig. 1A). Their cell walls were 1.34 ± 0.53 μm-thick and were more stained (Fig. 2B, E and H) than those of the pepper that received the HHP T1 treatment (Fig. 2A, D and G), though less structured than those of the untreated pepper (Fig. 1E). The middle lamella could be seen in some areas (Fig. 3B). HHP T4 also caused cell membrane breakdown (Fig. 3E) in some areas, but without the membrane withdrawal to the centre of the cell observed in the HHP T1 pepper (Fig. 2E). Chromoplasts could be seen in the interior of the cell (Fig. 2E), as in the untreated pepper (Fig. 1B), but the membranes of these organelles had dissolved in some areas (Fig. 3B and E). The disintegrated chromoplasts seemed to be associated with the lipid bodies (Fig. 3B and E), which appeared as independent structures in the untreated pepper (Fig. 1D).

The thermal treatment (PA) also led to structural modifications in the pepper tissue compared with the untreated pepper (Fig. 1). However, it led to less breakdown of the parenchymal tissue than the HHP treatments. The cells, with a mean area of 12127 ± 2208 μm^2, were more lightly stained (Fig. 2C) than in the untreated pepper (Fig. 1A). The cell walls, which were 0.75 ± 0.25 μm-thick, similar to those of the untreated pepper, presented a high degree of fibril bundling (Fig. 3C) and appeared more structured than those of the HHP-treated peppers (T1 and T4). The cell walls could be seen to be ruptured in the areas of plasmodesmata (Fig. 3C). Middle lamellae were present in many areas (Fig. 3C), as was also the case in the pepper parenchyma subjected to HHP T4. In the pepper parenchyma that underwent PA treatment, separation between the cell walls of adjoining cells only took place in some areas giving place to irregular intercellular spaces (Fig. 2C and F). The plasmalemma was separated (Fig. 2I) and broken down in some areas (Fig. 3C), as with the HHP T4 treatment. Chromoplasts were observed in the symplast areas in the PA-treated pepper, as in the untreated and HHP T4 peppers. However, as in the HHP T4 treatment, the lipid bodies seemed to be associated with the chromoplasts that had lost their membrane (Fig. 3C and F).

3.2. Total, insoluble, and soluble dietary fibre

Table 1 shows the total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) content results for the red sweet peppers with the different HHP and PA preservation treatments. In

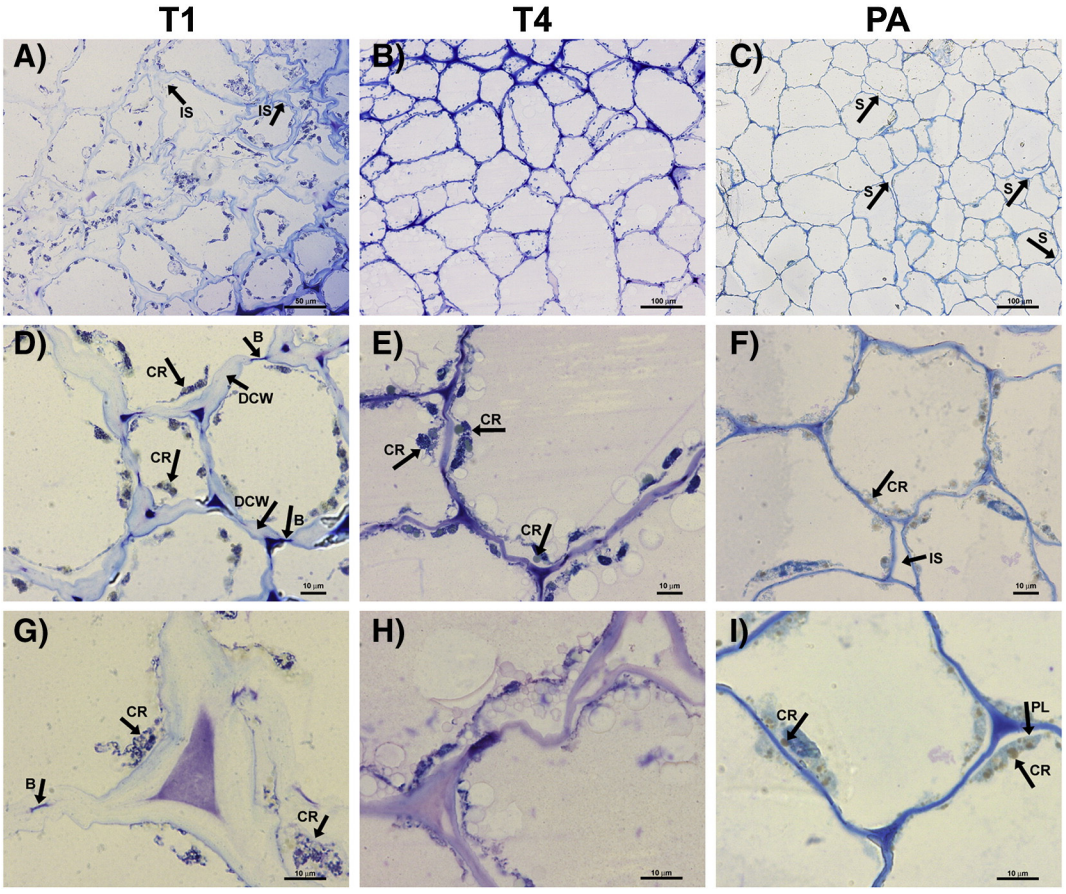

Fig. 2. Light microscopy micrographs of red Lamuyo sweet pepper subjected to HHP at 100 MPa (A, D, G) and 500 MPa (B, E, H), and pasteurized (C, F, I). B, bridges of remnants of cell wall material; CR, chromoplast; DCW: degraded cell wall; IS, intercellular space; PL, plasmalemma; S, separation between cell walls. Magnification: 20x (A), 10x (B and C), 60x (D, E, F), and 100x (G, H, I).

general, both types of treatment led to a significant reduction in TDF content ($p < 0.05$). The reduction was less pronounced with the PA and HHP T4 treatments, and no significant differences between these two treatments were found ($p > 0.05$). Nor was any statistically significant difference ($p > 0.05$) in TDF content found between the different HHP treatments studied, although the differences between the PA-treated peppers and those to which HHP treatments T1, T2 and T3 had been applied were significant ($p < 0.05$).

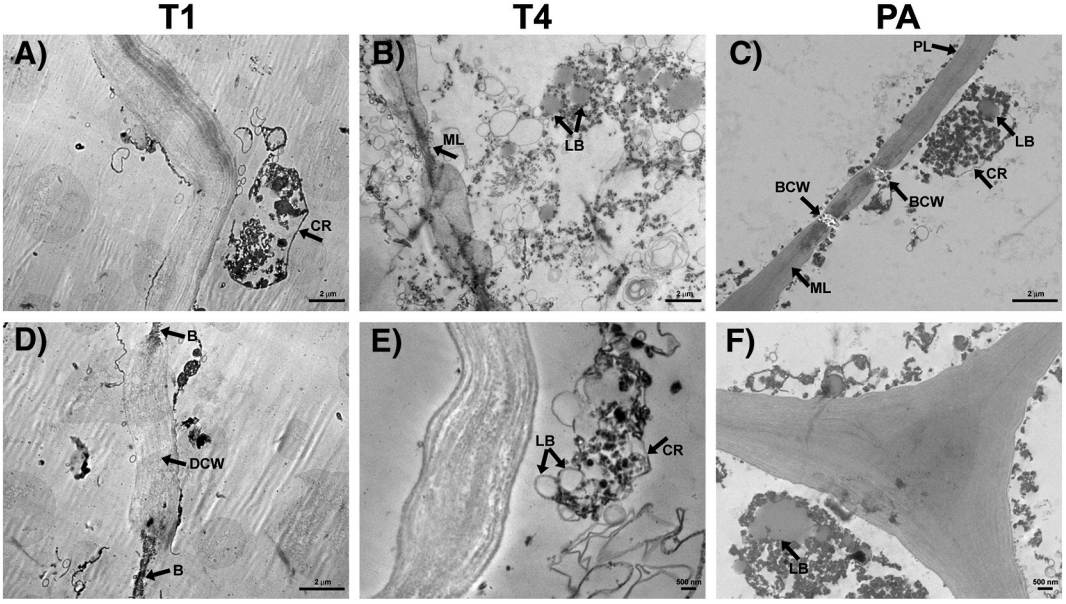

Fig. 3. Transmission electron microscopy micrographs of red Lamuyo sweet pepper subjected to HHP at 100 MPa (A and D) and 500 MPa (B and E), and pasteurized (C and F). B, bridges of remnants of cell wall material; BCW, broken cell wall; CR, chromoplast; DCW, degraded cell wall; LB, lipid body; ML, middle lamella; PL, plasmalemma. Magnification: 1200x (A and B), 1500x (C and D), and 2000x (E and F).

Table 1
Total, insoluble and soluble dietary fibre and pH of untreated (Control), HHP-treated (T1, T2, T3, and T4) and pasteurized (PA) red Lamuyo sweet pepper.

	TDF (g/ 100 g d.w.)	IDF (g/ 100 g d.w.)	SDF (g/ 100 g d.w.)	pH
Control	20.982^{a} (0.081)	12.706^{a} (0.433)	8.276^{a} (0.514)	4.923^{a} (0.076)
T1	18.020^{b} (0.016)	12.748^{a} (0.099)	5.271^{b} (0.084)	4.393^{b} (0.127)
T2	18.349^{b} (0.096)	11.830^{ab} (0.708)	6.519^{bc} (0.804)	4.540^{c} (0.026)
T3	18.320^{b} (0.673)	12.581^{a} (0.333)	5.739^{b} (0.961)	4.567^{c} (0.021)
T4	18.726^{bc} (0.444)	11.424^{b} (0.697)	7.302^{ac} (0.518)	4.740^{d} (0.060)
PA	19.526^{c} (0.326)	12.202^{ab} (0.564)	7.325^{ac} (0.238)	4.987^{a} (0.059)

d.w.: dry weight.
The values in parenthesis are the standard deviations.
In the same column, means without the same letter reveal significant differences ($p < 0.05$) according to the LSD multiple range test.

Table 2
Carotenoid content and antioxidant activity of untreated (Control), HHP-treated (T1, T2, T3, and T4) and pasteurized (PA) red Lamuyo sweet pepper.

	Carotenoid content (mg β-carotene/100 g f.w.)	Antioxidant activity [Trolox] (μmol/g f.w.)
Control	7.724^{a} (0.906)	16.750^{a} (0.567)
T1	5.335^{b} (0.383)	14.533^{b} (0.586)
T2	6.768^{c} (0.086)	14.42^{b} (0.921)
T3	6.692^{c} (0.140)	15.629^{c} (1.058)
T4	6.406^{cd} (0.182)	16.099^{ac} (0.884)
PA	5.685^{bd} (0.041)	16.629^{a} (0.557)

f.w.: fresh weight.
The values in parenthesis are the standard deviations.
In the same column, means without the same letter reveal significant differences ($p < 0.05$) according to the LSD multiple range test.

As regards the IDF content (Table 1), only the HHP T4 treatment caused a statistically significant reduction ($p < 0.05$) in this measurement, although no statistically significant differences ($p > 0.05$) in IDF content were observed between treatments HHP T4, HHP T2 and PA. Both PA and HHP T4 appeared to degrade the polysaccharides that make up the insoluble fibre. As found when studying the microstructure, although the high pressure (500 MPa) used in the HHP T4 treatment caused less tissue breakdown in general than HHP T1,T2 and T3 it did affected cellulose fibrils and 'glues' in the cell walls, as hemicellulose and lignine.

Looking now at the SDF content (Table 1), in this case HHP treatments T1, T2 and T3 did lead to a significant reduction in soluble fibre content ($p < 0.05$). Also, the differences between these HHP treatments were not significant ($p > 0.05$). The HHP T4 and PA treatments did not produce statistically significant differences ($p < 0.05$) compared to the untreated pepper. Nor were the differences between these two treatments significant ($p > 0.05$). In the microstructure study, it was found that even though the HHP T1, T2 and T3 treatments did not subject the sample to very high pressures, they did cause considerable changes in the pepper tissues. These structural changes seem to be more related to the SDF content than to the IDF content.

Kutoš, Golob, Kač, and Plestenjak (2003) studied the effect of high-temperature thermal processing on canned beans and found that it solubilised certain polysaccharides (hemicelluloses and pectic substances) and reduced the TDF content, mainly owing to loss of SDF. Elleuch et al. (2011) concluded that the changes in TDF content brought about by the thermal treatment depended on the type of cell material and the conditions under which the treatment was carried out. The changes in TDF and SDF content can also be explained by the variations in tissue pH brought about by the different treatments. Rodríguez, Jiménez, Fernández-Bolaños, Guillén, and Heredia (2006) established that fibre component solubilization increases as the pH rises. In Table 1 it will be seen that the treatments in which the pepper presented higher pH values (PA) had a higher SDF content than those where the pH was lower (T1).

3.3. Carotenoid content

The carotenoid content of untreated red Lamuyo-type sweet peppers (Control) subjected to the two preservation treatments – high hydrostatic pressure (HHP) and pasteurization (PA) – is shown in Table 2. These preservation treatments caused a statistically significant reduction ($p < 0.05$) in the carotenoid content of the peppers. The treatments that presented significantly lower carotenoid contents ($p < 0.05$) were HHP at 100 MPa (T1) and PA. There was no statistically significant difference between these two treatments ($p > 0.05$). The HHP treatments at 200 MPa (T2), 300 MPa (T3) and 500 MPa (T4) affected the carotenoid content least, suggesting that treatment at pressures above 100 MPa appear to preserve the carotenoid content. However, it should also be pointed out that no statistically significant differences ($p > 0.05$) in carotenoid content between treatments T4 and PA were found, suggesting that both treatments had the same effect on the carotenoid content of red Lamuyo-type sweet peppers.

The effect of HHP on the bioactive compound content of red sweet peppers has not received much attention. However, studies have been made in other plant product matrices. On studying the effect of HHP in tomatoes (Butz et al., 2002), orange, lemon and carrot juice (Butz et al., 2003), gazpacho [a cold tomato soup] (Plaza, Sánchez-Moreno, De Ancos, & Cano, 2006) and carrot and broccoli (McInerney, Seccafien, Stewart, & Bird, 2007), these authors found no statistically significant differences ($p > 0.05$) in carotenoid content between the HHP-treated samples and the controls. Other authors (Fernandez-Garcia, Butz, & Tauscher, 2001), while not finding differences in tomato carotenoid content (β-carotene or lycopene) between untreated and HHP-treated samples, did find a significant drop ($p < 0.05$) in the total carotenoid content of the samples subjected to thermal or HHP treatments in comparison to the control. They associated these differences with the modifications that take place in the tomato pulp microstructure during processing, which can induce changes in the exposure of hydrophilic structures or cellular decompartmentalization, affecting the arrangement of the internal membranes. This could cause changes in the accessibility of the carotenoids, which are located in the chromoplasts. For their part, Barba, Esteve, and Frígola (2010) studied the effect of HHP at 100, 200, 300 and 400 MPa on the total carotenoid content of a plant product-based beverage and established that the treatments at 100 and 400 MPa led to a significant decrease in carotenoid content ($p < 0.05$). Patras, Brunton, Da Pieve, Butler, and Downey (2009) encountered similar results in tomato purees on applying pressures of 400 and 500 MPa for 15 min. This loss of carotenoid content could be related to carotenoid polyene chain breakdown during processing. As a result of processing, these compounds can undergo isomerization and oxidation, the main causes of carotenoid breakdown (Rodríguez-Amaya, 1997).

Persimmons seem to behave differently when subjected to HHP treatments. Several authors (Hernández-Carrión et al., 2014; Plaza, Colina, De Ancos, Sánchez-Moreno, & Pilar Cano, 2012) have obtained statistically significant ($p < 0.05$) increases in carotenoid content compared to the control by applying different HHP treatments to persimmons. It would appear, therefore, that the effects of HHP on carotenoid content are closely related to the plant material to which this technology is applied and no general conclusions can be drawn.

As regards PA, its negative effect on the levels of various bioactive compounds, including carotenoids, has been reviewed extensively. For instance, Rawson et al. (2011) found that various authors had reported reductions in the content of bioactive compounds such as anthocyanins, ascorbic acid and carotenoid, in mulberry (Aramwit, Bang, & Srichana, 2010), durian juice (Chin et al., 2010), pineapple juice (Rattanathanalerk, Chiewchan, & Srichumpoung, 2005) and apple and cashew juice (Zepka & Mercadante, 2009).

It is important to note that of all evaluated treatments, T1 and PA presented the highest colour differences, obvious for the human eye, 10.15 and 9.14, respectively, agree with the lowest carotenoid content obtained in these samples in comparison with untreated sweet pepper (Table 2). Furthermore, T3 and T4 showed the lowest colour differences, 1.56 and 2.52, respectively, both not appreciated by the human eye which is consistent with the highest carotenoid content present in these samples. These results suggest the existence of a close relationship between the carotenoid content and colour of the red sweet pepper samples, indicating that the lower colour difference regarding the untreated sweet pepper, the greater the carotenoid content.

3.4. Antioxidant activity

Table 2 shows the antioxidant activity of red Lamuyo-type red peppers, both untreated and after application of HHP or PA. The results show that of all the treatments tested, HHP at 100 and 200 MPa (T1 and T2) induced significantly ($p < 0.05$) the greatest drop in antioxidant activity compared to the untreated peppers. The treatments with the least effect on antioxidant activity were HHP at 500 MPa (T4) and PA, where the antioxidant activity did not differ significantly ($p > 0.05$) from that of the untreated pepper. It would appear, therefore, that both T4 and PA have a similar effect on the antioxidant activity of red Lamuyo-type sweet peppers, which would corroborate the similar effects of both treatments on the carotenoid content, as noted in the previous section.

Various studies on the effects of HHP and thermal treatments on different food products have encountered similar results to those of the present study. For instance, Clariana, Valverde, Wijngaard, Mullen, and Marcos (2011) studied the effects of HHP treatments (200, 400 and 600 MPa) on turnips. On increasing the working pressure, they found that the loss of antioxidant activity decreased, to the point where no significant differences ($p > 0.05$) compared to the control sample were observed with the 600-MPa treatment. In the same way, the authors of studies on the effects of HHP on tomato juice (Fernandez-Garcia et al., 2001) or tomatoes and carrots (Butz et al., 2002) concluded that HHP treatments at 600 MPa did not bring about significant changes ($p > 0.05$) in the antioxidant activity of the treated samples compared to those that had not been treated. Nor did they observe significant differences ($p > 0.05$) in antioxidant activity compared to the control sample when the carrots or tomatoes were treated thermally (95 ºC, 5 min) (Butz et al., 2002). Again, Butz et al. (2003) found no significant changes ($p > 0.05$) in the antioxidant activity of various HHP-treated samples (orange, carrot, apple and tomato, and orange, lemon and carrot juices) compared to the control. Sánchez-Moreno et al. (2005) studied the effects of HHP (400 MPa) and PA (70 ºC) on orange juice and found that these treatments did not significantly influence antioxidant activity ($p > 0.05$).

It should be noted that the antioxidant activity results obtained in various foods subjected to different thermal or HPP treatments differ according to the product under study. For instance, McInerney et al. (2007) studied the effects of HHP on the antioxidant activity of different vegetables and found that they depended on the vegetable: the antioxidant activity of the broccoli was not affected significantly ($p > 0.05$), whereas that of the carrots fell significantly ($p < 0.05$) when working with pressures below 400 MPa. Keenan et al. (2010) studied the effects of HHP (450 MPa for 1, 3 and 5 min) and a thermal treatment (70 ºC, 10 min) on the antioxidant activity of a commercial smoothie elaborated with apple, apple juice, strawberry, orange and banana. They found that the HHP treatments reduced the antioxidant activity of the sample significantly ($p < 0.05$), whereas the PA treatment did not ($P > 0.05$). On applying 600 MPa for 1 min or 110 ºC for 8.6 s to a mango nectar, Liu, Wang, Li, Bi & Liao (in press) found no significant differences in the antioxidant activity of the treated samples compared to the control.

3.5. Texture properties

Table 3 shows the texture property values for the epicarp (outer skin) of red Lamuyo-type sweet peppers subjected to the preservation treatments applied in the study.

All the treatments studied (HHP treatments T1, T2, T3 and T4, and PA) led to a significant reduction ($p < 0.05$) in firmness (Table 3). The treatments that led to the least reduction in the values for this property were PA and HHP T4, in that order. Moreover, significant differences were found between all the treatments under study ($p < 0.05$), except between HHP T1 and T3.

The differences in shear force (Table 3) between the untreated samples and those subjected to PA were not significant ($p > 0.05$) but the HHP-treated peppers presented significantly lower shear force values ($p < 0.05$). T4 (500 MPa) was again the HHP treatment that produced a less sharp reduction in this property.

Texture profile analysis (TPA) revealed that the hardness, chewiness and gumminess values differed significantly ($p < 0.05$) between the different treatments (Table 3), but all the preservation treatments studied caused significant falls ($p < 0.05$) in the hardness, chewiness and gumminess values. PA was the treatment that caused them to fall the least, while the hardness, chewiness and gumminess values registered for the HHP-treated peppers were significantly lower ($p < 0.05$).

As regards cohesiveness, no significant differences ($p > 0.05$) were observed between the untreated and PA samples, but all the HHP treatments significantly reduced ($p < 0.05$) the values for this property, although T4 and T3 did so to a lesser extent. None of the preservation treatments studied had a significant influence on the texture property of springiness ($p > 0.05$).

The statistical study to examine the effects of each HHP treatment on the hardness, chewiness and gumminess of the red pepper epicarp (Table 4) showed statistically significant differences ($p < 0.05$) between the different treatments. The treatment with the highest hardness, chewiness and gumminess values was T4.

Table 5 shows the texture property values for the endocarp of red Lamuyo-type sweet peppers subjected to the different preservation treatments. The PA treatment did not influence the firmness of the pepper but all the HHP treatments reduced the tissue firmness values to a significant degree ($p < 0.05$). The loss was not as great with 200 MPa and 500 MPa (T2 and T4, respectively).

As regards shear force (Table 5), the PA pepper presented significantly higher values ($p < 0.05$) than the control pepper, while all the HHP treatments induced statistically significant reductions ($p < 0.05$). HHP T4 was the treatment with the lowest drop in shear force. The TPA results obtained from the measurements of the pepper endocarp (Table 5) reflected a statistically significant ($p < 0.05$) loss of hardness in the peppers subjected to HHP or PA, although less in the latter case than in the HHP peppers.

On analysing the cohesiveness of the pepper samples (Table 5), no statistically significant differences ($p > 0.05$) were observed between the control and PA peppers, but significantly lower values ($p < 0.05$) were found in the HHP-treated samples. T4 was the HHP treatment with the least impact on cohesiveness. No significant differences were found between T4 and PA ($p > 0.0.5$).

As regards springiness (Table 5), T1 and T3 led to significant rises in this property compared to the control ($p < 0.05$). The other treatments (T1, T4 and PA) did not have a significant effect on springiness in comparison with the control ($p > 0.05$). Table 5 shows that the chewiness and gumminess measurements of the pepper endocarp did not change

Table 3
Texture properties measured in the epicarp of untreated (Control), HHP-treated (T1, T2, T3, and T4) and pasteurized (PA) red Lamuyo sweet pepper.

	Firmness (N)	Shear force (N)	TPA analysis				
			Hardness (N)	Cohesiveness (%)	Springiness	Chewiness (N)	Gumminess (N)
Control	10.838[a]	41.555[a]	5.478[a]	73.700[a]	0.882[a]	3.642[a]	3.902[a]
	(1.097)	(4.869)	(1.500)	(4.283)	(0.088)	(1.047)	(0.920)
T1	1.050[b]	0.673[b]	0.844[b]	54.036[b]	0.909[a]	0.287[b]	0.343[b]
	(0.246)	(0.222)	(0.270)	(8.199)	(0.074)	(0.090)	(0.088)
T2	2.411[c]	2.167[bc]	0.639[b]	54.869[b]	0.913[a]	0.335[b]	0.343[b]
	(0.676)	(0.870)	(0.286)	(8.233)	(0.092)	(0.135)	(0.141)
T3	1.451[b]	5.209[c]	0.782[b]	60.908[c]	0.874[a]	0.445[b]	0.561[b]
	(0.519)	(1.508)	(0.337)	(4.927)	(0.086)	(0.195)	(0.211)
T4	5.598[d]	9.197[d]	1.407[b]	61.700[c]	0.857[a]	0.535[b]	0.780[b]
	(0.962)	(3.111)	(0.577)	(6.028)	(0.115)	(0.230)	(0.386)
PA	8.018[e]	43.675[a]	2.757[c]	73.750[a]	0.913[a]	1.718[c]	1.958[c]
	(0.762)	(6.202)	(1.521)	(6.689)	(0.088)	(0.889)	(0.883)

The values in parentheses are the standard deviations.
In the same column, means without the same letter reveal significant differences ($p < 0.05$) according to the LSD multiple range test.

to a statistically significant degree ($p > 0.05$) when the sample was pasteurized (PA), but both fell significantly ($p < 0.05$) in the HHP-treated peppers.

Looking separately at the effects of the HHP treatments on hardness, measured in the pepper endocarp (Table 4), T4 was found to present significantly higher values than T1 ($p < 0.05$). Separate analysis of the HHP treatments' effect on the chewiness and gumminess measured in the pepper endocarp (Table 4) showed that the values for these properties were significantly higher ($p < 0.05$) in the high pressure treatment (500 MPa) than when low pressure (100 MPa) was applied.

Generally speaking, PA and HHP T4 can be said to be the treatments that best preserve the texture properties of red Lamuyo-type sweet peppers. When using HHP to preserve the samples, the texture was affected less by high pressures, reaching 500 MPa, than when the samples were subjected to lower pressures (100, 200, 300 MPa). As found also when studying the microstructure, increasing the working pressure reduced the damage to the sweet pepper cell tissues. Consequently, out of the HHP treatments studied, T4 was the one that best preserved the texture properties. This bears out the results of the microstructure study, in which T4 was the HHP preservation treatment that best maintained tissue integrity.

During high pressure treatment, the substrates, ions and enzymes that are located in different compartments within the cell can be released and can interact with each other, leading to enzyme and non-enzyme reactions that bring about changes in texture in the foods subjected to these treatments (Oey, Lille, Van Loey, & Hendrickx, 2008). For example, pectin is broken down by enzymes such as pectin methyl esterase (PME), polygalacturonase (PG) and pectate lyase (PL). Thermal treatments bring about changes in the action of these enzymes, as do high pressure treatments. They can speed up or slow down chemical reactions, stimulate, delay, inactivate or stabilise pectin enzymes or dissociate enzyme inhibitors. All this induces changes in the texture properties of plant product tissues (Jolie et al., 2012; Sila et al., 2008). Red sweet peppers present high PG enzyme activity but no measurable PME activity (Arancibia & Motsenbocker, 2006; Castro et al., 2008; Ni, Lin, & Barrett, 2005).

In the present study, the damage to the texture was less noticeable with the PA and HHP T4 treatments, probably because they provided suitable conditions for inactivating enzymes such as PG. Houben et al. (2013) verified that when high temperatures (70 ºC) were applied for a relatively long time (10 min), PG was almost entirely inactivated. Crelier, Robert, Claude, and Juillerat (2001) studied PG inactivation in tomato juice and confirmed that this enzyme is sensitive to high pressure treatments, so its activity was drastically reduced by treatment with 400 MPa at 30 ºC and became almost non-existent when the pressure was raised to 500 MPa at the same temperature. Other studies (Houben et al., 2013; Jolie et al., 2012; Rodrigo et al., 2006) have found that PG activity diminishes sharply with pressures above 300 MPa at ambient temperature, falling to almost zero when working with pressures of 500 MPa or above. Rodrigo et al. (2006) found that in tomatoes, 15 min of treatment at ambient temperature with a pressure of 500 MPa totally inactivated the PG.

4. Conclusions

All the preservation treatments studied, whether PA or HHP, caused structural modifications in red Lamuyo-type sweet pepper tissues, but HHP T4 and PA were the treatments that had the least impact on the microstructure. These same treatments (HHP T4 and PA) were also the ones that least affected the bioactive compound content and the texture. High pressure treatment at 500 MPa could provide an alternative to pasteurization, the traditional thermal treatment for sweet pepper preservation, as the texture properties and bioactive compound content (fibre, carotenoids and antioxidant activity) of the red pepper tissues were found to be similar. Owing to their high bioactive compound

Table 4
Hardness, chewiness, and gumminess measured in the epicarp and endocarp of HHP-treated (T1, T2, T3 y T4) red Lamuyo sweet pepper.

	Epicarp			Endocarp		
	Hardness (N)	Chewiness (N)	Gumminess (N)	Hardness (N)	Chewiness (N)	Gumminess (N)
T1	0.844[a]	0.287[a]	0.343[a]	0.766[a]	0.262[a]	0.327[a]
	(0.270)	(0.090)	(0.088)	(0.249)	(0.104)	(0.155)
T2	0.639[a]	0.335[ab]	0.343[a]	0.963[ab]	0.405[ab]	0.413[ab]
	(0.286)	(0.135)	(0.141)	(0.395)	(0.143)	(0.168)
T3	0.782[a]	0.445[bc]	0.561[b]	1.009[ab]	0.558[bc]	0.599[bc]
	(0.337)	(0.195)	(0.211)	(0.473)	(0.231)	(0.282)
T4	1.407[b]	0.535[c]	0.780[c]	1.282[b]	0.712[c]	0.790[c]
	(0.577)	(0.230)	(0.386)	(0.498)	(0.329)	(0.330)

The values in parenthesis are the standard deviations.
In the same column, means without the same letter reveal significant differences ($p < 0.05$) according to the LSD multiple range test.

Table 5
Texture properties measured in the endocarp of untreated (Control), HHP-treated (T1, T2, T3, and T4) and pasteurized (PA) red Lamuyo sweet pepper.

	Firmness (N)	Shear force (N)	TPA analysis				
			Hardness (N)	Cohesiveness (%)	Springiness	Chewiness (N)	Gumminess (N)
Control	4.840^{a} (0.611)	29.690^{a} (4.673)	5.136^{a} (2.553)	71.993^{a} (7.702)	0.829^{a} (0.140)	2.996^{a} (1.024)	3.261^{a} (1.572)
T1	0.528^{b} (0.138)	0.997^{b} (0.287)	0.766^{b} (0.249)	52.238^{b} (8.278)	0.943^{b} (0.067)	0.262^{b} (0.104)	0.327^{b} (0.155)
T2	1.221^{c} (0.563)	1.564^{bc} (0.403)	0.963^{b} (0.395)	46.462^{c} (3.693)	0.909^{ab} (0.099)	0.405^{b} (0.143)	0.413^{b} (0.168)
T3	0.670^{b} (0.271)	5.256^{c} (1.774)	1.009^{b} (0.473)	59.185^{d} (6.450)	0.927^{b} (0.092)	0.558^{b} (0.231)	0.599^{b} (0.282)
T4	1.668^{c} (0.355)	16.447^{d} (5.137)	1.282^{b} (0.498)	64.467^{e} (6.565)	0.888^{ab} (0.124)	0.712^{b} (0.329)	0.790^{b} (0.330)
PA	4.452^{a} (0.572)	51.644^{e} (7.382)	3.775^{c} (1.639)	67.625^{ae} (6.025)	0.893^{ab} (0.110)	2.499^{a} (1.337)	2.588^{a} (1.105)

The values in parenthesis are the standard deviations.
In the same column, means without the same letter reveal significant differences ($p < 0.05$) according to the LSD multiple range test.

content, these pasteurized and HHP-treated at 500 MPa sweet peppers would be also a useful ingredient for formulating new functional foods.

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