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Impact of high hydrostatic pressures on the structure, diffusion of soluble compounds and textural properties of persimmon 'Rojo Brillante'

J.L. Vázquez-Gutiérrez *, M. Hernández-Carrión, A. Quiles, I. Hernando, I. Pérez-Munuera

Grupo de Microestructura y Química de Alimentos, Departamento de Tecnología de Alimentos. Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

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ABSTRACT

The use of high hydrostatic pressure (HHP) provides a reliable way of obtaining high quality food. The aim of this work was to analyze the effect of high pressure treatments on the microstructure and textural properties of astringent and non-astringent (95–98% CO_2, 24 h, 24 °C) persimmon 'Rojo Brillante'. Samples were submitted to different HHP treatments (200 MPa and 400 MPa during 1, 3 and 6 min). The microstructural changes observed by Low Temperature Scanning Electron Microscopy and Light Microscopy were related to the improvement in the diffusion and extractability of tannins and acid compounds of this fruit. Some textural properties (firmness and cohesiveness), pH and titratable acidity were also analyzed. Cell wall disruption and migration of the cell content to the intercellular spaces took place when 200 MPa was applied. Precipitated tannins could be observed in the intercellular spaces of the astringent samples, while they remained inside the tannic cells of the non-astringent ones. The cellular degradation was more obvious and the concentration of solutes in the intercellular spaces increased when 400 MPa was applied. In this way, HHP favored the diffusion of tannins and other soluble components to the intercellular spaces, which could be related to a higher extractability of these nutritional compounds in persimmon 'Rojo Brillante'. The tissue from non-astringent samples was more affected by HHP treatments. Despite the greater diffusion of soluble compounds, the application of HHP provoked undesirable effects on texture, such as a decrease in flesh firmness and cohesiveness in both astringent and non-astringent samples. pH increased in both astringent and non-astringent samples when 200 MPa was applied due to the insolubilization of tannins, while titratable acidity decreased for the same reason. According to the pH values obtained, extractability of acid compounds could be improved when 400 MPa is applied.

1. Introduction

When fruits and vegetables are processed to prolong their shelf life, they can undergo structural and sensory changes. The use of high hydrostatic pressure (HHP) provides a reliable way of obtaining food products with a high preservation of sensory quality. Since covalent bonds are not affected by pressure, this technology is expected to be less harmful to low molecular weight food compounds such as flavoring agents, pigments, vitamins, etc., than other treatments (Oey, Van der Plancken, Van Loey, & Hendrickx, 2008). However, according to kinetic and thermodynamic laws, pressure can influence rates and equilibria of reactions that involve changes in the total molar volume (Cheftel, 1992). In this way, biochemical reactions can be induced under pressure and quality attributes, such as texture or nutritional value, could be affected.

Availability of compounds can also be affected by HHP treatment due to changes in the molecular organization of the lipid–peptide complex and disruption of the phosphatidic acid bilayer membrane structure. This would lead to changes in the function of membrane-bound proteins that control ion permeability (Dörnenburg & Knorr, 1993). For instance, HHP treatment is known to influence the vitamin stability and the extraction yield of some bioactive compounds, such as ascorbic acid in green peas (Quaglia, Gravina, Paperi, & Paoletti, 1996), carotenoids and vitamin A in orange juice (De Ancos, Sgroppo, Plaza, & Cano, 2002), lycopene in tomato puree (Krebbers et al., 2003; Qiu, Jiang, Wang, & Gao, 2006), carotenoids in gazpacho and tomato puree (Plaza, Sánchez-Moreno, De Ancos, & Cano, 2006; Sánchez-Moreno, Plaza, De Ancos, & Cano, 2004), ascorbic acid in carrot and tomato juices (Dede, Alpas, & Bayindirli, 2007) and flavonols in onion (Roldán-Marín, Sánchez-Moreno, Lloría, de Ancos, & Cano, 2009). Carotenoids and vitamin A content have also been studied in persimmon puree (De Ancos, González, & Cano, 2000).

Texture of processed fruit and vegetable relates to microstructural quality and can be attributed mainly to the structural integrity of the cell wall and middle lamella, as well as to turgor pressure (Jackman & Stanley, 1995). Changes in texture are strongly related to transformations in cell wall polymers due to enzymatic and non-enzymatic reactions (Sila et al., 2008). Food is a complex system and the compounds responsible for sensory properties coexist with enzymes, metal ions, etc. Therefore, the effect of HHP treatment on structure and

* Corresponding author. Tel.: +34 963879965; fax: +34 963877369.
E-mail address: jovazgu@upvnet.upv.es (J.L. Vázquez-Gutiérrez).

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sensory characteristics can differ depending on the system to which it is applied. Substrates, ions and enzymes, which are located in different compartments in the cells, can be liberated and interact with each other during HHP treatment due to cell disruption and many different enzymatic and chemical reactions can take place (Oey et al., 2008). Therefore, it is important to analyze the microstructural changes during processing in order to understand their implications in the textural and nutritive properties of the product.

By means of Low Temperature Scanning Electron Microscopy (Cryo-SEM) and freeze-fracture it is possible to visualize the interior of the cell enclosed by the membrane and the three-dimensional organization with exceptional clarity. This technique avoids any risk of artifacts caused by fixation. On the other hand, Light Microscopy (LM) provides a way to localize specific compounds and membranes in the structure. Therefore, both techniques can provide complementary information about the structure of plant tissue.

The aim of this work was to analyze the effect of high pressure treatments on the microstructure and textural properties of persimmon 'Rojo Brillante'. The microstructural changes observed were related to the improvement in the diffusion and extractability of tannins and the acid compounds of this fruit.

2. Materials and methods

2.1. Sample preparation

Persimmon fruit cv. 'Rojo Brillante' were harvested in Carlet (Spain) at the beginning of November of 2009. The maturity index used is a visual observation of the external color of the fruit (Salvador et al., 2007) and six maturity stages are accordingly defined, ranging from I (yellow-green) to VI (orange-red). Stage III of this scale was studied in this work. Half of the batch was treated to remove astringency in closed containers with a 95% CO_2 atmosphere for 24 hours at 24 °C (non-astringent samples). After the treatment, the fruits were stored at 4 °C, together with the fruit that was not treated for astringency (astringent samples).

Cubes (15 mm) were taken from the equatorial area and heat-sealed in 110×220 mm plastic bags (Doypack type, Amcor, Spain). Each bag contained approximately 80 g of sample. The bags were placed inside a hydrostatic pressure unit with a 2350 ml capacity and water was used as the pressure medium (GEC Alsthom ACB 900 HP, type ACIP 665, Nantes, France). The pressures employed in the treatments were 200 and 400 MPa, during 1, 3, and 6 min, respectively, at 25 °C.

2.2. Microstructural analysis

2.2.1. Low Temperature Scanning Electron Microscopy (Cryo-SEM)

A JSM-5410 SEM microscope (JEOL, Tokyo, Japan) was used with a Cryo CT-1500 C unit (Oxford Instruments, Witney, UK) for the Cryo-SEM observation. Samples (1 mm thick pieces from the persimmon cubes) were placed in the holder, fixed with nitrogen slush ($T \leq -210$ °C), then transferred frozen to the Cryo unit, fractured, etched (−90 °C) and gold-coated (10^{-2} bar and 40 mA). Samples were then transferred to the microscope and examined at 15 kV, −130 °C and at a working distance of 15 mm.

2.2.2. Light Microscopy

For the Light Microscopy (LM) observation, samples were fixed with a 25 g L^{-1} glutaraldehyde solution (0.025 M phosphate buffer, pH 6.8, at 4 °C, 24 h), postfixed with a 20 g L^{-1} OsO_4 solution (1.5 h), dehydrated using a graded acetone series (300, 500, 700 and 1000 g kg^{-1}), contrasted in 40 g L^{-1} uranyl acetate dissolved in acetone and embedded in epoxy resin (Durcupan, Sigma–Aldrich, St. Louis, MO, USA). The samples were cut using a Reichert Jung ultramicrotome (Leica Mycrosystems, Wetzlar, Germany). Semithin sections (1.5 μ) were stained with 2 g L^{-1} toluidine blue and examined in a Nikon Eclipse E800 light microscope (Nikon, Tokyo, Japan).

2.3. Textural properties

Flesh firmness and cohesiveness were determined at room temperature with a TA.XTplus Texture Analyzer (Stable Micro Systems). Flesh firmness was expressed as the load in newtons (N) required to break the flesh of the persimmon cubes with a 4 mm diameter flat-tipped cylindrical probe at 1 mm s^{-1} test speed. A texture profile analysis was performed to determine cohesiveness. The samples were axially compressed in two consecutive cycles at 1 mm s^{-1} test speed and 75% compression, three seconds apart, with a 50 mm diameter flat plunger. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Firmness and cohesiveness values were an average of the measurements from eight cubes.

2.4. pH and titratable acidity

Juice was prepared in triplicate for pH and titratable acidity (TA) evaluation. 30 g of persimmon cubes were blended for each juice. TA was determined by titration of fruit juice with standardized 0.1 N sodium hydroxide, using phenolphthalein as an internal indicator. The TA value was expressed as g malic acid/100 ml of juice, according to A.O.A.C official method (A.O.A.C., 1990).

2.5. Statistical analysis

Data was subjected to variance analysis (ANOVA), using the least significant difference (LSD) test with a 95% confidence interval for the comparison of the test averages (Statgraphics Plus 5.1, Manugistics, Inc., Rockville, MA, USA).

3. Results and discussion

3.1. Microstructural study

By Cryo-SEM, untreated samples present a compact tissue where the parenchymatic cells are rounded and almost totally occupied by a large vacuole full of soluble material (Fig. 1A). In non-astringent samples (Fig. 1B), tannins have polymerized due to the treatment with CO_2 and they can be observed by Cryo-SEM as a compact mass inside the tannic cells (Gottreich & Blumenfeld, 1991). When astringent samples are examined by Light Microscopy, cell walls and membranes can be observed stained in blue with toluidine blue (Fig. 1C). However, the staining is less marked in the non-astringent samples (Fig. 1D), probably due to the degradative effect of CO_2 on the membranes (Salvador et al., 2007). In these samples, tannins can be observed stained in dark blue inside the tannic cells.

When 200 MPa was applied for 1 min to the astringent samples, cell wall disruption and migration of the soluble material from the cells to the intercellular spaces take place (Fig. 2A). These effects are greater in non-astringent samples (Fig. 2B). When LM sections are stained with toluidine blue, precipitated tannins can be found in the intercellular spaces of the astringent samples (Fig. 2C). However, tannins remain inside the tannic cells of the non-astringent samples because they had already polymerized with the CO_2 treatment, before the application of HHP. Moreover, deformation of cells is greater in non-astringent samples (Fig. 2D), more sensitive to the compression effect of the HHP treatment. As the treatment time increases (200 MPa/6 min), tannins can be observed evenly distributed in the intercellular spaces of astringent samples (Fig. 2E). Higher cell deformation and tissue compression are observed in both astringent and non-astringent samples (Fig. 2E and F) in comparison with the ones treated at 200 MPa for 1 min (Fig. 2C and D).

By Cryo-SEM, the diffusion of the intracellular liquid to the intercellular spaces becomes more obvious in the samples treated at 200 MPa/6 min. Precipitated tannins can be observed inside the vacuole of some tannic cells and in some intercellular spaces of the astringent samples (Fig. 3A). Tannins remain inside the tannic cells of the non-