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Use of image analysis to evaluate the effect of high hydrostatic pressure and pasteurization as preservation treatments on the microstructure of red sweet pepper

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ABSTRACT

The aim of this work was to evaluate the effect of HHP treatment and PAST on the microstructure of red Lamuyo-type sweet peppers using image analysis and to determine the parameters that allow characterizing the changes observed on the structure using different magnifications (100×, 200×, and 350×). The results show that all the preservation treatments evaluated caused structural modifications on the microstructure of red sweet pepper, but HHP at 500 MPa and PAST had less impact. Fractal dimension texture, contrast, inverse difference moment, and entropy are texture features that are appropriate for characterizing the effect of HHP and PAST on red pepper texture. In this context, it is important to consider the magnification at which red pepper texture is evaluated because cell damage caused by treatments is best observed at low magnification. Consequently, image analysis could be used in future studies to relate microstructure to the functionality of products subject to HHP.

Industrial relevance: Red sweet peppers (*Capsicum annuum*) are an excellent source of essential nutrients and bioactive compounds. High hydrostatic pressure (HHP) applied during food processing can improve the retention of food quality attributes and nutritional and organoleptic properties better than pasteurization (PAST). Image analysis is a non-invasive technique that allows to provide objective evaluations from digitalized images. There are no studies that quantify the effect of HHP and PAST using image texture parameters and that evaluate the effect of the magnification used on these texture features. These features are critical factors that determine food acceptance or rejection by the consumers. Thus, texture measurement has gained much attention from food science and industry. Therefore, it would be interesting to study the effect of these treatments on the microstructure of red sweet pepper tissue using image analysis. Thereby, it would be possible to relate the image information to structural modifications and to the extractability of bioactive compounds or acceptance of preservation processes by consumers.

1. Introduction

Red sweet peppers (*Capsicum annuum* L) are an excellent source of essential nutrients such as carbohydrates, vitamins, and minerals (Faustino, Barroca, & Guiné, 2007). In addition to being a good source of essential nutrient, pepper is rich in fiber and other bioactive compounds such as carotenoids, which possess antioxidant and anti-inflammatory activity, phenols, and flavonoids (Deepa, Kaur, George, Singh, & Kapoor, 2007; Duma & Alsina, 2012; Zhuang, Chen, Sun, & Cao, 2012). Moreover, fresh pepper is considered to be one of the vegetables with the highest content of vitamin C within the plant kingdom (Serrano et al., 2010). Additionally, pepper prevents atherosclerosis and hemorrhages, improves scar formation and stamina, and improves blood cholesterol levels (Faustino et al., 2007).

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). HHP facilitates the production of food products that have the quality of fresh foods but the convenience and profitability associated with shelf life extension (McClements, Patterson, & Linton, 2001). HPP can be applied to a range of different foods, including juices and beverages, fruits and vegetables, meat-based products, fish, and precooked dishes, with meat and vegetables being the most popular applications (Norton & Sun, 2008).

On the other hand, image analysis can be particularly a useful tool for characterizing food morphology because the highly irregular structures of many food materials elude precise quantification by conventional means. This technique allows to obtain measurements from digitalized images. These measurements provide objective evaluations of the

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morpho-colorimetric features of samples, a method that is more quantitative and less biased than the common method of visual perception, which is prone to variation due to the personal opinions of inspectors (Russ, 2007; Sonka, Hlavac, & Boyle, 2008; Sun, 2008). Nowadays, many software programs for image processing and analysis are available on the market able to analyze digital images in real time providing precise and accurate measurements of the size, shape, color, and texture of the objects studied. Many studies have demonstrated the utility of image analysis for the study of morphometric and colorimetric characteristics in fruits and vegetables (López-García, Andreu-García, Blasco, Aleixos, & Valiente, 2010; Rocha, Hauagge, Wainer, & Goldenstein, 2010), in legumes and durum wheat kernels (Venora, Grillo, Ravalli, & Cremonini, 2009; Venora, Grillo, & Saccone, 2009; Venora, Grillo, Shahin, & Symons, 2007), in and bakery products (Abdullah, Aziz, & Dos Mohamed, 2000; Grillo et al., 2014).

Image analysis can evaluate different morphometric characteristics, also known as morphometric features. Morphometric features can be divided into three groups: dimensionality, which refers to measuring various characteristics of the object such as area, perimeter, Feret diameter, and maximum length, among others, using specific computer programs; form, which refers to the graphical representation of the study object as an approximate reference to an Euclidean geometric figure, including circular and elliptical form factors, eccentricity, sphericity, and convexity; and texture, which refers to the information needed to describe the regularity of an object, e.g., its compactness, roughness and sinuosity (Aguilera, 2007).

Texture is one of the important characteristics used in identifying objects or regions of interest in an image, whether the image be a photomicrograph, an aerial photograph, or a satellite image (Haralick, Shanmugam, & Dinstein, 1973). The gray level co-occurrence matrix (GLCM) proposed by Haralick et al. (1973) is an image processing technique that has been widely used for measuring of texture in images. It first generates a gray level co-occurrence matrix that is defined as the distribution of co-occurring values at a given offset over a given image then calculate a set of textual features (usually called Haralick features) from the matrix that can reflect the image texture. Different textural features can be obtained from an image using image analysis, such as angular second moment, contrast, correlation, inverse difference moment, and entropy.

Although numerous publications can be found about the effect of different preservation treatments, such as high hydrostatic pressure and pasteurization (PAST) on the microstructure and on the size and/or shape parameters of different plant tissues (Hernández-Carrión, Hernando, & Quiles, 2014; Hernández-Carrión, Vázquez-Gutiérrez, Hernando, & Quiles, 2014; Vázquez-Gutiérrez, Hernández-Carrión, Quiles, Hernando, & Pérez-Munuera, 2012; Vázquez-Gutiérrez et al., 2013) and about the effect of different processing techniques such as drying on the color of sweet pepper using image analysis (Romano, Argyropoulos, Nagle, Khan, & Müller, 2012), there are no studies that quantify the effect of HHP and PAST using image texture parameters and that evaluate the effect of the magnification used on these texture parameters. In this sense, the study of the effect of HHP and PAST on the microstructure of red sweet pepper tissue using image analysis is essential and would enable to relate the image information to structural modifications and to the extractability of some bioactive compounds, such as carotenoids, that could affect the functionality of the selected plant tissue. Also, it is important to start to develop appropriate decision algorithms, methods, and magnifications that allow acceleration and optimize industrial processes that can be evaluate by image analysis, particularly in the case of important products as sweet pepper.

The aim of this work was (1) to evaluate the effect of HHP treatment and PAST on the microstructure of red Lamuyo-type sweet peppers using image analysis and (2) to determine the parameters that allow characterizing or quantitatively describe the changes observed on the structure using different magnifications.

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 × 200 mm plastic bags (Doypack type, Amcor, Spain). Each bag contained approximately 100 g of red sweet pepper. One batch was not subjected to any treatment (CNT). The second, third, fourth, and fifth batch were treated by HHP at different pressures (100, 200, 300, and 500 MPa) according to Hernández-Carrión et al. (2014). The last batch was pasteurized (PAST) in a water bath at 70 °C for 10 min (come-up time to temperature = 30 min) (Hernández-Carrión et al., 2014). The bags were then stored at 4 °C until they were analyzed. The microstructure was analyzed 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 °C .

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} OsO_4 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 °C 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. Scanning electron microscopy (SEM)

Pieces (3 mm wide) from raw and treated red sweet pepper were frozen at −20 °C and then freeze-dried at 1 Pa for 3 days (LIOALFA- 6, Telstar, Barcelona, Spain). Then red sweet pepper samples were vacuum sealed in vials in the same freeze-drier so that they would remain stable (Llorca et al., 2001). After that, they were individually placed on SEM slides with the aid of colloidal silver and then gold-coated with (SCD005, Baltec, Germany) at 10^{-2} Pa and an ionization current of 40 mA for 120 s. The samples were observed in a scanning electron microscope (JSM-5410, Jeol, Japan) at an acceleration voltage of 15 kV. The microscope was equipped with an integrated program for digital image capture (INCA 4.09, Oxford Instruments, England). Magnifications of 100×, 200×, and 350× were used.

2.4. Image analysis

2.4.1. Morphometric analysis

Image processing was carried out according to Pedreschi, Mery, Mendoza, and Aguilera (2004). Images of the red sweet pepper of 1280 × 1024 pixels were captured using a light microscopy and stored as bit maps (gray scale with brightness values between 0 and 255) (Quintanilla-Carvajal et al., 2011) by using the ImageJ software (Rasband, W.S., ImageJ v.1.43 s, National Institute of Health, Bethesda, Maryland, USA). The following morphological parameters were determined for each treatment: area, perimeter, circularity, and Feret diameter. With these results, the cell size distribution was evaluated.