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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

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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