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# Impact of thermal and high pressure processing on quality parameters of beetroot (*Beta vulgaris L.*)

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ABSTRACT

In this work, high-hydrostatic pressure (HHP) treatments of 650 MPa at different processing times (3, 7, 15 and 30 min) were applied on beetroot slices (var. *Red cloud*) as an alternative to blanching pre-treatment (90 °C for 7 min). Polyphenol oxidase (PPO) and peroxidase (POD) activity, physical (texture and color) and nutritional (betanins retention, total phenolic content, antioxidant capacity and ascorbic acid) parameters were evaluated. Differently from blanching, HHP led only to partial inactivation of PPO (10–25%) and POD (25%) at each tested time. Total phenol content and FRAP value were found to be statistically similar ($p > 0.05$) in HHP-treated and blanched beetroot. An increase of betanins content was observed in HHP-treated samples, resulting in 6-fold higher content in comparison to the raw. However, the higher the time-exposure to high pressure, the higher pigments degradation, as similarly observed for ascorbic acid. Finally, texture properties such as hardness and chewiness were better retained in HHP samples than in thermally treated ones, showing an increase according to the time of pressure exposure. Overall, the HHP processes improved the nutritional quality of the products, as revealed from the principal component analysis.

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## 1. Introduction

Red beet (*Beta vulgaris* L.) is a member of the Chenopodiaceae family, cultivated for its large roots, although leaves are also utilizable. Seeds, roots and leaves of the plant are rich of polyphenols and a water soluble nitrogen pigments group named betalains. The betalains family represents the principal pigment in red beet and the characteristic red–violet color is regarded as major attribute for beetroot quality and acceptability. In particular, two classes of betalains are well-known: the red–violet betacyanins (400–2100 mg/kg fresh weight), which include the important dye betanin, and the yellow–orange betaxanthins (200–1400 mg/kg fresh weight) (Ninfali & Angelino, 2013). Similar to other natural pigments, betalains are very sensitive to degradation by heat, light, enzymes and oxygen; producing a discoloration of pigment (Martinez-Parra & Munoz, 2001). Betalains have been widely studied for their nutritional and functional health benefits; they are considered as useful cancer preventive agents and presented a high radical scavenging antioxidant activity (Kanner, Harel, & Granit, 2001; Lever & Slow, 2010; Ninfali & Angelino, 2013). Beetroot is the only source of betanin approved in the United States and in the European Union as food additive (E-162) for coloring low acid food, and commercially exempt from batch certification (Moreno, García-Viguera, Gil, & Gil-Izquierdo, 2008). Beetroot is commonly consumed fresh as well as cooked, pickled, or canned. Preservation of vegetables usually involves thermal treatments such as blanching, which prevent microbial growth and led to the inactivation of heat resistant enzymes like peroxidase (POD) and polyphenoloxidase (PPO). Also, blanching results in a more stable product prior to other processing steps such as freezing or canning. A controlled blanching step contributes to the retention of vitamins and nutrients for further processed foods. However, heat sensitive molecules may be lost, and, in water blanching, soluble compounds may be leached.

Several pretreatment approaches have been used to enhance

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vegetable quality. Among them, nonthermal technologies are gaining widespread acceptance throughout the food industry. Pressure-assisted thermal processing (PATP) or high hydrostatic processing (HHP) are alternative technologies for the preservation of low acid vegetables as they produce minimal deterioration effects on quality (Medina-Meza, Barnaba, Villani, & Barbosa-Cánovas, 2015; Rastogi, Nguyen, & Balasubramaniam, 2008). During HHP process, food is subjected to isostatic pressures between 100 and 1000 MPa for several minutes with temperature ranges from −20 °C to 60 °C in order to inactivate enzymes, microorganisms or spore (Oey, Lille, Van Loey, & Hendrickx, 2008). Large molecules, microbial cell structures and enzymes are generally more sensitive to pressure, whereas small molecules such as vitamins and flavor components (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014) as well as pigments (Krebbers, Matser, Koets, & Van den Berg, 2002) are unaffected.

Improved antioxidant and nutritional activities are often observed on HHP treated vegetables (Patras, Brunton, Da Pieve, & Butler, 2009); however, several enzymatic activities like PPO and POD are reported to be even more enhanced, probably because of the extensive release of enzymes from the cells (Gonzalez-Cebrino, Duran, Delgado-Adamez, Contador, & Ramirez, 2013), which leads to a quick loss of quality during shelf-life (Suthanthangjai, Kajda, & Zabetakis, 2005). On the other hand, texture is an important sensorial attribute dominating a product quality that can affect the acceptance of consumers. Several studies have shown that blanching controlled conditions can enhance the texture of the product by liberating pectinmethylesterase, the enzyme responsible for increased firminess (Basak & Ramaswamy, 1998; De Roeck, Mols, Duvetter, Van Loey, & Hendrickx, 2010). Similar effects on texture has been achieved using high pressure pretreatments (200-Mpa–500 Mpa for 10–15 min) (Rastogi et al., 2008) or combined temperature-high pressure pretreatments (Sila, Smout, Vu, Van Loey, & Hendrickx, 2005). However, the degree of cell disruption is not only dependent of the pressure but also on the type of plant cell and temperature applied. Thus, pressures of 1000 MPa at 75 °C for 80s in green beans and 600 MPa at 80 °C up to 90 min in carrot disks shown pronounced texture preservation in contrasts with 200 to 400 MPa at 20 °C for 20 min in cherry tomatoes (Oey et al., 2008).

A fundamental understanding of the correlations between pigment degradation and quality attributes of beetroot is a crucial for obtaining a product with high quality for further processing in the food industry. The aim of this work is to assess high pressure processing (650 MPa, from 3 to 30 min) as an alternative to blanching (90 °C, 7 min) for beetroot pretreatment, evaluating the effect on the enzymatic (PPO and PDO), physical (texture and color) and nutritional (betanin retention, total phenolic content, antioxidant capacity and ascorbic acid) attributes of the processed product.

## 2. Materials and methods

### 2.1. Sample preparation

Red beetroots *(B. vulgaris L.* var. Red cloud) harvested in Mexico (2014) were purchased from a local market (Pullman, WA, USA). Roots were cleaned, peeled and cut in slices of 10 ± 1 mm thickness and 80 mm diameter. This sample geometry was chosen in order to be comparable to products available in the market. The samples are renamed in the rest of the paper as raw (R), blanched (BL), whereas the HHP treatments are identified as HHP3, HHP7, HHP15 and HHP30 for 3, 7, 15 and 30 min of 650 MPa pressure treatment, respectively.

### 2.2. Pretreatments

#### 2.2.1. Blanching

Conventional thermal blanching was performed according to Latorre, de Escalada Plá, Rojas, and Gerschenson (2013) by immersion of the beetroot slices (100 g) into a water bath at 90 ± 2 °C for 7 min in a sample/water ratio of 1:5. After, samples were placed in ice water bath for 2 min. Temperature was recorded along the entire process for the water bath and the central point of each slice by using a thin-wire copper-constantan type T thermocouple.

#### 2.2.2. High hydrostatic pressure treatment

A pilot plant scale 2 L high hydrostatic press (Engineering Pressure Systems, Inc., Andover, MA, USA) was used to pressurize the red beet slices. Samples (100 g) were vacuum sealed inside flexible (75 μm thickness) plastic pouches (Ultravac Solutions, Kansas City, MO, USA). A 100 g/L Hydrolubic 123B soluble oil water solution (Houghton Oil Valley Forge, PA, USA) was used as the pressure medium. The plastic pouches were then placed inside a cylindrical pressure vessel (0.1 m internal diameter, 0.25 m internal height). The unit was operated with a hydraulic intensifier pump (Hochdruck-Systeme GmbH, AP 10-0670-1116, Sigless, Austria) that allows to reach the operating pressure in a few seconds. Come-up time was between 0.7 and 0.8 min. The HHP treatment was at 650 MPa for 3, 7, 15 and 30 min. These conditions were chosen on the basis of preliminary experiments and also according to the significant enzymatic inactivation on fruit and vegetables achieved by our group and other authors in the range of 600–700 MPa (Duong & Balaban, 2014; Gonzalez-Cebrino et al., 2013; Medina-Meza et al., 2015). Experiments were conducted at room temperature (20 ± 1 °C). The temperature increase due to compression was not higher than 2–3 °C/100 MPa. Three pouches were processed and analyzed for each time of treatment.

### 2.3. Chemical and enzymatic assays

After treatments, samples were finely grounded with a domestic blender (Oster® 12-Speed Blender, Osterizer, Milwaukee, WI), then 20 g were used for each analysis. All the spectrophotometric determinations were performed with a UV–Vis Spectronic 20 Genesys spectrophotometer (Spectronic Instruments,Inc, Rochester NY, USA). All analyses were performed in triplicate.

#### 2.3.1. Enzymatic activity

Raw and treated grounded beetroot slices were used for the evaluation of polyphenol oxidase (PPO) according to Medina-Meza et al. (2015). The activity was evaluated using catechol (0.1 mg/mL) as substrate, and following the formation of the brownish compound *o*-quinone at 420 nm for 2 min. Peroxidase (POD) activity was performed accorded to Carvajal, Martinez, Jamilena, and Garrido (2011), using guaiacol (10 mg/mL) as a donor and $H_2O_2$ (40 mM) as a substrate. The increase in absorbance at 470 nm was followed for 2 min. PPO and POD activity were calculated using the slope of a linear segment absorbance-time (Adams, Brown, Ledward, & Turner, 2003). Percentage variations were calculated in comparison to the raw sample.

#### 2.3.2. Betanin content

Betanin extraction was conducted according to the method of von Elbe (2001) with slight modifications. The grounded material was washed with distilled water till complete discoloration of the samples. Betanin was quantified on the aqueous extract measuring the absorbance at 538 nm. The results were expressed as mg pigments/g dw based on a calibration curve prepared using a standard solution of betanin (Sigma Aldrich, St. Louis, MO, USA) dissolved in