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High hydrostatic pressure blanching of baby spinach (*Spinacia oleracea* L.)

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Polyvinylpyrrolidone (PubChem CID: 6917)
Serum albumin (PubChem CID: 16132389)
Catechol (PubChem CID: 289)
Guaiacol (PubChem CID: 460)
Hydrogen peroxide (PubChem CID: 784)
Ethanol (PubChem CID: 702)
2,2-diphenyl-1-picrylhydrazyl (DPPH) (PubChem CID: 2735032)
Gallic acid (PubChem CID: 370)
Ascorbic acid (PubChem CID: 54670067)
Acetone (PubChem CID: 180)

ABSTRACT

Given the high susceptibility of baby spinach leaves to thermal processing, the use of high hydrostatic pressure (HHP) is explored as a non-thermal blanching method. The effects of HHP were compared with thermal blanching by following residual activity of polyphenol oxidases and peroxidases, colour retention, chlorophyll and carotenoids content, antioxidant capacity and total polyphenols content. Spinach subjected to 700 MPa at 20 °C for 15 min represented the best treatment among the conditions studied due to its balanced effect on target enzymes and quality indices. The latter treatment reduced enzyme activities of polyphenol oxidases and peroxidases by 86.4 and 76.7%, respectively. Furthermore, leaves did not present changes in colour and an increase by 13.6% and 15.6% was found in chlorophyll and carotenoids content, respectively; regarding phytochemical compounds, retentions of 28.2% of antioxidant capacity and 77.1% of polyphenols content were found. Results demonstrated that HHP (700 MPa) at room temperature, when compared with thermal treatments, presented better retention of polyphenols, not significantly different chlorophyll and carotenoids content and no perceptible differences in the instrumental colour evaluated through ΔE value; therefore, it can be considered a realistic practical alternative to the widely used thermal blanching.

1. Introduction

In recent years there has been an increasing consumer demand for nutritious products with high sensorial quality and acceptable shelf life. This demand has driven research and development in non-thermal food processing technologies, amongst which, the use of high hydrostatic pressure (HHP) processing is believed to have considerable potential with some innovative applications, such as improving the intake of nutrient and non-nutrient phytochemicals, and development of new products and ingredients with extended life and keeping quality (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). Additionally, HHP has been increasingly investigated in the last decade for lowering enzyme activity.

Most vegetables that are canned, frozen and dehydrated cannot be stored for long periods without blanching which typically occurs in water at high temperatures (75–95 °C) for relatively short times (1–10 min) (Gökmen, 2010). Although thermal treatments are effective in terms of reducing enzymatic activity and microbial load, they affect levels of antioxidants, polyphenols, vitamins, carotenoids and flavonoids, and deteriorate sensory properties (Medina-

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Meza, Barnaba, Villani, & Barbosa-Cánovas, 2015). Rastogi et al. (2007) proposed HHP as a non-thermal blanching method which, contrary to thermal treatments, has limited influence on covalent bonds of low molecular weight components such as some nutrients, colour and flavour compounds (Oey, Van der Plancken, Van Loey, & Hendrickx, 2008). Among these attributes, colour has a strong influence on consumers' acceptance and purchase intention (Gökmen, 2010; Medina-Meza et al., 2015). It is thought that enzymatic browning induced by peroxidases (POD) and polyphenoloxidases (PPO) contributes the most to colour deterioration (Steet & Tong, 1996). Enzymes selected as indicators of the blanching adequacy may vary from one product to another. Between these enzymes, POD and PPO activities are usually chosen to indicate the extent of thermal blanching (Whitaker, 1991), in addition these enzymes are amongst the most resistant to pressure.

Several studies have focused on the effects of HHP on enzyme activity in vegetables and vegetable-based products, but very few are *in situ* studies. Moreover, HHP processing as a blanching method for leafy vegetables, has only been studied in the case of white cabbage (Alvarez-Jubete, Valverde, Patras, Mullen, & Marcos, 2014). There is virtually no information on the effects of HHP on the quality characteristics of baby spinach leaves (*Spinacia oleracea* L.) despite its high commercial demand and increasing consumption. It is noteworthy that spinach leaf production doubled in Asia since 2002 and its worldwide production exceeded 20 Mt in 2013 (FAOSTAT, 2015). The present study compares traditional hot water blanching of baby spinach leaves with HHP blanching with respect to residual enzyme activities as well as other physico-chemical characteristics, with a view to establish whether HHP blanching is a realistic practical alternative to the widely used thermal blanching.

2. Materials and methods

2.1. Raw material

Baby spinach leaves (*Spinacia oleracea* L.), cv. Monza, were directly obtained from suppliers within the Dorset county, UK. The leaves were harvested in June–August and were transported at refrigerated temperatures to the University of Reading where they were stored at 4 °C before being processed. Five leaves weighing 0.5–1.0 g each were selected and packaged (Multivac® A300, Germany) in PA-PE (Polyamide-Polyethylene) bags under vacuum (−85 kPa gauge).

2.2. HHP treatments

The HHP equipment (Stansted Fluid Power Ltd., UK) with canister dimensions: 37 mm diameter and 246 mm length, had a maximum working pressure of 900 MPa and used a 30 g/100 mL solution of 1,2-Propanediol (Sigma-Aldrich, UK) for transmitting pressure. The temperature was controlled by a thermostatic device circulating distilled water through the jacket and registered with an external sensor device. The samples were placed in the product canister, for processing, following which they were immediately cooled in a cold water bath (4 ± 2 °C) and stored refrigerated (4 ± 2 °C). The come up time for pressurization was 30–60 s depending on pressure applied, and depressurization time was less than 30 s.

Processing conditions based on preliminary studies were selected: 700 MPa, 70 °C, 15 min; 700 MPa, 20 °C, 15 min; and 800 MPa, 20 °C, 15 min. Temperature and pressure conditions were monitored during HHP processing (Fig. 1), and temperatures mentioned are the average temperatures (±3 °C) of the pressure transmitting fluid. Untreated samples were considered as controls.

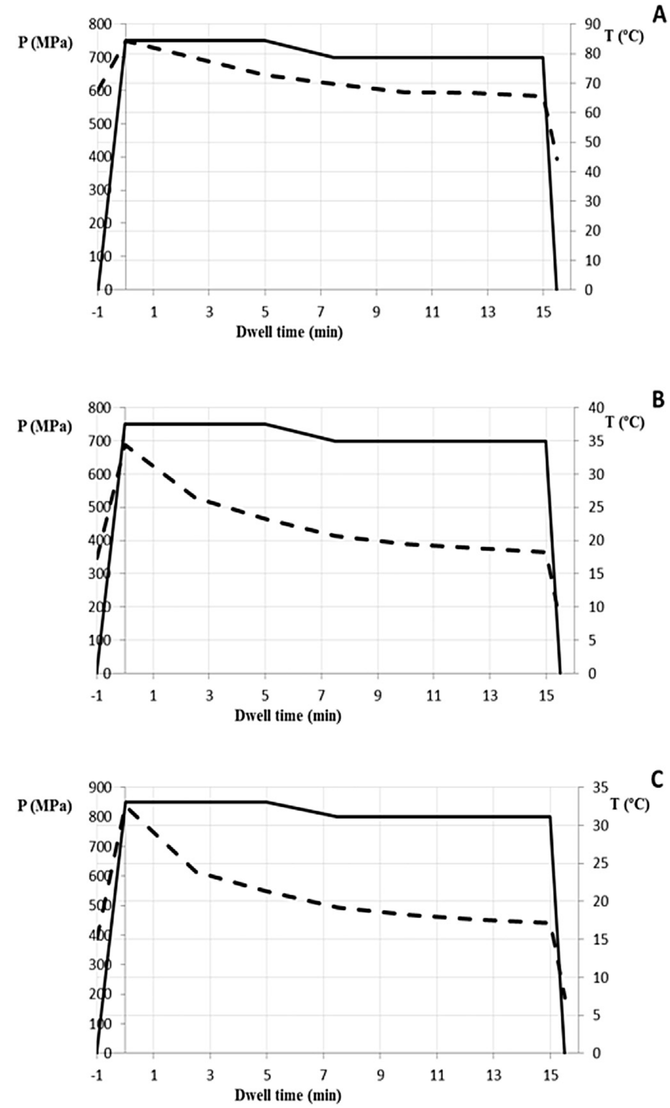

Fig. 1. Pressure (P) and temperature (T) change with respect to time during HHP processing. **A.** Treatment **P1** (700 MPa; 70 °C; 15 min) **B.** Treatment **P2** (700 MPa; 20 °C; 15 min) **C.** Treatment **P3** (800 MPa; 20 °C; 15 min). Solid and dotted lines represent pressure and temperature changes, respectively.

2.3. Thermal blanching

Beakers, each containing spinach leaves dipped in distilled water (ratio 1:200 g:mL), were placed in a temperature controlled water bath for a given time, after which the samples were immediately cooled by immersion in cold water (0–4 °C for 3 min). Based on preliminary experiments with spinach leaves, treatments at 70 °C for 15 min and 90 °C for 0.5 min were chosen because these conditions represent a mild thermal blanching and regular blanching treatment, respectively.

2.4. Chemical and physical analysis

2.4.1. Extraction method for PPO (E.C.1.10.3.1) and POD (E.C.1.11.1.7)

A combination of methods described by Arnnok, Ruangviriyachai, Mahachai, Techawongstien, and Chanthai (2010); Kim, Kim, Chung, and Moon (2014); and Wang et al. (2013) was used to extract PPO and POD from spinach leaves. All the enzyme extraction steps were carried out at 4 °C. Briefly, 5.00 ± 0.10 g of