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Study on high pressure homogenization and high power ultrasound effectiveness in inhibiting polyphenoloxidase activity in apple juice

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ABSTRACT

High pressure homogenization (HPH) and ultrasound with (US_{ct}) or without (US) temperature control were applied to apple juice individually or in combination for inactivating polyphenoloxidase (PPO). Ten passes HPH at 150 MPa were needed to achieve 50% PPO inactivation. US_{ct} led to 90% PPO decrease at the longest time (45 min), whereas total enzyme inactivation was achieved by subjecting samples to 6 min US. Results showed that temperature affected enzyme inactivation rather than the process applied. Moreover, the HPH-US_{ct} and HPH-US combined treatments led to enzyme residual activities similar to those caused by the application of HPH and US_{ct}, and US individual treatments, respectively. US provided to the apple juice less energy density to obtain PPO inactivation than US_{ct} and HPH, due to the contribution of the *in situ* generated heat. Also, US showed the lowest energy consumption, thus confirming its appropriateness.

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1. Introduction

Polyphenoloxidase (PPO) is a widely distributed enzyme in nature and plays an important role in catalyzing the hydroxylation of monophenols to *o*-diphenols and dehydrogenation of *o*-diphenols to *o*-quinones in the presence of oxygen (Espin et al., 1998). As known, the aforementioned final products are responsible for the formation of browning compounds and thus cause quality loss of vegetable products. Traditionally, PPO inactivation is achieved by the application of thermal treatments, which, however, may cause loss of sensory and nutritional quality of vegetable products. To tackle these issues, non-thermal technologies have gained significant interest over the last decades for their ability of reducing enzyme activity while minimizing detrimental effects on food quality. A number of studies has been reported on the effects of high pressure homogenization (HPH) and high power ultrasound on this food quality-related enzyme, due to their ability to change the enzymatic activity by the application of mechanical stresses

and cavitation phenomena to a fluid (Lacroix et al., 2005; Liu et al., 2009a, 2009b; Suarez-Jacobo et al., 2012; Terefe et al., 2015; Tribst and Cristianini, 2012). Both increase and decrease in PPO activity in fruit juices and model systems subjected to HPH or ultrasound treatments are described in the literature, due to differences in equipment, process conditions, enzyme source, among others (Costa et al., 2013; Liu et al., 2009a, 2009b; Silva et al., 2015; Suarez-Jacobo et al., 2012; Yu et al., 2013). As a rule, PPO inactivation can be obtained by applying intense HPH and ultrasound processes, that can be achieved by providing the matrix with very high pressures/number of passes and long times (Abid et al., 2014; Suarez-Jacobo et al., 2012). It is noteworthy that these process conditions might not fit the industrial needs as they can contribute to increase the ownership total cost. In the attempt to overcome these drawbacks, combined technologies have been taken into consideration. As an example, the simultaneous application of ultrasound with mild heat (thermosonication) and pressure (200–500 kPa; manothermosonication) or UV light (photosonication) has been demonstrated to improve ultrasound efficacy in inactivating PPO (Abid et al., 2014; Başlar and Ertugay, 2013; López et al., 1994; Sulaiman et al., 2015; Terefe et al., 2015). However, from these data a clear indication on the most suitable treatment for PPO inactivation can be hardly obtained in terms of energy efficiency

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and applicability at the industrial level. Therefore, the objective of this research work was to compare the effectiveness of HPH and ultrasound processes in inactivating PPO in apple juice. As heat may be generated during ultrasonication, its contribution to enzyme inactivation was also considered. To this purpose, apple juice was subjected to HPH or ultrasound treatments with and without temperature control. Moreover, the effect of combined HPH and ultrasound processes on the enzyme activity was studied for the first time. Processes efficiency was evaluated in terms of energy density transferred to the juice during treatments and electrical energy consumption of the HPH and ultrasound devices.

2. Materials and methods

2.1. Apple juice preparation

A 20 kg batch of fresh apples (*Malus domestica* Borkh., cv. *Golden Delicious*) were purchased at the local market and maintained at 7 °C until use. Apples were peeled and the juice was extracted using a household table top juice extractor (Ariston Hotpoint Slow Juicer, Fabriano, Italy). The extract was filtered through a filter cloth to remove impurities and coarse particles, centrifuged at 4000 g for 5 min at 4 °C (Beckman Avanti tm J-25, Beckman Instruments Inc., Palo Alto, CA, USA) and filtered again by using a filter cloth. Apple juice was prepared fresh for every trial from the same batch of fruits to minimize sample variability. The resulting clear apple juice having a soluble solid content of 14.5 ± 0.2 °Brix and pH of 3.6 ± 0.2 was immediately subjected to HPH and/or ultrasonication with or without temperature control.

2.2. HPH and ultrasound treatments

The methodology of Bot et al. (2017) was followed. Briefly, HPH processing was performed by means of a continuous lab-scale high-pressure homogenizer (Panda Plus, 2000, GEA Niro Soavi Spa, Parma, Italy) supplied with two Re + type tungsten carbide homogenization valves, with a flow rate of 2.5 cm³/s. Aliquots of 150 mL of apple juice were subjected to increasing pressures from 0 (control) to 150 MPa, or for up to 10 successive passes at 150 MPa. Ultrasound treatments were carried out with (US_{ct}) and without (US) temperature control by using an ultrasonic processor (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) operating at 24 kHz frequency and 100 μm amplitude, and equipped with a titanium horn tip diameter of 22 mm. During the ultrasonication experiment, the temperature was either controlled using a cryostatic bath, to dissipate the heat generated during treatment, or uncontrolled, leaving the temperature to rise due to heat dissipation. The US_{ct} and US treatments were performed on 150 mL apple juice for increasing time periods up to 45 and 7 min, respectively. Following the treatments, the samples were cooled in an ice bath.

Further experiments were carried out by subjecting 150 mL apple juice to HPH at 150 MPa followed by ultrasound with (HPH-US_{ct}) and without (HPH-US) temperature control for up to 15 and 4 min, respectively. The time between the two treatments did not exceed 30 s. Samples were cooled in an ice bath at the end of the second treatment.

2.3. Thermal treatment

The total temperature-time combination received by the sample during ultrasonication was applied to the sample in the absence of the ultrasound treatment. To this purpose, aliquots of 150 mL of apple juice were introduced into 250 mL capacity glass vessels and heated in a thermostatic water bath (Ika Werke, MST BC, Staufen,

Germany) under continuous stirring, by mimicking the same temperature profile produced during ultrasound treatment with (TT_{ct}) and without (TT) temperature control. Following the treatments, the samples were cooled in an ice bath.

2.4. Temperature measurement

The sample temperature was measured just before and immediately after (i.e. before the cooling step) each treatment by a copper-constantan thermocouple probe (Ellab, Hillerød, Denmark) immersed in the fluid, connected to a portable data logger (mod. 502A1, Tersid, Milan, Italy). In addition, during ultrasound and thermal treatments, the temperature was recorded as a function of time, by immersing (50 mm) the thermocouple tip in the fluid, half way between the solution centre and the inside wall of the vessel.

2.5. Energy density computation

The energy density (E_v , MJ/m³) transferred from the homogenization valve to the sample during HPH treatment was computed as described by Stang et al. (2001), according to eq. (1):

$$E_v = \Delta P \quad (1)$$

where ΔP is the pressure difference operating at the nozzles.

As the power density (P_v , W/m³) transferred from the probe to the sample during ultrasound treatment is markedly affected by temperature (Raso et al., 1999), this parameter was first determined calorimetrically by means of eq. (2),

$$P_v(T) = \frac{mc_p(\partial T/\partial t)}{V} \quad (2)$$

where m is the sample mass (kg), c_p is the sample heat capacity (3870 J/kg K as given by Ashrae, 2014), T is temperature (K), V is the sample volume (m³), and t (s) is the time frame of treatment considered. Temperature values were recorded in quasi-adiabatic conditions at various temperature levels as suggested by Raso et al. (1999). The energy density was then estimated by integration according to eq. (3) on the whole treatment time:

$$E_v = \int P_v(T)dt \quad (3)$$

The energy density of multiple passes HPH and combined treatments was calculated as the sum of the energy density values of the corresponding single pass HPH and HPH plus US_{ct} or US (Calligaris et al., 2016). The energy density of the thermal treatment was estimated according to eq. (4):

$$E_v = \frac{mc_p\Delta T}{V} \quad (4)$$

2.6. Electrical energy consumption measurement

The measurement of electrical energy consumption was performed as reported in Bot et al. (2017). The energy requirement was estimated by measuring the electrical consumption at the mains supply. The high pressure homogenizer was supplied with three-phase 400 V electrical power, thus a three-phase energy logger was inserted (Kilo Box, Electrex, Reggio Emilia, Italy) to measure the electrical consumption (MJ/m³). The ultrasonic processor was supplied with single-phase 230 V electrical power, and a power meter (PC-300, Lafayette, Taiwan) was connected to measure the electrical power and thus calculate the electrical energy (MJ/m³) for

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