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Inactivation of peroxidase and polyphenoloxidase in coconut water using pressure-assisted thermal processing

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ABSTRACT

The effect of pressure assisted thermal processing (PATP) was evaluated on the inactivation kinetics of polyphenol oxidase (PPO) and peroxidase (POD) and selected quality attributes of coconut water. Coconut water from green young coconuts was treated at 200, 400 and 600 MPa, 40–90 °C, and 60–1800 s of holding time. The activities of PPO and POD were determined using spectrophotometric methods. No enzymatic activity was detected for both enzymes within 300 s at 90 °C/400–600 MPa. The combination of 400 MPa/90 °C/300 s yielded POD and PPO inactivation, and could be used in the industrial development of PATP treated-coconut water. The POD showed to be more pressure-temperature resistant than the PPO in coconut water. The pressure-temperature inactivation kinetics of PPO and POD in coconut water were well described by the Weibull model. The activation energy for the inactivation of POD and PPO were 107–192 and 41–191 kJ mol^{-1}, respectively, while the activation volume varied from −13.2 to 10.2 and −37 to 9.2 cm^3 mol^{-1}, respectively. Total phenolic content extractability significantly increased after PATP treatments at all conditions evaluated compared to the control. Low ΔE values of PATP treated coconut water were obtained, indicating imperceptible change of color.

Industrial relevance: Pressure Assisted Thermal Processing (PATP) is an emerging technology that requires further research. The results of this study highlighted for the first time the potential of PATP on polyphenoloxidase and peroxidase inactivation of coconut water, maintaining color characteristics of coconut water. The pink color after PATP treatment was not observed. In addition, the use of kinetic models helped to determine the optimal conditions for enzyme inactivation. The outcomes of this study can be used for further industrial development of PATP treated-coconut water.

1. Introduction

Coconut water is a clear and colorless liquid inside young green coconuts. This liquid is mildly sweet when freshly extracted from young green coconuts (Purkayastha et al., 2012). Due to its low-calorie content (17.4 kcal/100 g) and relative high concentration of minerals, coconut water has been proposed as a natural beverage alternative to sport drinks. In addition, coconut water is rich in many beneficial bioactive compounds, including vitamin C, vitamin B, potassium, sodium, magnesium, calcium, arginine, alanine, lysine, and glutamic acid (Cappelletti et al., 2015; Reddy, Das, & Das, 2005).

Inside the hermetic cavity of the coconut, the liquid is sterile and its composition depends on the maturity of the coconut (Luengwilai, Beckles, Pluemjit, & Siriphanich, 2014). However, when the coconut water is removed from its cavity, it spoils within a day because of external contamination by microorganisms during extraction, refrigeration, and packaging. Leite, de Assis, da Silva, Sant'Anna, and de Santana (2000) found a population of up to 5-log of *Bacillus cereus* in fresh coconut water stored under refrigeration. Currently, there is a major challenge to assure microbiological safety while retaining fresh-like quality attributes without using preservatives and additives. The activity of peroxidase (POD) and polyphenoloxidase (PPO) present in coconut water also affects its quality, including discoloration, off-flavor, turbidity, and short shelf-life (Ciou, Lin, Chiang, & Charles, 2011). Coconut water is classified as a low-acid drink (pH = 5.0–5.5) and therefore severe thermal conditions are mandatory to warrant microbiological safety (Luengwilai et al., 2014). The commercial production of coconut water employs ultra-high-temperature pasteurization (UHT,

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130–150 °C for at least 4 s). Unfortunately, such thermal treatment eliminates the delicate flavor characteristics, which limits the marketability of the product (Jayanti, Rai, Dasgupta, & De, 2010).

Alternatively, hydrostatic pressure levels of 400–600 MPa at ambient temperature have been effective in inactivating pathogenic and spoilage vegetative cells (Martínez-Monteagudo & Balasubramaniam, 2016). Pressure pasteurized coconut water is already on the market in North America with an estimated shelf-life of 12 d under refrigeration. There is industrial interest to extend the shelf-life of coconut water longer than that of high pressure processing to increase its marketability and further distribution (Gordon & Jackson, 2017). However, pressure alone at moderate temperature (~60 °C) cannot inactivate spores. Pressure-assisted thermal processing (PATP) is an emerging sterilization technology that consists in applying high hydrostatic pressure (100–600 MPa) to a preheated sample (75–90 °C) over certain time (3–10 min) (Martínez-Monteagudo & Saldaña, 2014). Samples compressed hydrostatically rise their temperature, allowing rapid and uniform heating at target process temperatures of 90–120 °C. During PATP, the temperature of the food material increases due to physical compression under pressure. Upon decompression, the product cools volumetrically to its initial temperature. The PATP was first developed to inactivate bacterial spores and achieve commercially sterility of low-acid foods (Sizer, Balasubramaniam, & Ting, 2002). In this study, it was hypothesized that PATP can be used to obtain shelf stable coconut water with fresh like attributes. Therefore, the main objective of this study was to investigate the effect of PATP on the inactivation of PPO and POD enzymes, use different models to predict inactivation as a function of pressure, temperature and holding time, and evaluate the change of total phenolic content and color of coconut water treated by PATP.

2. Materials and methods

2.1. Coconut

Ten green coconuts imported from Vietnam were purchased from a local supermarket (Sobeys, Edmonton AB, Canada). After sanitizing the monocarp with 10% ethanol solution, a sterilized drill was used to perforate the coconut monocarp to remove the coconut water. This water was filtered using a filter cloth, and manually swirly. The coconut water was then stored at −18 °C for further PATP treatments.

2.2. Pressure-assisted thermal processing

A four-vessel system (Apparatus U111 Unipress, Warszawa, Poland) was used for the inactivation of PPO and POD in coconut water. Each vessel has a capacity of 8 mL. The vessels were heated with a circulator thermostat (Lauda Proline RP 855 Low Temperature, Lauda-Konigshofen, Germany) using propylene glycol as the pressure transmission fluid. Polypropylene tubes (Cryogenic vial, Fisher Scientific, Pittsburgh, PA) of 3 mL were filled with untreated coconut water. Samples were pressurized to 200, 400, and 600 MPa at temperatures of 40, 60, 80, and 90 °C with holding times of 60, 120, 300, 600, 900 and 1800 s. The samples were pressurized at a rate of 10 MPa s^{-1}. At the end of the holding time, the vessels were decompressed, and the samples were removed immediately from the high-pressure vessels, cooled down with ice and stored at −18 °C for further analysis. Earlier, this experimental system was validated for kinetic studies (Martinez-Monteagudo & Saldaña, 2015a,b, Martínez-Monteagudo & Saldaña, 2014). All experiments were performed at least in triplicate.

2.3. Enzyme activity

The POD activity was measured using a UV-VIS spectrophotometer (Jenway 6320D, Standford, United Kingdom) according to the method described by Matsui, Gut, De Oliveira, and Tadini (2008), with slight modifications. A test tube containing 3.5 mL of buffer ($Na_2HPO_4{\cdot}2H_2O$ + KH_2PO_4, pH 6), 0.4 mL of ABTS (2.2 azino-bis 3–ethylbenzthiazoline-6-sulfonic acid) solution (0.02 mol/L) and 0.4 mL of hydrogen peroxide (0.1% v/v) was placed in a water bath at 25 °C for 300 s. Then, 1 mL of coconut water was added to this solution and transferred to a cuvette where the absorbance solution was measured at 405 nm and recorded every 10 s for 300 s. The reference value of the POD was determined using a blank solution containing 3.5 mL of buffer ($Na_2HPO_4{\cdot}2H_2O$ + KH_2PO_4, pH 6.0), 0.4 mL of ABTS solution (0.02 mol/L), 0.4 mL of hydrogen peroxide (0.1% v/v) and 1 mL of distilled water.

The PPO activity was measured using the same UV-VIS spectrophotometer according to the method described by Matsui et al. (2008), with slight modifications. A test tube containing 2.25 mL of sodium phosphate buffer (0.2 mol/L, pH 6.0) and 0.75 mL of 0.2 mol/L pyrocatechol solution was placed in a water bath at 25 °C for 300 s. Coconut water sample (1 mL) was added to this solution and the absorbance was measured at 425 nm and recorded every 10 s for 5 min. The reference value of PPO was determined using a blank solution containing 0.75 mL of pyrocatechol, 2.25 mL of sodium phosphate buffer (0.2 mol/L, pH 6) and 1 mL of distilled water. All POD and PPO analyses were carried out at least in triplicate. For both enzymes, absorbance was plotted against time and the values of enzymatic activity were calculated from the slope of the initial linear part of the curves following the method reported by Matsui et al. (2008). The relative activity in coconut water samples was determined using Eq. (1):

$$\text{Relative activity of enzyme} = \left(\frac{A_t}{Ao}\right) x100\% \quad (1)$$

where, A_t is the mean of the enzyme activity after PATP treatment at specific processing conditions, and A_o is the mean of the initial enzyme activity before the PATP treatment.

2.4. Quality parameters

2.4.1. Color

Color was determined using a colorimeter (CR210, Minolta, Osaka, Japan), following the methodology reported by Park, Balasubramaniam, and Sastry (2014). Briefly, the colorimeter was calibrated using a white standard plate (L^* = 97.83, a^* = 0.43, b^* = 1.98). The CIELAB L^*, a^* and b^* values represent the indicators of lightness, redness and yellowness, respectively. Total change in color (ΔE) was calculated using Eq. (2):

$$\Delta E = \sqrt{(L^*_{tret} - L^*_{raw})^2 + (a^*_{tret} - a^*_{raw})^2 + (b^*_{tret} - b^*_{raw})^2} \quad (2)$$

where, L^*_{raw}: lightness of coconut water before the PATP treatment, L^*_{tret}: lightness of coconut water after the PATP treatment, a^*_{raw}: redness of coconut water before the PATP treatment, a^*_{tret}: redness of coconut water after the PATP treatment, b^*_{raw}: yellowness of coconut water before the PATP treatment, and b^*_{tret}: yellowness of coconut water after the PATP treatment.

2.4.2. Total phenolic content

The Folin-Ciocalteu method was used to determine total phenolic content (Singleton & Rossi, 1965). Briefly, the sample aliquot (0.04 mL), distilled water (3.1 mL) and the Folin-Ciocalteu reagent (0.2 mL) were mixed. After 6 min, sodium carbonate (20% w/v; 600 µL) was added and the mixture was incubated for 2 h in dark at room temperature. The absorbance was measured at 765 nm. The final results were expressed as milligrams of gallic acid equivalents per gram of coconut water (mg GAE g^{-1}).

2.5. Modeling enzyme inactivation

The enzymatic inactivation models used for the experimental data are presented in Table 1. The parameters of each model were calculated

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