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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):

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

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):

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

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

Table 1
Kinetic equations used to represent the inactivation of peroxidase and polyphenoloxidase in coconut water treated with pressure-assisted thermal processing.

Model	Mathematical expression	Equation number
First-order	$\frac{A_t}{A_o} = exp^{(-k_i \cdot t)}$	(3)
Fractional	$A_t = A_\infty + (A_o - A_\infty) \cdot exp^{(-k_i \cdot t)}$	(4)
Weibull	$\frac{A_t}{A_o} = exp^{\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right)}$	(5)
Isozyme	$\frac{A_t}{A_o} = A_l \cdot exp^{(-k_l \cdot t)} + A_s \cdot exp^{(-k_s \cdot t)}$	(6)

A_t – enzyme activity at a given time; A_o – initial enzyme activity; k_i – inactivation rate constant for Eqs. (3) and (4); t – holding time; A_∞ - enzyme activity after prolonged processing; α, β – scale and shape parameters, respectively; A_l – enzyme activity of the labile fraction; As – enzyme activity of the stable fraction; k_l – inactivation rate constant of the labile fraction; and ks – inactivation rate constant of the stable fraction.

using Athena Visual Workbench, a powerful software package for modeling and parameter estimation (www.athenavisual.com). The predictive capability of the individual models was assessed by the coefficient of determination (R^2), the adjusted coefficient of determination (R^2_{Adj}), residual analysis, and average absolute percentage of residuals (E%).

The influence of temperature and pressure on the rate constant (k_i) was expressed by Arrhenius-type and Eyring-type models, respectively:

$$k_i = k_T \cdot exp^{\left[\frac{-E_a}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_r}\right)\right]} \tag{7}$$

$$k_i = k_P \cdot exp^{\left[\frac{-\Delta V^{\#}}{R \cdot T} \cdot (P - P_r)\right]} \tag{8}$$

where, k_T and k_P are the reaction rate constants at a reference temperature (T_r) and pressure (P_r), respectively; E_a is the apparent activation energy (kJ mol^{-1}); $\Delta V^{\#}$ is the activation volume (cm^3 mol^{-1}); T is the temperature (K); P is the pressure (MPa); and R is the universal gas constant (8.314 J mol^{-1} K^{-1}). The average values of the experimental temperatures and pressures were used as T_r and P_r, respectively. In the case of the Weibull model, the effect of temperature and pressure was evaluated using the mathematical model proposed by Martínez-Monteagudo and Saldaña (2014) and Martinez-Monteagudo and Saldaña (2015a,b).

$$\frac{1}{\alpha} = \frac{1}{\alpha_T} \cdot exp^{\left[\frac{-E_a}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_r}\right)\right]} \tag{9}$$

$$\frac{1}{\alpha} = \frac{1}{\alpha_P} \cdot exp^{\left[\frac{-\Delta V^{\#}}{R \cdot T} \cdot (P - P_r)\right]} \tag{10}$$

where, α is the scale parameter, α_T and α_P are the scale parameters at a reference temperature and pressure, respectively.

3. Results and discussion

3.1. Enzyme inactivation of coconut water treated by PATP

Fig. 1a–c shows the remaining activity of POD in coconut water treated with PATP (200–600 MPa; 40–90 °C; 0–1800 s). In general, the activity of POD gradually decreased over time, in which the final activity depends on the combination of temperature and pressure. At 40 °C and 1800 s, the remaining activity was 0.52 ± 0.02, 0.66 ± 0.03, and 0.74 ± 0.02 at 200, 400, and 600 MPa, respectively. A gradual decrease of the remaining activity of POD until reaching a plateau after 600 s was observed at 60 and 80 °C, independently of the pressure. The exception of this generalization was observed at 90 °C, where the remaining activity of POD was not detected after 120–300 s, regardless of the pressure used (200–600 MPa). Similar observations have been reported in litchi pericarp, where POD was completely inactivated after 90 °C for 600 s and after 100 °C for 60 s (Mizobutsi et al., 2010).

The remaining activity of PPO in coconut water treated with PATP is presented in Fig. 1d–f. At 40 °C and 1800 s, the remaining activity of PPO was 0.21 ± 0.01, 0.50 ± 0.02, and 0.62 ± 0.02 at 200, 400, and 600 MPa, respectively. These activity values are substantially lower than those observed for POD at the same pressure and temperature conditions (Fig. 1a–c), demonstrating that POD was more pressure-temperature resistant than PPO in coconut water. The remaining activity of PPO within the temperature range of 60–80 °C and pressure of 200–600 MPa was between 0.13 and 0.33. Similar results were reported in the remaining POD activity of strawberry puree and pineapple puree during thermal and high-pressure treatments (Chakraborty, Srinivasa, & Mishra, 2015; Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). The combination of 90 °C and 200–600 MPa yielded no detectable PPO activity after 300 s of treatment. A remarkable observation during the inactivation of PPO is the existence of antagonist and synergist effects as a result of different combinations of temperature and pressure. A noticeable antagonist effect was observed at a combination of 40 °C and 600 MPa, yielding an activity value of 0.60 ± 0.02 after 1800 s, while an activity value significantly lower (0.21 ± 0.02) was obtained at 40 °C and 200 MPa after 1800 s. The behavior of higher remaining activity at a lower temperature (40 °C) and high pressure (200–600 MPa) for both PPO and POD could be attributed to the partial rearrangements of the enzyme structures that enhanced the intramolecular interactions with the substrate, resulting in a higher catalytic performance. In contrast, a synergistic effect can be exemplified at a combination of 90 °C and 600 MPa, where the remaining activity of PPO was 0.08 ± 0.01 after 120 s, while the combined effect of 90 °C and 200 MPa, yielded an activity value of 0.20 ± 0.02 after 120 s. Inactivation of enzyme by combined action of pressure and temperature is a complex phenomenon. The primary structure of the enzyme could be minimally affected by pressure while the secondary structure suffers structural modifications only at very high pressures. The tertiary structure is greatly affected by pressure because pressure disrupts hydrophobic and electrostatic interactions (Ludikhuyze, Van Loey, Indrawati, & Hendrickx, 2003). Consequently, water solvates the exposed charge groups, leading to a volume reduction that inactivates the enzymes (Ludikhuyze et al., 2003).

3.2. Inactivation models

Table 2 shows the performance of different models used to represent the inactivation of POD and PPO in coconut water at PATP conditions. The first-order and fractional models failed to adequate represent the experimental data, judging by low values of R^2 (0.375–0.806 and 0.839–0.983, respectively) and high values of E (32.17–54.15% and 15.36–34.32%, respectively). The R^2_{Adj} and $E(\%)$ are parameters used for evaluating the fitting performance of kinetics models. The R^2 values indicate how well the model describes the experimental data, R^2_{Adj} values indicate the influence of the number of parameters on R^2, and $E\%$ compares the overall error in terms of percentage. The Weibull and Isozyme models presented the best fitting performance throughout the entire experimental domain (Table 2), with the highest R^2 and R^2_{Adj} values and the lowest E% values. The residual analysis (standardized residual against predicted values) of Weibull and Isozyme models revealed random patterns (graph not shown), which validates the estimation of parameters and prediction of new observations. The fitting performance (Table 2) indicated almost no distinction between Weibull and Isozyme models, with both models being statistically appropriate to describe the inactivation of POD and PPO in coconut water treated with PATP. The

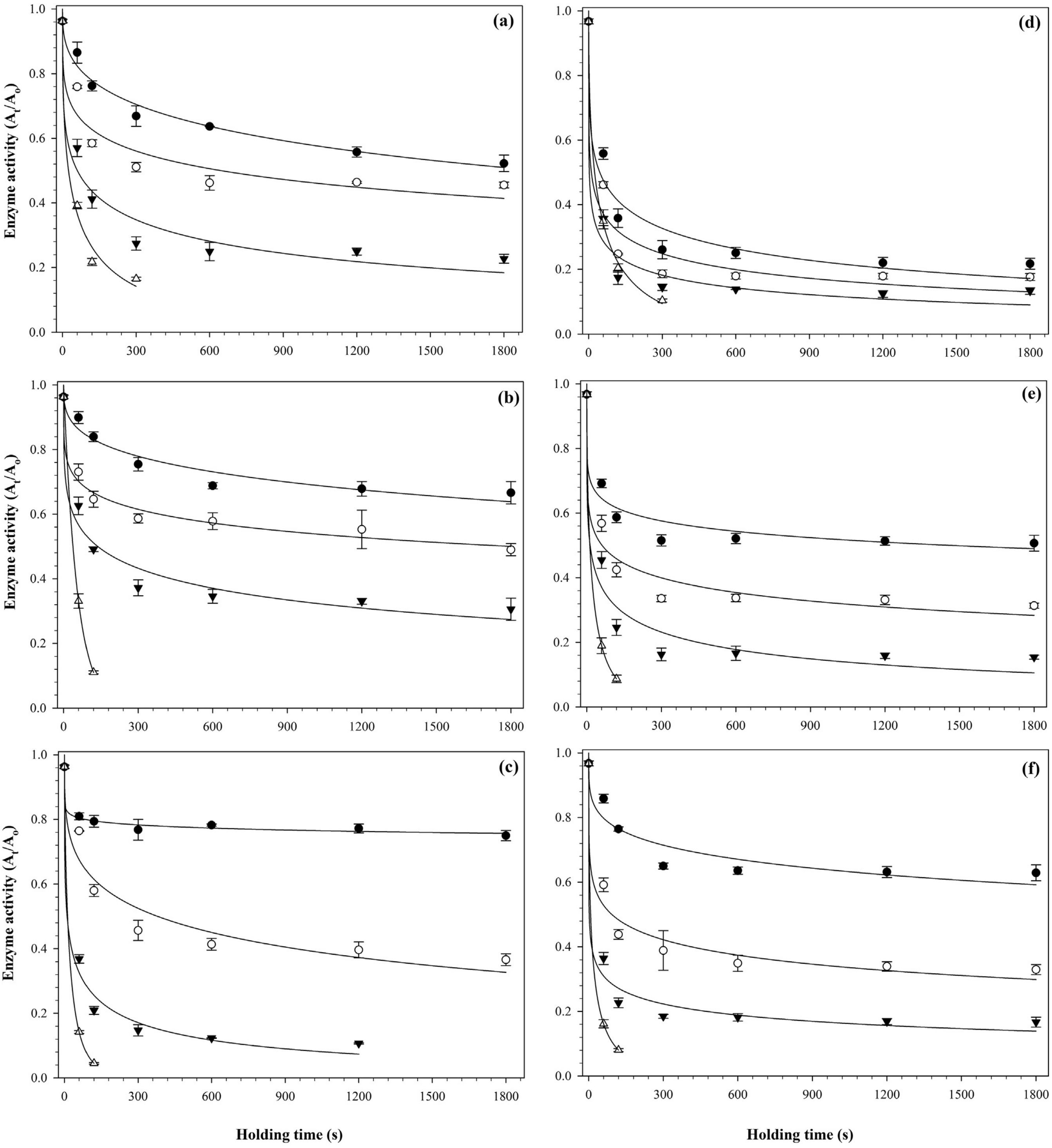

Fig. 1. Relative enzymatic activity during pressure-assisted thermal processing: peroxidase activity at: (a) 200, (b) 400, and (c) 600 MPa; and polyphenoloxidase activity at: (d) 200, (e) 400, and (f) 600 MPa. ● 40, ○ 60, ▼80, and Δ 90 °C. Curves represent data obtained with Eq. (11).

isozyme model accounts for the presence of isozymes with different stabilities, where the labile fraction is clearly identified from the stable fraction (Gökmen, 2010). However, our experimental data cannot confirm the existence of different fractions for both enzymes, which challenges the applicability of the Isozyme model. On the other hand, the application of the Weibull model for enzyme inactivation assumes that the inactivation followed an exponential probabilistic distribution (Cunha, Oliveira, & Oliveira, 1998). The information presented in Table 2 suggested that the inactivation of POD and PPO can be seeing as a failure phenomenon, where a fraction of an intact enzyme is reduced with time at isothermal and isobaric conditions, regardless of the inactivation mechanism as the entire process is governed by probability laws. Weibull model has been successfully used for modeling not only the temperature-pressure inactivation of spores (Couvert, Gaillard, Savy, Mafart, & Leguerinel, 2005; Fernandez, Collado, Cunhan, Ocio, & Martinez, 2002; Mafart, Couvert, Gaillard, & Leguerinel, 2002; Van

Table 2
Fitting performance of inactivation models for peroxidase and polyphenoloxidase in coconut water treated at pressure-assisted thermal processing conditions.

Parameters	Peroxidase (POD)			Polyphenoloxidase (PPO)		
	200 MPa	400 MPa	600 MPa	200 MPa	400 MPa	600 MPa
First-order model, Eq. (3)						
R^2	0.539	0.375	0.801	0.806	0.584	0.684
R^2_{Adj}	0.529	0.361	0.796	0.802	0.544	0.677
E (%)	35.55	36.54	32.17	54.15	45.32	41.84
Fractional model, Eq. (4)						
R^2	0.901	0.844	0.925	0.983	0.839	0.907
R^2_{Adj}	0.893	0.833	0.919	0.982	0.828	0.901
E (%)	16.81	20.54	26.65	15.36	34.32	27.17
Weibull model, Eq. (5)						
R^2	0.949	0.916	0.977	0.967	0.962	0.973
R^2_{Adj}	0.946	0.912	0.976	0.965	0.961	0.972
E (%)	12.27	15.36	13.61	19.33	15.48	13.32
Isozyme model, Eq. (6)						
$\mathbf{R^2}$	0.963	0.873	0.953	0.987	0.921	0.948
$\mathbf{R^2_{Adj}}$	0.959	0.858	0.947	0.986	0.912	0.921
E (%)	10.23	19.09	21.26	12.32	24.47	13.39

R^2 – coefficient of determination; R^2_{Adj} – adjusted coefficient of determination; E (%) – residual analysis, and average absolute percentage of residuals.

Boekel, 2002) and enzymes (Criado, Civera, Martinez, & Rodrigo, 2015; Deylami, Rahman, Tan, Bakar, & Olusegun, 2016; Sampedro & Fan, 2014) but also the retention of bioactive lipids (Guo, Bellissimo, & Rousseau, 2017; Martinez-Monteagudo & Saldaña, 2015).

3.2.1. Modeling the inactivation of POD and PPO

The temperature-pressure inactivation was evaluated by combining Eq. (7) with Eq. (9), and Eq. (8) with Eq. (10). The resulting equations resemble that of Arrhenius model (Eq. (11)) and Eyring model (Eq. (12)).

$$\frac{A_t}{A_o} = exp\left[-\left(\frac{t}{\alpha_T}\cdot exp\left(\frac{-E_a}{R}\cdot\left(\frac{1}{T}-\frac{1}{T_r}\right)\right)\right)^{\beta}\right] \tag{11}$$

$$\frac{A_t}{A_o} = exp\left[-\left(\frac{t}{\alpha_P}\cdot exp\left(\frac{-\Delta V_a^{\#}}{R\cdot T}\cdot(P-P_r)\right)\right)^{\beta}\right] \tag{12}$$

The influences of temperature and pressure on the fitting and kinetic parameters are provided in Tables 3 and 4, respectively. The fitting performance of both equations suggests that both models and their associated parameters can be used to satisfactorily model the inactivation of POD and PPO within the experimental domain. The scale parameter at a reference temperature (α_T) varied from 789 to 3640 for POD and from 54 to 229 for PPO. On the other hand, the scale parameter at a reference pressure (α_P) varied within the range of 35–2750 and 25–360 for POD and PPO, respectively. A general observation for both enzymes was that α_T increased with pressure, while α_P decreased with temperature. The α_T and α_P were inversely proportional to the frequency or pre-exponential factor of Arrhenius and Eyring equation. The pre-exponential factor can be seen as the reaction rate when no energetic barrier is present (Martínez-Monteagudo & Saldaña, 2014).

Table 3
Fitting parameters of Arrhenius-type Eq. (11) for the inactivation of peroxidase and polyphenoloxidase in coconut water treated with pressure-assisted thermal processing.

Parameter	Arrhenius-type Eq. (11)					
	200 MPa		400 MPa		600 MPa	
	POD	PPO	POD	PPO	POD	PPO
α_T	788.9 ± 214.6	54.4 ± 9.5	3169.8 ± 262.9	144.1 ± 53.9	3640.4 ± 81.1	228.5 ± 66.5
β	0.25 ± 0.05	0.23 ± 0.04	0.18 ± 0.06	0.16 ± 0.04	0.26 ± 0.05	0.18 ± 0.04
E_a	107.2 ± 20.4	40.8 ± 12.1	172.9 ± 25.6	144.5 ± 37.3	192.2 ± 34.6	190.5 ± 43.1
R^2	0.949	0.967	0.916	0.965	0.977	0.973
R^2_{Adj}	0.945	0.965	0.912	0.961	0.976	0.971
E (%)	12.27	19.33	15.56	15.48	13.61	13.32

The error was calculated from the 95% confidence interval. POD – peroxidase; PPO – polyphenoloxidase; α_T – scale parameter at a reference temperature; β – shape parameter; E_a – apparent activation energy (kJ mol^{-1}); R^2 — coefficient of determination; R^2_{Adj} — adjusted coefficient of determination; *E(%)* – average absolute percentage of residuals.

Table 4
Fitting parameters of Eyring-type Eq. (12) for the inactivation of peroxidase and polyphenoloxidase in coconut water treated with pressure-assisted thermal processing.

Parameter	Eyring-type Eq. (12)							
	40 °C		60 °C		80 °C		90 °C	
	POD	PPO	POD	PPO	POD	PPO	POD	PPO
α_P	2750 ± 230	360 ± 20	298 ± 15	258 ± 45	176 ± 35	31 ± 11	35 ± 9	25 ± 9
$\Delta \boldsymbol{V_a}^{\#}$	−13.2 ± 4.6	−37.7 ± 9.4	4.1 ± 2.1	−16.2 ± 4.2	13.3 ± 3.1	−4.1 ± 1.7	10.2 ± 3.6	9.2 ± 3.2
β	0.28 ± 0.06	0.21 ± 0.05	0.24 ± 0.05	0.18 ± 0.04	0.24 ± 0.07	0.19 ± 0.04	0.53 ± 0.09	0.47 ± 0.01
R^2	0.879	0.951	0.895	0.946	0.967	0.967	0.988	0.993
R^2_{Adj}	0.873	0.948	0.881	0.944	0.961	0.965	0.987	0.991
E (%)	4.44	8.91	9.27	14.15	18.5	18.51	16.69	14.25

The error was calculated from the 95% confidence interval. POD – peroxidase; PPO – polyphenoloxidase; α_P – scale parameter at a reference pressure; β – shape parameter; $\Delta \boldsymbol{V_a}^{\#}$ – activation volume (cm^3 mol^{-1}); R^2 — coefficient of determination; R^2_{Adj} — adjusted coefficient of determination; *E(%)* - average absolute percentage of residuals.

The values for the shape parameter (β) were within the range of 0.18–0.53 and 0.16–0.47 for POD and PPO, respectively, and no systematic influence of temperature and pressure was observed. The estimated parameters for β were lower than 1, indicating deviation of the first-order reaction. The main assumption of a first-order reaction is that the reactants (enzymes) are unaffected by the food matrix, which is very unlikely in this case. Thus, the inactivation of POD and PPO in coconut water was expected to deviate from the first-order reaction. The parameters of activation energy (E_a) and activation volume ($\Delta \boldsymbol{V_a}^{\#}$) are oftentimes considered the most relevant kinetic information. The inactivation of POD and PPO yields activation energies of 107–192 and 41–191 kJ mol^{-1}, respectively. On the other hand, the activation volume of POD and PPO varied from −13 to 13 and −38 to 9 cm^3 mol^{-1}, respectively. Although a systematic relationship (E_a increases with pressure and $\Delta \boldsymbol{V_a}^{\#}$ increases with temperature) can be observed, such relationship could not be statistically confirmed due to the large variability of the activation parameters.

3.2.2. Activation modes

The inactivation of enzymes is a very complex phenomenon involving a reversible partial unfolding, followed by an irreversible modification that leads to the inactivation. The step of unfolding-refolding is negligible due to its difficulty to measure experimentally. Thus, the inactivation is governed by the irreversible step that is thought to follow the Arrhenius and Eyring law when temperature and pressure is applied. Thus, the activation parameters (E_a and $\Delta \boldsymbol{V_a}^{\#}$) are of great importance since they provide essential information for process development and optimization. However, a theory explaining the influence of pressure and temperature on the E_a and $\Delta \boldsymbol{V_a}^{\#}$, respectively, has not been developed. An attempt was made to provide a meaningful interpretation of the activation modes during the inactivation of POD and PPO under PATP conditions. Therefore, it is convenient to express the E_a as a dimensionless number (Arrhenius number, $\frac{E_a}{R\cdot T_r}$) as a function of pressure (Fig. 2a). Arrhenius number is used for the analysis and optimization of complex reactions. The Arrhenius number is the ratio of activation energy to the average kinetic energy ($R{\cdot}T_r$). Reactions having small Arrhenius number are less sensitive to temperature, thus a relative large increase in temperature is needed to accelerate the reaction. On the other hand, large values of Arrhenius number indicate the reaction sensitivity towards temperature, and a relative small raise in temperature would result in substantial increase in the reaction rate. The Arrhenius number varied from 37 to 67 for POD and from 14 to 65 for PPO. For both enzymes, the Arrhenius number increased with pressure. A possible interpretation is that pressure levels of 600 MPa may induce irreversible modifications of the enzyme structure that favor the inactivation by temperature. Contrary, such modifications may occur to a lesser extend at pressure levels of 200 MPa. Consequently, the unfolding-refolding step becomes predominant and higher temperatures are needed to overcome such physical barriers. Fig. 2b shows the dimensionless activation volume ($\frac{\Delta V_a^{\#}\cdot P_r}{R\cdot T_r}$) as a function of temperature. The inactivation of POD yielded $\frac{\Delta V_a^{\#}\cdot P_r}{R\cdot T_r}$ values of −2.3 to 1.3, increasing with temperature. Similar tendency was observed for the denaturation of PPO, where the $\frac{\Delta V_a^{\#}\cdot P_r}{R\cdot T_r}$ values increase with temperature from −5.1 to 1.2. In general, the $\frac{\Delta V_a^{\#}\cdot P_r}{R\cdot T_r}$ for both enzymes revealed that the denaturation process (***native → inactive***) is characterized by negative values. A possible interpretation is that at a given temperature, for instance 40 °C, the net effect of pressure is predominant over the thermal effect. As the temperature increased (80–90 °C), the dimensionless activation volume became positive, meaning that the thermal effect is now the predominant force. These observations exemplify the complexity of inactivation mechanisms of enzymes under pressure and temperature. It also indicates that the overall denaturation process is favored with pressure at a given temperature. The forces affected by pressure in the denaturation process are hydrogen-bondings, hydrophobic interactions, and electrostatic forces. Interestingly, the extent of such forces increases with temperature. Indeed, increasing the temperature result in positive values of the $\frac{\Delta V_a^{\#}\cdot P_r}{R\cdot T_r}$ for both enzymes (Fig. 2b), meaning that the denaturation process is slightly inhibited by pressure. The influence of temperature on the $\frac{\Delta V_a^{\#}\cdot P_r}{R\cdot T_r}$ is much more complex than the relationship of Arrhenius number and pressure because the $\frac{\Delta V_a^{\#}\cdot P_r}{R\cdot T_r}$ may also represent inherit change in the volume as the enzyme unfolds. The $\Delta \boldsymbol{V_a}^{\#}$ is a reflection of all molecular changes occurring during the progression of all steps involved during the denaturation, folding-unfolding and irreversible inactivation. The enzyme denaturation is a complex interplay process, where time, pressure, and temperature play a significant role. We have postulated a denaturation process where both activation modes are considered:

$$\textbf{\textit{Native}} \overset{\Delta V_a^{\#}}{\longleftrightarrow} \textbf{\textit{Unfolded}} \overset{E_a}{\rightarrow} \textbf{\textit{Inactivated}}$$

It is thought that the folding-unfolding step is mainly controlled by pressure. Hydrogen bonds are related to solvent interactions, which may result in negative $\Delta \boldsymbol{V_a}^{\#}$ values (Weemaes, Ludikhuyze, Van den Broeck, & Hendrickx, 1998). Hydrophobic interactions involve the exposure of a non-polar component of the structure with similar structural

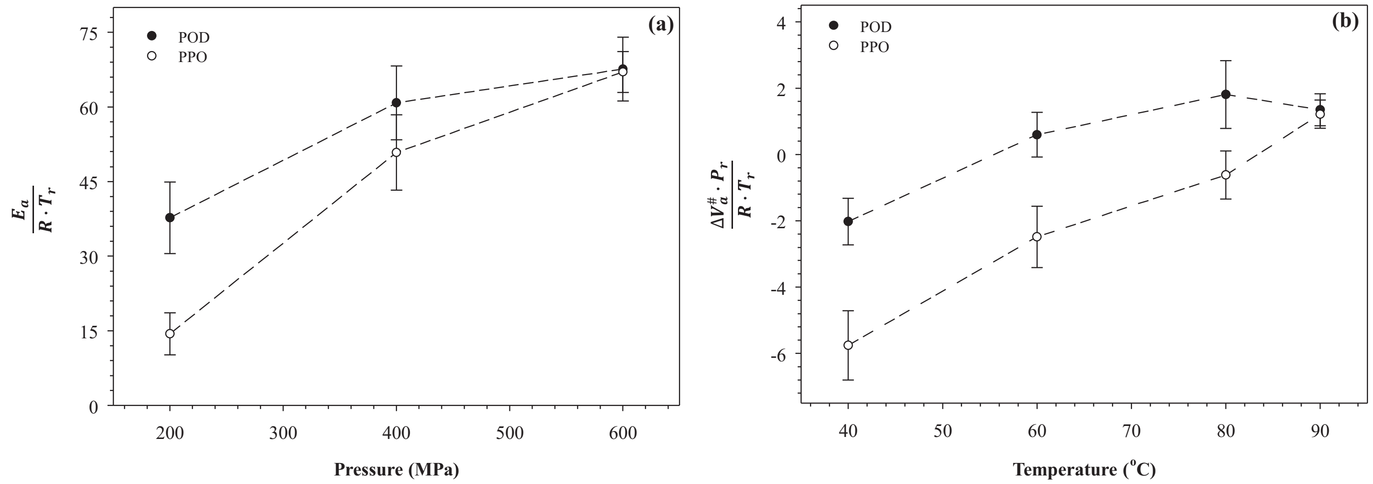

Fig. 2. Dimensionless activation parameters for the inactivation of peroxidase and polyphenoloxidase: (a) Influence of pressure on the dimensionless activation energy, and (b) influence of temperature on the dimensionless activation volume. The error bars were calculated from the 95% confidence interval.

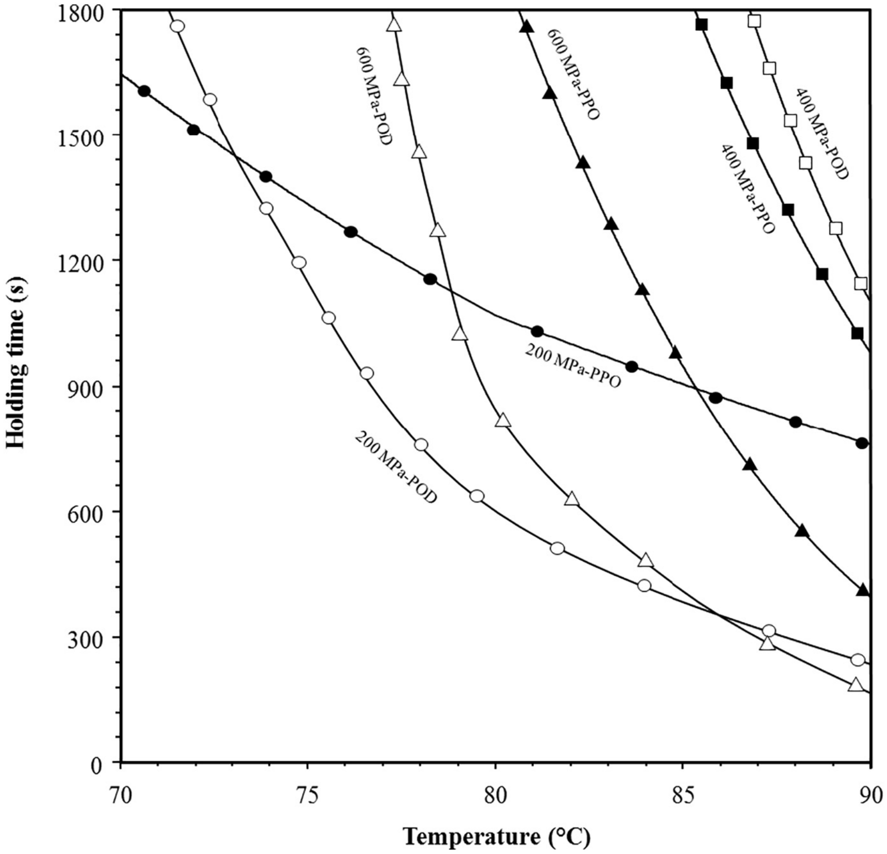

Fig. 3. Inactivation of peroxidase (POD) and polyphenoloxidase (PPO) in coconut water treated with pressure assisted thermal processing. The contour lines represent the require conditions to inactivate 90% of the enzyme activity.

units, rather than to its exposure to solvation by water. The net effect of such interactions results in positive $\Delta V_a^{\#}$ values (Damodaran, 1996). Electrostatic interactions include ion-pairing of ionizable groups, which ionic dissociation greatly impact the conformational stability of the enzyme. Ionization volumes of synthetic proteins (polylysine) are negative because the protein undergoes uncoiling of the helical conformation, which results in a reduction of the molecular volume (Mozhaev, Lange, Kudryashova, & Baldy, 1996).

3.2.3. PATP working conditions for 90% PPO and POD inactivation

The combined effect of pressure and temperature on the inactivation of POD and PPO is illustrated through kinetic contour plot (Fig. 3). The contour lines are the combinations of temperature and time at a given pressure that led to at least 90% of enzyme inactivation. Such kinetic plot can be used for developing a PATP process for coconut water. For instance, the combination of 600 MPa and 85 °C for 1000 s yields > 90% of POD and PPO inactivation. Designing a process that extends the shelf-life of coconut water can be derived from the information presented in Fig. 3 in combination with other quality parameters like phenolic content and color change. It should be mentioned that the conditions illustrated in Fig. 3 cannot guarantee commercial sterilization of the coconut water since no information on spore inactivation is available. In the absence of such information, the inactivation of *Bacillus amyloliquefaciens*, a pressure-resistant spore, reported elsewhere (Ahn, Balasubramaniam, & Yousef, 2007; Martínez-Monteagudo, Gänzle, & Saldaña, 2014) was used for interpretation of the kinetic data. In general, temperatures higher than 100 °C in combination with 400–600 MPa for 5 min (3000 s) yielded between 4 and 5 log reduction of *Bacillus amyloliquefaciens*, achieving commercial sterilization. These processing conditions would yield complete inactivation of PPO and POD in coconut water.

3.3. The effect of other quality parameters on coconut water treated by PATP

Color is an important parameter that influences product quality and purchase desire of the consumers (Gökmen, 2010). The color related parameters of the coconut water, control and samples after treatment at 40 and 80 °C, and 400 and 600 MPa, are shown in Tables 5 and 6. The values of L* parameter ranged from 84.43 ± 0.04 (coconut water treated at 80 °C, 400 MPa for 300 s) to 87.14 ± 0.04 (coconut water treated at 80 °C, 600 MPa for 1800 s). No significant difference was observed in L* values compared to the control at 40 °C/400–600 MPa and 80 °C/400 MPa. However, a slightly increase of L* value was observed after 600 s at 80 °C/600 MPa compared to the control. Similarly, Garcia-Parra, Gonzalez-Cebrino, Delgado, and Ramirez (2016) reported a slight increase of L* values of pumpkin puree, from 53.5 to 57.2 compared to the control, after PATP treatment (600 MPa/80 °C/300 s). Cappelletti et al. (2015) reported a slight decrease of L* values of coconut water, from 99.59 to 98.35 compared to the control, employing high pressure carbon dioxide (12 MPa, 45 °C, 300–1800 s) and a slight decrease of L* values of coconut water from 99.59 to 98.35 compared to the control, employing heat pasteurization (90 °C, 60 s).

The PATP treatment resulted in an increase of a* values in coconut water samples. The most intense red color (a*) parameter was observed in coconut water samples treated at 80 °C, 400 MPa for 600 and 1200 s

Table 5
Color parameters of coconut water treated by PATP at 400 MPa.

Time	Color parameters							
	40 °C/400 MPa				80 °C/400 MPa			
(s)	L*	a*	b*	ΔE	L*	a*	b*	ΔE
C	85.52 ± 0.01^{a}	0.07 ± 0.01^{c}	−2.28 ± 0.01^{e}	–	85.52 ± 0.01^{a}	0.07 ± 0.01^{f}	−2.28 ± 0.01^{g}	–
60	85.18 ± 0.01^{a}	0.20 ± 0.01^{a}	−1.67 ± 0.01^{b}	0.71 ± 0.01^{a}	84.76 ± 0.01^{a}	0.23 ± 0.05^{d}	−1.88 ± 0.01^{f}	0.87 ± 0.01^{f}
120	85.18 ± 0.05^{a}	0.19 ± 0.02^{a}	−1.71 ± 0.01^{c}	0.67 ± 0.02^{d}	86.35 ± 0.05^{a}	0.35 ± 0.01^{b}	−0.82 ± 0.01^{c}	1.70 ± 0.0S^{c}
300	85.26 ± 0.02^{a}	0.22 ± 0.01^{a}	−1.56 ± 0.01^{a}	0.78 ± 0.01^{b}	84.43 ± 0.04^{a}	0.21 ± 0.05^{e}	−1.78 ± 0.01^{e}	1.21 ± 0.01^{e}
600	85.26 ± 0.01^{a}	0.13 ± 0.01^{b}	−1.57 ± 0.01^{a}	0.76 ± 0.01^{b}	86.85 ± 0.01^{a}	0.47 ± 0.01^{a}	−0.43 ± 0.05^{b}	2.31 ± 0.01^{a}
1200	84.48 ± 0.03^{a}	0.20 ± 0.03^{a}	−1.74 ± 0.01^{c}	1.18 ± 0.01^{a}	86.09 ± 0.03^{a}	0.47 ± 0.01^{a}	−0.37 ± 0.05^{a}	2.03 ± 0.02^{b}
1800	85.37 ± 0.01^{a}	0.22 ± 0.01^{a}	−1.68 ± 0.01^{bc}	0.64 ± 0.01^{d}	85.31 ± 0.01^{a}	0.29 ± 0.05^{c}	−0.85 ± 0.01^{d}	1.46 ± 0.01^{d}

Values are means of at least two replications. C: Control; L*: lightness; a*: red for positive values and green for negative values; b*: yellow for positive values and blue for negative values, ΔE: total change in color, different letters are significantly different at $p < 0.05$.

Table 6
Color parameters of coconut water treated by PATP at 600 MPa.

Time	Color parameters							
	40 °C/600 MPa				80 °C/600 MPa			
(s)	L*	a*	b*	ΔE	L*	a*	b*	ΔE
C	85.52 ± 0.01^{a}	0.07 ± 0.01^{e}	−2.28 ± 0.01^{f}	–	85.52 ± 0.01^{c}	0.07 ± 0.01^{d}	−2.28 ± 0.01^{e}	–
60	85.21 ± 0.01^{a}	0.25 ± 0.02^{b}	−1.37 ± 0.01^{b}	0.98 ± 0.01^{c}	85.75 ± 0.01^{c}	0.31 ± 0.01^{c}	−1.03 ± 0.01^{b}	1.29 ± 0.01^{c}
120	84.83 ± 0.02^{a}	0.20 ± 0.02^{c}	−1.96 ± 0.01^{e}	0.77 ± 0.01^{f}	85.60 ± 0.01^{c}	0.34 ± 0.01^{b}	−1.03 ± 0.01^{c}	1.28 ± 0.01^{c}
300	85.28 ± 0.03^{a}	0.20 ± 0.01^{c}	−1.51 ± 0.05^{c}	0.82 ± 0.02^{e}	85.40 ± 0.01^{c}	0.31 ± 0.01^{c}	−1.11 ± 0.02^{d}	1.20 ± 0.05^{d}
600	84.85 ± 0.04^{a}	0.23 ± 0.01^{b}	−1.68 ± 0.01^{d}	0.91 ± 0.01^{d}	86.48 ± 0.03^{a}	0.37 ± 0.01^{a}	−0.67 ± 0.02^{b}	1.90 ± 0.05^{b}
1800	85.98 ± 0.01^{a}	0.29 ± 0.02^{d}	−1.00 ± 0.02^{d}	1.15 ± 0.01^{b}	87.14 ± 0.04^{a}	0.38 ± 0.01^{a}	−0.48 ± 0.01^{a}	2.44 ± 0.01^{a}

Values are means of at least two replications. C: Control; L*: lightness; a*: red for positive values and green for negative values; b*: yellow for positive values and blue for negative values, ΔE: total change in color, different letters are significantly different at $p < 0.05$.

(0.47 ± 0.01), while the lowest a* values obtained was 0.13 ± 0.01 at 40 °C, 400 MPa for 600 s. Similarly, Cappelletti et al. (2015) reported an increase of a* values in coconut water samples from 0.003 (control) to 0.07 and 0.11 after high pressure carbon dioxide (12 MPa, 45 °C, 300–3600 s) and heat pasteurization treatments (90 °C, 60 s), respectively.

The blueness of processed coconut water ranged from −1.96 ± 0.01 to −0.37 ± 0.01. The PATP caused increase in b* values of coconut water for all treatment conditions evaluated. However, high temperature conditions, 80 °C/600 MPa, resulted in increased b* values compared with the PATP conditions, 40 °C/600 MPa. Similarly, Terefe, Matthias, Simons, and Versteeg (2009) observed that the highest temperature evaluated (60 °C) resulted in the highest b* values of strawberries treated under PATP (300–600 MPa, 600 s). Cappelletti et al. (2015) also reported an increase of b* values of coconut water from 0.52 (control) to 1.02 after high pressure carbon dioxide (12 MPa, 45 °C, 300–1800 s) and from 0.52 (control) to 1.36 after heat pasteurization treatments (90 °C, 60 s). It is thought that the increase of b* values might be related to phenolic compounds oxidation of coconut water samples.

Another important parameter is ΔE which is used to denote difference between the colors of two products. It is assumed that a consumer can distinguish the color of two samples when $\Delta E \geq 5$ units (Perez-Magarino & Gonzales-Sanjose, 2003). The values of ΔE shown in Tables 5 and 6 of coconut water ranged from 0.67 ± 0.02 (coconut water treated at 40 °C, 400 MPa for 120 s) to 2.44 ± 0.01 (coconut water treated at 80 °C, 600 MPa for 1800 s). The PATP treatment of coconut water resulted in ΔE values ≤5 units. Cappelletti et al. (2015) reported ΔE values of 5.1 and 8.1 for coconut water after high pressure carbon dioxide (12 MPa, 45 °C, 300–1800 s), and heat pasteurization treatments (90 °C, 60 s), respectively. However, Liu, Zhang, Zhao, Wang, and Liao (2016) showed that cucumber juice treated under high pressure processing (HPP) (500 MPa/300 s) and high temperature short time (HTST, 110 °C/8.6 s) resulted in ΔE values of 0.75 and 2.99, respectively. Similarly, Barba, Esteve, and Frigola (2013) reported that blueberry juice treated under HPP (200–600 MPa, 0–42 °C, 300–900 s) resulted in ΔE values in the range of 0.83–1.44. The PATP treated samples had low ΔE values, resulting on imperceptible change of color of coconut water samples.

3.3.1. *Total phenolic content of coconut water*

Total polyphenol content (TPC) of raw coconut water and treated coconut water samples at 40–120 °C and 400–600 MPa are shown in Fig. 4. The TPC in the raw coconut water was 0.07 mg GAE/g. The TPC values significantly increased up to 68% after PATP treatment at 120 °C/600 MPa/120 s (0.15 mg GAE/g) and up to 69% (0.16 mg GAE/g) after treatment at 120 °C/400 MPa/1800 s. The increase of TPC can be attributed to the disruption of cellular walls and hydrophobic bonds during PATP treatment, increasing extractability of antioxidant compounds (Sharma et al., 2015). These results are in agreement with previous studies reporting increase of TPC values after PATP. Garcia-Parra et al. (2016) found that the combination of 600 MPa/70 °C/60 s increased up to 65% TPC of pumpkin puree compared to the control. Kaushik, Rao, and Mishra (2016) also reported that total phenolic content of mango pulp increased by 7–27% relative to the untreated sample after PATP (400 MPa/60 °C/300 s). Terefe et al. (2009) found no significant difference on TPC values of strawberries compared with the control after PATP treatment (600 MPa/60 °C/600 s). These results demonstrated the potential of PATP to increase extractability of total phenolic content of coconut water.

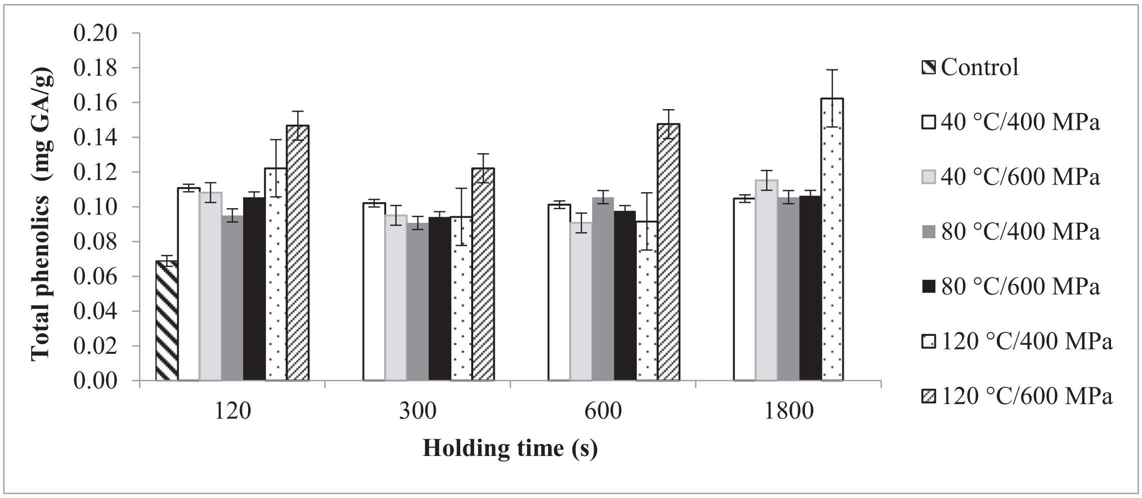

Fig. 4. Total phenolic content of coconut water after PATP treatment.

4. Conclusions

No enzymatic activity was detected for PPO and POD enzymes of green coconut water after 120 s at 90 °C/400–600 MPa. Both enzymes exhibited inactivation behavior as a function of increasing temperature from 40 to 90 °C. The POD enzyme of coconut water showed higher resistance to PATP treatment than the PPO enzyme. The recommended conditions to ensure > 90% inactivation of POD and PPO in coconut water are 400 MPa/90 °C/300 s. The pressure assisted thermal inactivation kinetics of coconut water PPO and POD were well described by the Weibull model, contributing to identify the PATP optimum conditions to inactive PPO and POD of coconut water. The PATP treatment resulted in ΔE values lower than 5 units, indicating that the original color of coconut water was maintained. The extraction of total phenolic content of coconut water significantly increased after PATP treatment. These results highlight the potential use of PATP for inactivation of PPO and POD enzymes of coconut water to maintain its quality.

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