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Effects of ohmic pasteurization of coconut water on polyphenol oxidase and peroxidase inactivation and pink discoloration prevention

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ABSTRACT

Ohmic pasteurization was used to inhibit polyphenol oxidase (PPO) and peroxidase (POD) in coconut water (CW). The electrical conductivity of CW is naturally high, making it ideal for the ohmic heating process. At 70 and 80 °C, the PPO activity decreased continuously with holding time and decreased faster at a higher electric field strength. At 90 °C, PPO activity decreased to about 10% of its initial activity at only 3 min, and then remaining constant. PPO inhibition was well described using a biphasic model and the heat-stable PPO fraction varied from 0.07 to 0.15. POD activity was promoted at 70 °C, but decreased with holding time at 80 °C and was completely inactivated at 90 °C. The CW treatment using a traditional process for 3 or 6 min increased the PPO activity during cold storage and the CW turned light pink on day 14. In contrast, no pink color was observed in the ohmic-heated CW. The results suggested that ohmic heating was effective and could be used to prevent pink discoloration in CW.

1. Introduction

The aromatic water of the coconut (*Cocos nucifera* L.) is a popular isotonic drink that has a pleasant sensory characteristic, a low calorific value and contains important amino acids and high amounts of nutrients, especially potassium, manganese and iron (Halim et al., 2018; Prades et al., 2011). Though coconut water is stable when it is inside the coconut fruit, the inconvenience aspects of transporting and drinking are the main reasons that coconut water must be extracted from the fruit and kept in a suitable commercial container. Therefore, pasteurization is a necessary process to preserve as much as possible of the coconut water quality and sensory characteristics.

One of the most important problems of coconut water manufacturers is off color due to enzymatic browning which takes place as soon as the coconut water has been extracted and exposed to the air by the actions of two enzymes: polyphenol oxidase (PPO) and peroxidase (POD) (Fox, 1991; Prades et al., 2012; Thaisakornphun and Tongchitpakdee, 2018). Thus, many studies have focused on the inactivation of PPO and POD using different pasteurization methods (Abedelmaksoud et al. 2018a; Abedelmaksoud et al. 2018b; Campos et al., 1996; Jakób et al., 2010; Marrufo-Hernández et al., 2017; Ribeiro et al., 2017; Silva et al., 2015).

Heat treatment is a method widely used to pasteurize fruit juices including coconut water. Tan et al. (2014) found that the *D*-values at 83.8 °C of PPO and POD in coconut water collected from young coconut fruits were 500 and 140 s, respectively, which suggested that the heat stability of PPO was much higher than POD. Similar results were reported by Thaisakornphun and Tongchitpakdee (2018) who found that POD was completely inhibited after heating at 85 °C for 3 min while PPO activity was still detected even after heating at 90 °C for 14 min. Murasaki-Aliberti et al. (2009) suggested that PPO in coconut water comprised two isoforms: heat labile and heat stable. Thus, it was possible that the residual heat-stable PPO could respond to the physicochemical changes in pasteurized coconut water during cold storage and limit the shelf life of the product. Consequently, study is required on a new process that can effectively control the activity of PPO in pasteurized coconut water to prevent pink discoloration.

Ohmic heating is a heating method whereby electrical energy is passed through food materials (Icier, 2012). The process heats food quickly and uniformly and is highly energy efficient (Knirsch et al., 2010; Li and Sun, 2002). Castro et al. (2004) studied the sensitivity of several important food-related enzymes toward ohmic heating and reported that temperature, heating time and electrical field strength affected enzyme inhibition. Their results showed that the electrical field had an additional effect on the inhibition of lipoxygenases and polyphenoloxidase. They suggested that the action of electric field on metallic prosthetic groups of lipoxygenases and polyphenoloxidase

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could be the main reason that the enzymes were inactivated. Similar results were reported by Jakób et al. (2010) who stated that ohmic heating enhanced the rate of inactivation of pectin methylesterase in fruit juices, and of alkaline phosphatase in milk and POD vegetable juices. The effect of ohmic heating on the inactivation of PPO in sugarcane juice (Saxena et al., 2017), mango juice (Abedlmaksoud et al., 2018a) and apple juice (Abelmaksoud et al., 2018b) were studied. They found that ohmic heating caused the effective reduction of residual PPO activity in short processing times. However, ohmic heating effects are not known about the PPO activity in coconut water and pink discoloration that results from storage under commercial storage. Thus, the objectives of this research work were to determine the effects of ohmic heating on the activity of PPO and POD in coconut water during heating and cold storage at 4 °C, and to assess the changes in color of the pasteurized coconut water during storage.

2. Material and methods

2.1. Coconut water sample preparation

Young, green dwarf “Nam Hom aromatic coconut” fruits (*Cocos nucifera* L.) were obtained from coconut trees in a local plant at Kasetsart University, Nakorn Pathom province, Thailand. The coconut fruit maturity was about 7 months. All fresh coconut water samples collected from the fruits were well-mixed to ensure uniformity of the sample. The coconut water was filtered through a stainless sieve (opening size about 0.5 mm) and packed in plastic bags, 150 mL each pack. The samples were immediately frozen at −18 °C to retard chemical reactions. Each sample was left in a water bath until its temperature reached 4±1 °C before it was used.

2.2. Pasteurization process

Pasteurization was performed using a laboratory scale ohmic heating system which consisted of a voltage regulator (0–250 V, 50 Hz), a rectangular ohmic cell and a temperature controller equipped with a 3-wire RTD platinum sensor (Fig. 1). The inner dimension of the ohmic cell (height × width × length – in mm) were 40 × 80 × 100. A pair of stainless electrode (100 mm × 40 mm) were carefully installed so that its surface was attached to the cell wall to ensure that coconut water sample was heated uniformly. The temperature, voltage and electric current were recorded using a data logger (Sangchaimeter, RP3430, Thailand). A PID controller (Primus, PT-03, Thailand) operated in on-off mode was used to control sample temperature. A volume of 150 mL of coconut water was poured into the ohmic cell and the temperature sensor was fixed in the middle of the cell. Then, the sample was ohmically heated and held at a constant temperature (70, 80 or 90 °C) under two different electric field strength (10 or 20 V/cm) for 3–15 min. The electric field strength selection was based on the change in the coconut water color. If the electric field strength was higher than 20 V/cm, the color of coconut water became darker very fast after ohmic process began for few minutes. After heating, the sample was rapidly cooled in an ice-water bath. Samples were stored in a refrigerator at 4 °C for a maximum 21 days. During storage, samples were randomly collected for quality assessment.

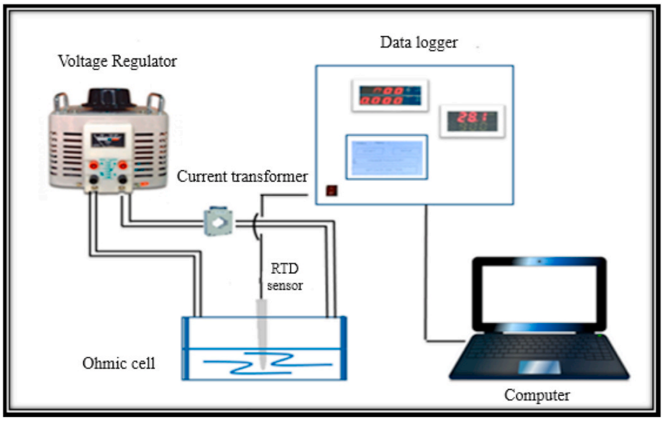


Fig. 1. Ohmic pasteurizer system.

For a comparison purpose, coconut water was pasteurized using a conventional method. A volume of 150 mL of coconut water was poured into a clean glass container. Then, the container was put in a stainless pot that contained warm water (initial temperature about 70±2 °C). The water in the pot was heated using an electric heater. During heating, coconut water in the container was continuously stirred using a stirring rod and the temperature of the sample was carefully monitored and controlled by switching the electric heater on/off so that the temperature profile of the coconut water heated using a conventional method was the same as that heated using ohmic process.

2.3. Electrical conductivity determination

As mentioned, the electric current and voltage were recorded during the pasteurization process. Electrical conductivity of a sample was calculated using Equation (1):

$$\sigma = IL/VA \tag{1}$$

where I, V, L and A are the electric current (A), voltage (V), distance between electrodes (7.5 cm) and sample contact area of electrodes (20 cm^2), respectively.

2.4. Polyphenol oxidase (PPO) activity assay

PPO activity was determined using a method modified from Campos et al. (1996). A volume of 5.5 mL of phosphate buffer (pH 6.0) was mixed with 1.5 mL of 0.2 M pyrocatechol. Then, 2 mL of coconut water were added into the mixture and mixed thoroughly using a vortex mixer. For blank test, 2 mL of deionized distilled water was mixed with phosphate buffer solution and pyrocatechol. Change in the absorbance at a wavelength of 425 nm monitored using a UV-VIS spectrophotometer (Shimadzu, UV-1800, Japan), after subtraction with the blank, was used to determine PPO activity.

2.5. Peroxidase (POD) activity assay

The method used to determine POD activity was modified from Campos et al. (1996). A sample of 7 mL of phosphate buffer (pH 5.5) was mixed with 1.5 mL of 0.05 %v/v guaiacol and 0.5 mL of 0.1 %v/v hydrogen peroxide solutions in a test tube. Then, 2 mL of coconut water (or distilled water for the blank) was added to the test tube and mixed thoroughly. The test tube was then warmed in a water bath until the temperature reached 35 °C. POD activity was determined from changes in absorbance at a wavelength of 470 nm (after subtraction with the blank) using the UV-VIS spectrophotometer.

2.6. Enzyme inhibition kinetic models

A biphasic model was used to analyze the kinetic behavior of the enzyme as shown by Equation (2) and the decimal reduction time or D value of the enzyme (the time needed for 90% inactivation of initial activity of enzyme) was calculated using Equation (3): (Murasaki-Aliberti et al., 2009; Saxena et al., 2017):

$$A_t / A_0 = (1-\alpha)\exp(-k_l t) + \alpha \exp(-k_s t) \tag{2}$$

$$D = 2.303/k \tag{3}$$

where A_t and A_0 are activity at time “t” and initial activity of the enzyme, k_l and k_s are the inactivation rate constant of the heat-labile and heat-stable components and α is the ratio between the heat-labile and heat-stable fractions. The *z* value was also calculated as the negative reciprocal of the slope of the graph of log D versus temperature.

2.7. *Color measurement*

Color changes in the coconut water were analyzed based on the *L*a*b** color system using a spectrophotometer (HunterLab, Miniscan, Germany). The spectrophotometer was calibrated using a white calibration plate. A volume of 15 mL of the sample was placed in an open, transparent glass Petri dish and at least three readings of each sample were done against a white background.

2.8. *Total plate count and yeast and mold measurement*

Total plate count (TPC) and yeast and mold numbers in each sample were determined using the "pour plate" technique (AOAC, 2000). Plate count agar and potato dextrose agar were used to determine the TPC and yeast and mold counts of coconut water, respectively. Coconut water samples were serially diluted in sterilized distilled water. Approximately 15–20 mL medium were poured into sterile Petri plates in a laminar hood and allowed to solidify. One mL of sample was then plated onto the medium using the spread plate method under aseptic conditions. All samples were then incubated. The incubation condition for TPC was 37 °C for 48–72 h, and it was 25 °C for 5 days for yeast and mold count. All experiments were repeated three times and average values were reported.

2.9. *Statistical analysis*

All experiments involved at least three independent replicates. Analysis of variance was performed to determine significant differences between the means. Duncan's new multiple range test was used to compare means at a confidence level of 0.95 (significance level = 0.05). Results from the experiments were recorded as the mean ± standard deviation.

3. Results and discussion

3.1. *Coconut water electrical conductivity*

Fig. 2 shows the electrical conductivity (EC) of the coconut water samples which were collected from different coconut fruits to test the uniformity of the sample. The relationship between the EC and temperature was linear. The EC of coconut water was in the range between 0.7 and 2.4 S/m at 25–90 °C which was relatively high compared to several kinds of fruit juice: 0.2–1.1 S/m at 20–85 °C for pomegranate juice (Darvishi et al., 2013), 0.4–0.8 S/m at 30–80 °C for grape juice (Icier et al., 2008) and 0.4–1.0 S/m at 20–74 °C for lemon juice (Darvishi et al., 2011). Since the heating rate is directly proportional to the EC of the food, the high EC suggested that coconut water was suitable for ohmic heating. Slightly different EC values among samples were observed. Halim et al. (2018) reported that mature coconut water contained more electrolytes while young coconut water was sweeter because of the higher sugar content. Therefore, in this research, the coconut water samples collected from different fruits of different bunches were mixed together to ensure uniformity of samples before they were used in the pasteurization process and analyzed for their properties.

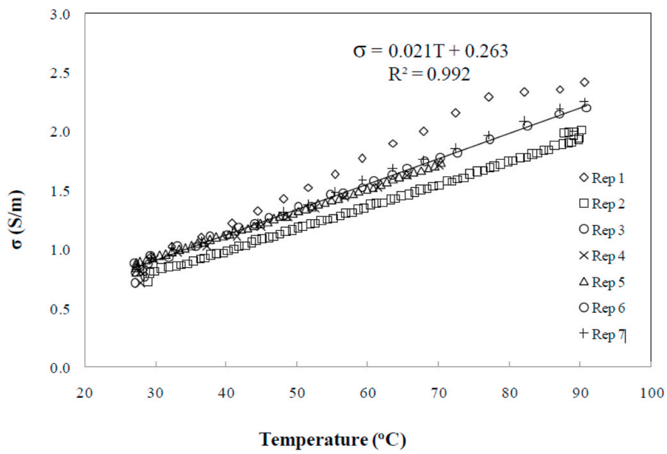
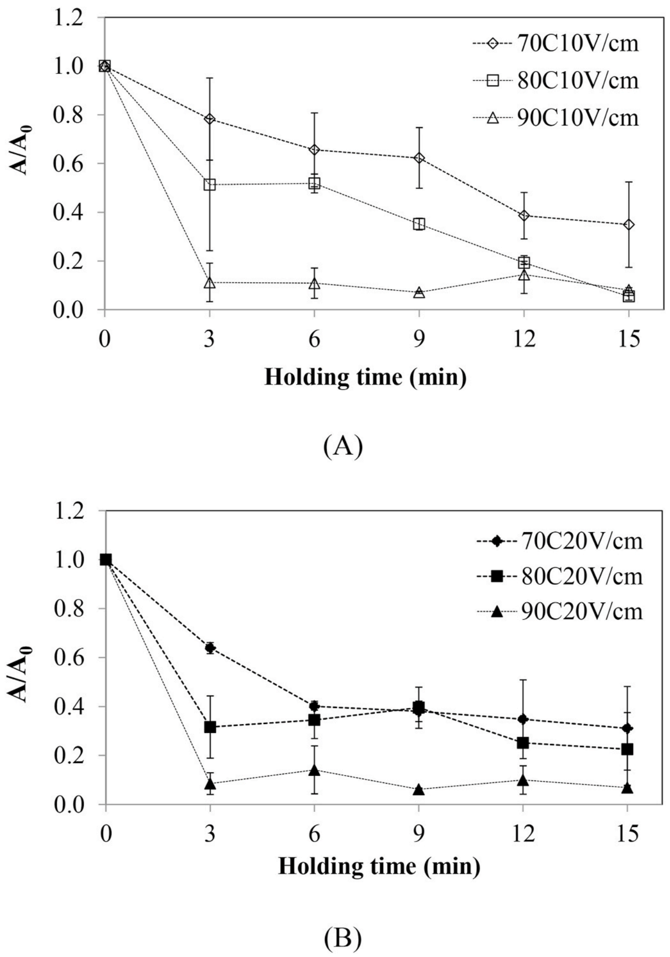


Fig. 2. Electrical conductivity of fresh coconut water samples collected from different coconut fruits.

3.2. *Polyphenol oxidase activity*

PPO is an enzyme that plays an important role in the enzymatic browning reaction in food. The effects of ohmic heating at 10 V/cm for a maximum of 15 min on inactivation of PPO in coconut water are shown in Fig. 3A. When the sample temperature reached 70 °C, the PPO activity decreased with holding time at an average reduction rate of 0.04/min. The effect of thermal treatment on PPO inactivation has been widely reported (Saxena et al., 2017; Tan et al., 2014; Thaisakornphun; Thaisakornphun and Tongchitpakdee, 2018; Toralles et al., 2005). Iqbal et al. (2019) suggested that possible mechanisms of PPO inactivation were: conformational changes of the secondary structures from an α-helix to a β-sheet in the PPO molecules, aggregation between the molecules and distortion of tertiary structure of PPO molecules during



(A)

(B)

Fig. 3. Decreasing of polyphenol oxidase activity with processing time during ohmic heating at 70, 80 and 90 °C at A) 10 V/cm and B) 20 V/cm.

processing, either by thermal or non-thermal. Similar results were also mentioned by Baltacioglu et al. (2015) who studied the effects of thermal treatment on conformational change of PPO in mushroom and by Zhong et al. (2007) regarding the effects of a pulsed electrical field in decreasing α-helix structure and PPO inhibition.

At 80 °C, the PPO activity decreased continuously at a higher reduction rate (about 0.06/min) than at 70 °C. Enzyme activity rapidly decreased to the lowest point within 3 min at 90 °C and then remained stable, even though the samples were held at constant temperature for 15 min ($p > 0.05$), which suggested that the residual PPOs in coconut water were heat-stable. This result agreed with Murasaki-Aliberti et al. (2009) who reported that PPO activity rapidly decreased to 12% of the initial value after 1 min of heating at 86.9 °C but then the activity of PPO did not change. They concluded that PPO in coconut water comprised two groups, with the first group being sensitive to heat and denaturing quickly during heating, while the second group was heat-stable.

The PPO activity of the sample heated at 90 °C for 12 min was slightly higher than for 9 and 15 min. An increase in the enzyme activity during heating at constant temperature was also reported by Saxena et al. (2017) and Castro et al. (2004) who considered that the ohmic heat treatment increased inter-chain reactions which could result in a better enzyme-substrate interaction.

When the electric field strength was increased to 20 V/cm (Fig. 3B), the enzyme activity decreased more rapidly at 70 and 80 °C. Though the behavior of PPO at a temperature of 90 °C was similar for the sample ohmic heated at 10 V/cm, but it could be possible that the times required to heat sensitive PPO fraction at 10 and 20 V/cm were shorter than 3 min and were different. However, burns on the edges of both electrodes were observed when the ohmic heater was operated at 20 V/cm and 90 °C for a few minutes. This could have been due to the high electric field intensity around the edge of the electrode and the electrical conductivity of the coconut water, leading to an increase in the electric current flowing through the edges of the electrode. These burns adversely affected the color of the coconut water as the samples became darker with a longer holding time. The results indicated that an electric field strength of 10 V/cm was sufficient and more suitable for the system used in this research. It should be noted here that though the electric field strength applied in this research was only 10 and 20 V/cm, an optimization the process condition by increasing an electric field strength to a value between 10 and 20 V/cm could be interesting in order to decrease the process time which might preserve more nutrient in coconut water.

3.3. Peroxidase activity

In contrast to PPO, POD was completely inactivated at the maximum temperature. Fig. 4 shows the effect of the temperature, holding time and electric field strength on the POD inhibition. Clearly the holding time and temperature strongly affected the POD activity ($p < 0.05$). At 70 °C and 10 V/cm, the holding process did not deactivate POD but, in contrast, it promoted the POD activity. Toralles et al. (2005) studied the effect of pH on the activity of PPO and POD from Granada clingstone peaches and reported that PPO was mostly active at pH 6.0–6.5 while the maximum POD activity was at pH 4.8–5.5. The pH of the coconut water samples was in the range 5.42–5.46 which favored the POD activity. This could explain why the POD activity increased at a low heating temperature. However, the inactivation temperature slightly depended on the source of POD. For example, Jakób et al. (2010) mentioned that inactivation of POD in vegetable mixtures (carrot, broccoli and potatoes) at 70 °C was possible but would take quite a long time as it required 20 min to reduce 90% of the enzyme activity. Fortunately, the heat sensitivity of POD was high as POD activity rapidly decreased at 80 °C and POD activity was not detected when coconut water was heated at 90 °C for only 3 min, regardless of the electric field strength (10 or 20 V/cm). As commercial coconut water products are pasteurized at 80–95 °C, the pink discoloration in coconut water might depend mainly on the PPO activity residue in the pasteurized product, because POD activity was not detected after heating and during cold storage (see Fig. 8).

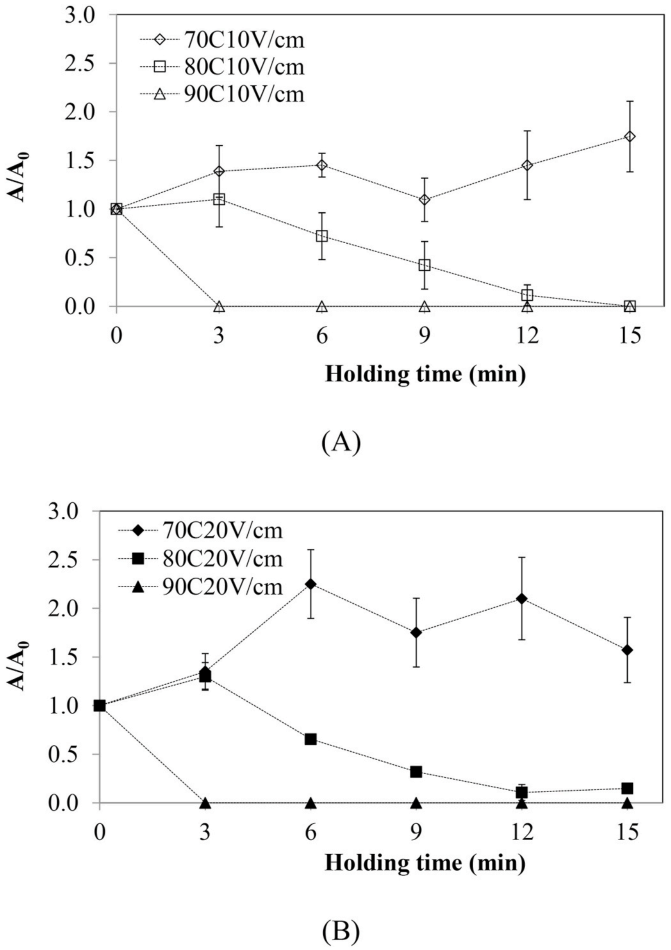


Fig. 4. Decreasing of peroxidase activity with processing time during ohmic heating at 70, 80 and 90 °C at A) 75 V and B) 150 V.

Makroo et al. (2020) had reviewed non-thermal effect of ohmic heating on POD inactivation. Electric field caused the alteration of POD surface charge and/or enzyme environment due to the ionization of solution components and distribution of their ions in an applied electric field during ohmic heating. They also mentioned that the severity of ohmic heating on POD inactivation depended on food materials. Gomes et al. (2018) also reported the effect of ohmic heating (at 80 °C and 160–180 V, electrode gap 5–6.8 cm) on an inactivation of POD in *Tetsukabuto* pumpkin and found that ohmic process decreased POD activity to 10% of its initial value faster than the conventional process by nearly two times and almost of POD was inactivated within 3 min. Compare to the current research, a holding time of 12 min was required to inactivate 90% of POD in coconut water at the same temperature (80 °C). From the result, it was recommended that the pasteurization temperature for coconut water should be in a range of 85–90 °C.

3.4. Kinetic models of enzyme inhibition during heating

Fig. 5 shows a decrease in the PPO activity during ohmic heating at 70 °C and 10 V/cm. The inactivation rate constant values for the biphasic model (k_l and k_s) and the ratio between heat-stable and heat-labile components of PPO in coconut water (α) are provided in Table 1 ($R^2 > 93\%$). The inactivation rate constant of the heat-labile component (k_l) was much higher than the heat-stable component (k_s) regardless of the temperature and electric field strength, suggesting that the heat-stable fraction was less inactivated than the heat-labile one. The

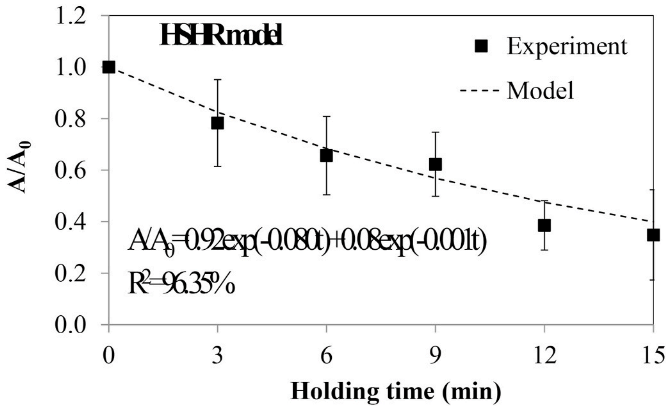


Fig. 5. Kinetic of polyphenol oxidase activity decreasing during ohmic heating at 70 °C and 10 V/cm.

Table 1
Kinetic parameters and ratio (**α**) between heat-stable and heat-labile components of polyphenol oxidase in coconut water.

Electric field strength (V/cm)	Temperature (°C)	k_l[a] (min^{-1})	k_s[a] (min^{-1})	α
10	70	0.080	0.001	0.08
	80	0.142	0.006	0.07
	90	6.790	0.010	0.11
20	70	0.162	0.001	0.10
	80	0.200	0.007	0.15
	90	6.600	0.011	0.08

[a] l = heat-labile; s = heat-stable.

heat-stability fraction can be estimated from the values of α which were around 7–11%. This could explain why PPO in coconut water could not be completely inactivated. From the data summarized by Iqbal et al. (2019) on the effects of thermal and non-thermal treatment on PPO inactivation in fruit juices and purees, the maximum PPO inactivation ability of the process depended on the fruit type, process and treatment conditions. However, no such process completely inactivated PPO. In the current study, ohmic heating at 90 °C for longer than 3 min produced the best result with a maximum of 93% PPO inhibition. Table 2 shows the *D* and *z* values of PPO in coconut water compared with data from the literature. The calculated values from the current study were comparable with the values reported by Saxena et al. (2017) and Tan et al. (2014). The *D* values of PPO at 70 and 80 °C decreased with an increase in the electric field strength. At 90 °C, the *D* values of the heat-sensitive component were only 0.3 min while the heat-stable ones were 209–230 min depending on the electric field strength. The data in Tables 1 and 2 can be used to estimate the required holding time. For example, the time needed for 99% PPO inactivation ($t_{99\%}$) ~ -ln $(0.01/\alpha)/k_s$ = 230 min which is too long and not practical because a high degree of product deterioration could occur. Thus, the best way to prevent the pink discoloration in coconut water would be perhaps not to completely inactivate PPO but to determine the minimum holding time that could control or retard the PPO activity during storage.

3.5. *Stability of pasteurized coconut water during cold storage*

3.5.1. *Total plate count and yeast and mold*

Fresh, unprocessed coconut water was kept at 4 °C for 21 days and tested for microbial growth. At only day 2, some yeast and mold spots were observed (11 CFU/mL) while the TPC increased to 97 CFU/mL on day 7 (data not shown). This indicated the necessity of the pasteurization process to prolong the coconut water shelf life.

To evaluate the efficacy of the pasteurization processes, ohmic heated samples were stored at 4 °C for a maximum storage time of 21 days. For comparative purposes, coconut water samples were also pasteurized using a traditional process (boiling) at 90 °C for 3, 6 and 9 min. The temperature of the sample was carefully controlled by adjusting the heat source which is an electric heater. Fig. 6 shows that the temperature profiles of a traditional process and ohmic heating were not different. The results showed that ohmic heating at 90 °C and 10 V/cm for 9 min could kill microbes efficiently. The TPC detected no activity in only the sample ohmic heated for 9 min while the TPC values in all other samples were very low, ranging from 1 to 5 CFU/mL. No yeast or mold activity was detected in any of the ohmic-heated samples compared to values of 1 CFU/mL in all the boiled samples.

Table 2
D and *z* values of polyphenol oxidase in ohmic-heated coconut water compared to other studies.

Parameter	Heating method[a]	Component			Source
		Heat-labile	Heat-stable	Not specified	(Sample)
D_{70} value (min)	OH 10 V/cm	32.9	2303.0	–	Current research (coconut water)
D_{80} value (min)		15.2	383.8	–	
D_{90} value (min)		0.3	230.3	–	
z value (°C)		10.1	15.9	–	
D_{70} value (min)	OH 20 V/cm	14.2	2305.0	–	
D_{80} value (min)		11.5	329.8	–	
D_{90} value (min)		0.3	209.4	–	
z value (°C)		12.8	20.0	–	
D_{80} value (min)	OH 32 V/cm	12.1	98.0	–	Saxena et al. (2017) (sugarcane juice)
z value (°C)		46.1	42.7	–	
$D_{83.8}$ value (min)	Boiling	–	–	9.8	Tan et al. (2014) (coconut water)
z value (°C)		–	–	23.8	

[a] OH = ohmic heating.

3.6. *PPO activity*

The activity of PPO in unprocessed coconut water during storage is shown in Fig. 7A. The PPO activity increased in the first two days but then continuously decreased with storage time. Fig. 7B, C and 7D show the activity of PPO in coconut water sample heated at 90 °C for 3, 6 and 9 min, respectively. The activities of PPO in the ohmic-heated samples were significantly lower than those in the control samples heated for the same time. The PPO activity in all pasteurized samples increased sharply in the first two days. From day 4 to day 21, the activity of PPO in samples

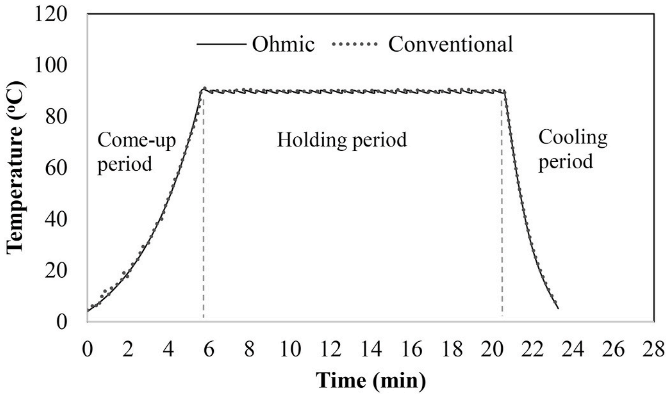


Fig. 6. Temperature profiles of coconut water during ohmic heating at 10 V/cm and heating using a conventional method.

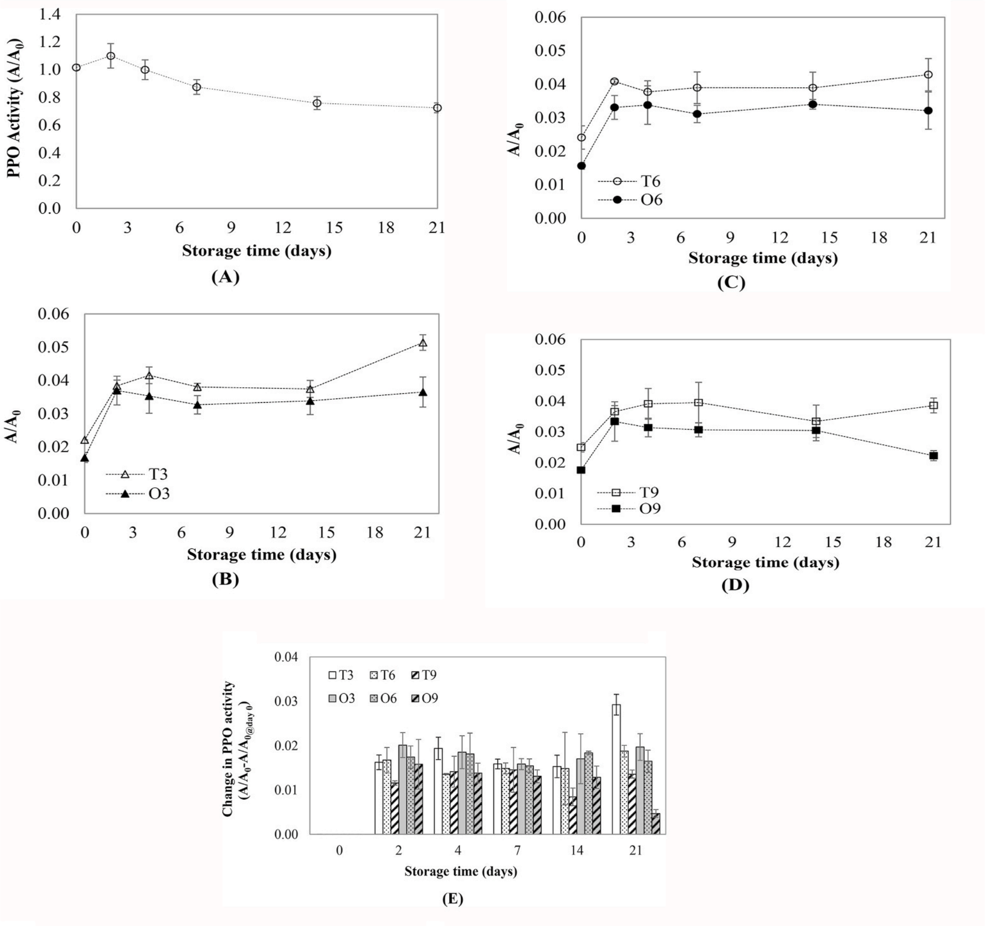


Fig. 7. Relative activity of polyphenol oxidase in (A) unprocessed coconut water and in coconut water samples heated at 90 °C by traditional process (denoted by "T") or ohmic heating (denoted by "O") at 10 V/cm for (B) 3 min, (C) 6 min and (D) 9 min and (E) changes in relative activity of polyphenol oxidase in samples during storage at 4 °C for 21 days.

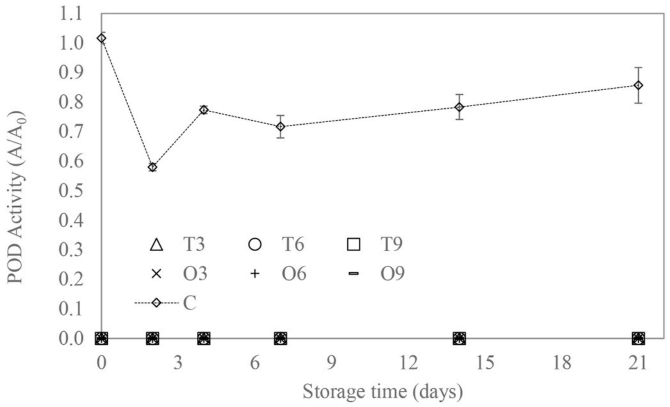


Fig. 8. Activity of peroxidase coconut water during storage at 4 °C for 21 days. "C" means control unprocessed sample. "O" and "T" denoted the sample was heated at 90 °C by ohmic heating at 10 V/cm or traditional process. The number followed the letter is the holding time.

T3 and T6 (pasteurized using a traditional process for 3 and 6 min) slowly increased with storage time, indicating that the heat treatment was insufficient to control the PPO activity. In contrast, the activity of PPO in samples O3 and O6 (ohmically heated for 3 and 6 min) increased at day 2 of cold storage but then remained constant until day 21. The difference behavior of PPO might be due to conformational changes of PPO structure in such way that it changed its behavior during cold storage. For the traditional process, a holding time of at least 9 min was necessary. A slow decrease in the PPO activity was also observed for sample O9. This could have been due to the disruption of the enzyme structure at low temperature. This finding agreed with Xu (2005) who reported that the activity of PPO in *Castanea henryi* chestnuts decreased by 20% from its initial activity after storage at 4 °C for 30 days. Fig. 7E shows an increase in enzyme activity during cold storage in the sample compared to the activity at day 0. It was clear that PPO activity in samples T3 increased at a faster rate at day 21 while PPO activity in samples O9 continuously decreases from day 2 to day 21.

3.7. *POD activity*

Fig. 8 shows the lack of POD activity during storage in any of the heated samples which meant that the heat treatment was adequate for POD inactivation. A similar result was reported by Thaisakornphun and Tongchitpakdee (2018) who indicated that POD in coconut water had been inactivated immediately after the heating process at 85–90 °C for at least 3 min.

3.8. *Color*

The values of L^* or lightness, a^* or redness and b^* or yellowness of fresh coconut water were 35.60 ± 0.07, −0.52 ± 0.02 and 0.76 ± 0.02, respectively. The values of L^* slightly decreased (from about −0.5 to −0.7%) while the values of b^* increased (from 15 to 25%) with heating time but did not significantly change from the initial values during storage for 21 days (data not shown). However, the value of a^*, which is an indicator for the pink color of coconut water, changed with storage time to varying degrees depending on the heating profile of the sample.

The higher positive value of a^* indicated that the sample had more pink color. Fresh coconut water was clear with no pink color ($a^* = -0.52 \pm 0.02$). The a^* values of the samples that were ohmic heated for 9 min were significantly lower than for the samples treated at 3 min. This could have been due to the destruction of sensitive pigments during heating (Saxena et al., 2017). At day 0, there was no significant difference between the a^* values of the ohmic- and conventional-heated samples that were treated for the same holding time (Fig. 9). The a^* value of all samples increased with storage time but at different rates depending on the ohmic heating conditions. The a^* values of samples O3, O6 and O9 (ohmic heating for 3, 6 and 9 min) increased by about 0.006 per day. On the last day of storage, there were no significant differences among the a^* values of sample O3, O6 and O9 which were -0.13 ± 0.02, -0.15 ± 0.04 and -0.16 ± 0.02, respectively. No pink color was observed in any of the ohmic-heated samples. Garcia et al. (2007) demonstrated that the pink discoloration of coconut water was influenced many factors including the level of PPO in coconut water. In their preliminary study, they reported that 7 month aromatic-green-dwarf coconut fruits (which is our case) had the highest PPO activity. They proposed that the mechanism that leads to the formation of pink colored compounds was contributed to copper binding site, which can be reduced by the reaction with the phenolic substrate. In our case electric field strength could affected on the metallic group of PPO, and acted on enzyme inactivation, leading to a reduction in formation of pink compounds.

In contrast, pink discoloration was found in samples that were processed using a traditional process for 3 and 6 min (T3 and T6). The a^* values of samples T3 and T6 increased by 0.019 and 0.018 per day, respectively. At day 14, the a^* values of samples T3 and T6 became positive and a light-pink color was observed in both samples. The degree of pink discoloration was higher on the last storage day when the a^* values of samples T3 and T6 reached 0.16 ± 0.01, and 0.11 ± 0.05, respectively. Though no pink discoloration was observed, on the last storage day, the a^* value for sample T9 had already increased to -0.06 ± 0.01 and had tended to increase with storage time.

4. Conclusion

Coconut water is a food material with a high electrical conductivity, making it suitable for the ohmic-heating process. PPO was more heat-stable than POD. The ohmic heating process completely inactivated POD at 90 °C for 3 min while about 10% of PPO activity was still detectable. The results showed that PPO in coconut water comprised two groups, with the first group in the majority being heat-sensitive and inactivated rapidly at 90 °C. In contrast, the second group was heat-stable and was not inactivated even when the holding time was extended to 15 min at 90 °C. While a pink color was observed in the samples pasteurized using the traditional process for 3 or 6 min and kept at 4 °C, there was no pink discoloration in the ohmic-heated samples. Observation of the relationship between the a^* value and PPO activity during cold storage indicated that the pink discoloration in coconut water could possibly have been due to the residual heat-stable component of PPO. The PPO activity levels in all ohmic-heated samples were significantly lower than those in the samples heated using the traditional process. The simplicity of the process makes ohmic heating a suitable, alternative method for coconut water pasteurization as it could reduce the production time and prevent pink discoloration of coconut water during cold storage under commercial conditions.

CRediT authorship contribution statement

Kobsak Kanjanapongkul: Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Veraya Baibua:** Visualization, Investigation.

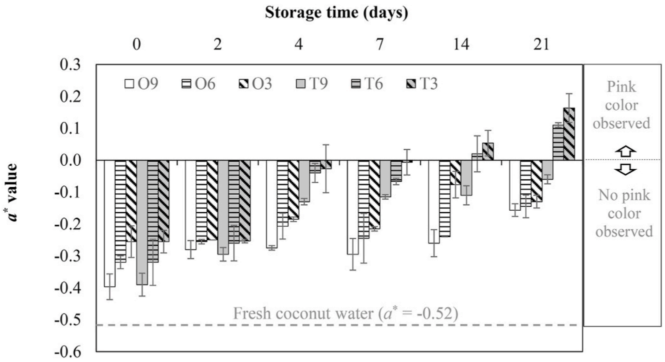


Fig. 9. Effectiveness of ohmic heating on pink discoloration prevention during storage. O and T stand for ohmic heating and traditional process. A number after the letter indicates the heating time in minutes.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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