

Effect of blanching on enzyme and antioxidant activities of rambutan (*Nephelium lappaceum*) peel

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Abstract

Rambutan (*Nephelium lappaceum*) peel is a potential source of antioxidant. As rambutan is a seasonal fruit, a proper heat treatment prior to storage is necessary. Thus, this study was conducted to determine the effect of water and steam blanchings on browning enzymes and antioxidant activities of rambutan peel extracts. Rambutan from the variety of 'Anak Sekolah' were peeled and the peel was blanched in boiling water for 0, 2.5, 5 min and by autoclaving for 0, 5, 10 and 15 min. The residual peroxidase (POD) and polyphenoloxidase (PPO) activities, antioxidant activity (2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity), total polyphenol content (TPC) and peel extract colour were determined. The results showed that both water and steam blanchings significantly reduced ($p < 0.05$) POD and PPO activities. The results also indicated that the increase in the blanching period did not significantly reduce the enzyme activities further. In terms of antioxidant activity, the thermal pretreatment caused no significant difference in the contents of phenolic compounds, as well as the antioxidant capacity of the final product.

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Introduction

Native to Southeast Asia, rambutan (*Nephelium lappaceum*) which belongs to the family of Sapindaceae, is a potential fruit to be commercialized since it is widely planted all over Malaysia, Thailand and other ASEAN regions. Initial studies showed that the red coloured peel of rambutan has high antioxidant activity (Okonogi *et al.*, 2006). Thus, rambutan peel, which usually is thrown away as waste, may serve as a source of useful antioxidant for extraction. However, rambutan has a short shelflife, where the quality of the fruits will drastically decrease if they are not well handled. According to O'Hare and Underhill (1994), chilled ripe rambutan fruits will undergo colour changes from red to horseradish and finally turn into brown. The change in colour of rambutan peel is believed to be due to the oxidation of antioxidant compounds via the enzymatic browning reaction (Zhang *et al.*, 2005). As rambutan is a seasonal fruit, an effective method of preserving the antioxidant compounds and reducing their oxidation is very critical. This will prolong the storage of rambutan peel, thus making it available for processing throughout the year.

Blanching is commonly used in food processing to inactivate enzymes and destroy microorganisms. It is a process of exposing vegetables or fruits to high temperatures for a short period. This process

not only prolongs the shelf life of vegetables by inactivating the enzymes responsible for browning (polyphenoloxidase, lipoxygenase and peroxidase), but also improves both colour and flavour (Miller and Rice, 1996). However, blanching is also accompanied by a reduction in sensory and nutrient qualities in many foods, mainly due to Maillard reaction (Nicoli *et al.*, 1991). Food material that has been exposed to high temperatures usually experiences an adverse effect on quality (loss of texture and undesired colour changes) and has a reduced content or bioavailability of some nutrients (Volden *et al.*, 2008). Hence, it is imperative to keep blanching conditions at a level just sufficient to cause inactivation of the deleterious enzymes but with minimal effect on other beneficial attributes. In general, peroxidase is recognized as being one of the most heat-stable enzymes in fruits and vegetables and is considered to be an indicator enzyme for adequacy of thermal process (Williams *et al.*, 1986). The presence of residual peroxidase in processed products caused quality changes such as in texture, colour, flavour, and nutritional components (Gonçalves *et al.*, 2007). PPO activities lead to the formation of undesirable brown pigments and off-flavoured products (Yemenicioglu *et al.*, 1999). Two kinds of reactions catalysed by PPO are the hydroxylation of monophenols to o-diphenol and the oxidation of o-diphenol to o-quinone (Toma's-Barbera'n and Espi'n, 2001). Conventionally,

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blanching is done using boiling water. This method is simple and inexpensive, but has the highest potential of leaching of water-soluble components.

Blanching using steam also has been practiced in the oils and fats industry. Oil palm fruits are commonly exposed to steam to inactivate enzymes before extraction (Dominguez *et al.*, 1994). Thermal processing also has a significant effect on natural antioxidants in the plant materials. For example, it has been reported that the antioxidant activities of kale, spinach and swamp cabbage were reduced significantly after 1 min of blanching. Phenolic compounds are also reported to be very sensitive to heat treatment even for short period of cooking (Ismail *et al.*, 2004). Due to the seasonal nature of rambutan and the inexistence of literature regarding blanching of rambutan peel, the present study was carried out to determine the effects of blanching using boiling water and steam on enzyme and antioxidant activity in rambutan peel.

Materials and Methods

Water blanching

Rambutan (*Naphelium lappaceum*) from the variety Anak Sekolah was sourced from Malaysian Agriculture Research Development Center (MARDI), Serdang at a maturity index of 4. The rambutan fruits were peeled by hand and the peel and flesh were separated. Rambutan peels were blanched for 0, 2.5 and 5 min in boiling water at 100°C. After blanching, the samples were immediately cooled in an ice bath to stop the heating process. About 10 g of rambutan peel was then blended in 50 ml distilled water. The homogenate was then filtered using cheese cloth and centrifuged for 15 min at 1000 rpm. The supernatant was collected and subjected to analyses for enzyme activity, DPPH free radical scavenging assay and total phenolic content.

Steam blanching

Rambutan peel was heated for 0, 5, 10 and 15 minutes using an autoclave at 100°C. Unblanched sample was used as a control. After blanching, the peels were subjected to the same treatment and analyses as for water blanching.

Enzyme activity

Peroxide (POD) activity was measured using guaiacol (Merck, Germany) and polyphenol oxidase (PPO) activity was measured using pyrocatechol (Sigma-Aldrich, Germany) according to Duckworth and Coleman (1970). The collected supernatant of the blanched rambutan peel were kept in an ice bath before being analysis. The activity of the PPO

was expressed as U/g (dw) by using $\epsilon_M = 1830 \text{ M}^{-1} \text{ cm}^{-1}$ for benzoquinone produced from catechol. The activity of POD was expressed as U/g (dw) by using $\epsilon_M = 2.66 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for H_2O . Enzyme activity was defined as the amount of extract capable of degrading 1 μmole substrate per minute.

DPPH assay

The antioxidant capacity of the supernatant was determined using DPPH free radical scavenging assay according to the method of Kriengsak *et al.* (2006) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using a spectrophotometer. A 150 μL of extracts were allowed to react with 2850 μL of the DPPH solution for 90 minutes in the dark. Subsequently the absorbance at 515 nm was recorded. Additional dilution of samples was carried out if the absorbance measured was over the linear range of the standard curve. Results were expressed in ascorbic acid equivalent antioxidant capacity per dry weight, AEAC / g (dw) (Lim *et al.*, 2006) as desired by the equation below.

$$\text{AEAC (mg AA/100g)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{(A_{\text{control}} - A_{\text{AA}})} \times [\text{Ascorbic Acid}] \times \left[\frac{\text{total sample volume}}{\text{sample vol} \times \text{vol ext}} \right] \times \left(\frac{100}{\text{g sample}} \right)$$

Total polyphenol content

Total polyphenol content (TPC) was determined using the Folin-Ciocalteu's reagent as reported by Lim *et al.* (2006). Gallic acid solutions at different concentration (10, 25, 50, 75 and 100 mg/L) were treated similarly and used to construct a standard curve. Total polyphenol contents were expressed in percent gallic acid equivalents per dry weight, % GAE / dw (g).

Total anthocyanin estimation

Total anthocyanin was determined using single pH method as described by Fuleki and Francis (1968). 10 g of rambutan peel was blended in 100 ml methanol: 1% hydrochloric acid at room temperature by using a Warring blender. The homogenate was then kept at 4°C in the dark overnight to improve extraction efficiency before filtration using Whatman paper no 1 in a Buchner funnel. The rambutan peel extracts were transferred into 100 ml volumetric flask and solvent volume made up. Maximum absorption was determined at 530 nm wavelength using a spectrophotometer UV-VIS (UV-2450 UNICAM, England). Total anthocyanin content were expressed in mg/100 g.

Peel extract colour

About 250 g unblanched and blanched rambutan peel using water and steam blanching were pressed using a hydraulic press (Mariwealth, Kajang, Selangor, Malaysia). The extract was collected in test tubes and left standing for 10 min at room temperature. Every 2 min, the extracts were measured for degree of lightness (L), redness (a* value) and chroma (C) using a chromameter (Minolta, Japan).

Statistical analysis

The data recorded on the sample were statistically analysed using ANOVA and Duncan multiple range test. The Statistical Analysis Software (SAS) package (version 8.2 of SAS Institute, Inc.) was used for the analysis.

Results and Discussion

Enzyme activity

Figure 1 shows the normalized value of peroxidase (POD) and polyphenol oxidase (PPO) activity in water blanched rambutan peel. As indicated, the POD activity in the rambutan peel was significantly reduced ($p < 0.05$) when blanched for 2.5 minutes. However, there was no significant difference between the 2.5 and 5 min blanching period on POD activity. For PPO activity, a significant difference ($p < 0.05$) was observed after blanching for 2.5 and 5 min. Both blanching methods showed a gradual reduction of POD and PPO activities with increasing blanching period up to 5 minutes. The experimental result is similar to Gao *et al.* (2012) where water blanching inhibits both PPO and POD effectively in sour cherry pulp.

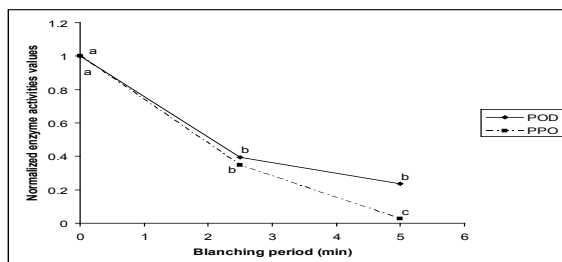


Figure 1. Normalized enzyme activities of rambutan peel extracts from different water blanching period. (POD: peroxidase; PPO: polyphenol oxidase).

^{a,b} Means within the same enzyme with different letters were significantly different ($p < 0.05$).

For steam blanching (Figure 2), results also showed both enzyme activities were decreased after blanching. There was significant differences ($p < 0.05$) between the different blanching times. Both POD and PPO demonstrated similar trends in both water and steam blanched samples where their activity significantly ($p < 0.05$) decreased after blanching compared to unblanched samples. Blanching using

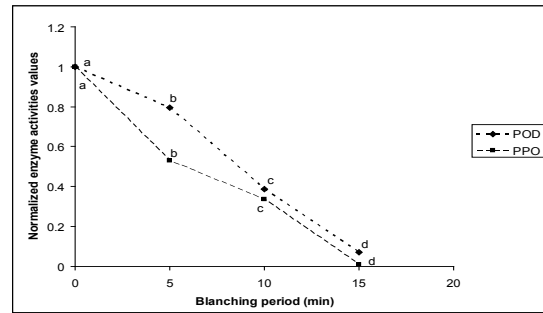


Figure 2. Normalized enzyme activities of rambutan peel extracts from different steam blanching period. (POD: peroxidase; PPO: polyphenol oxidase).

^{a,d} Means within the same enzyme with different letters were significantly different ($p < 0.05$).

steam produced a more drastic reduction in POD and PPO activities compared to water blanching after 5 minutes which may be due to the more efficient heat transfer.

DPPH assay and total phenolic content

As shown in Table 1, water blanched rambutan peel did not show any significant difference ($p < 0.05$) in antioxidant capacity after 2.5 and 5 mins of blanching. This trend has been observed before (Ismail and Wee, 2004), where cabbage did not show significant difference ($p < 0.05$) between the fresh sample with sample blanched for 5 and 10 minutes. Hunter and Fletcher (2002) have indicated that boiling processes at above 95°C would decompose the antioxidant components of vegetables. However, studies have shown that some of the antioxidant components in vegetables remain unchanged after cooking (Ewald *et al.*, 1999). Table 1 also shows the total polyphenol content of water blanched samples. Although a decreasing trend was observed when blanching was carried out up to 5 mins, these changes were not statistically significant.

Table 1. Normalized antioxidant activity (DPPH), total polyphenol content (TPC) and total anthocyanin of extracts from rambutan peel blanched using different method and time

Treatment	Time (min)	DPPH	TPC	Anthocyanin
Water blanching	0	1.00 ± 0.01 ^a	1.00 ± 0.01 ^a	1.00 ± 0.01 ^b
	2.5	0.97 ± 0.21 ^a	0.97 ± 0.10 ^a	1.39 ± 0.03 ^a
	5	0.97 ± 0.14 ^a	0.85 ± 0.15 ^a	1.04 ± 0.04 ^b
Steam blanching	0	1.00 ± 0.01 ^a	1.00 ± 0.01 ^a	1.00 ± 0.01 ^c
	5	0.98 ± 0.26 ^a	1.00 ± 0.35 ^a	1.64 ± 0.04 ^a
	10	0.87 ± 0.31 ^a	1.03 ± 0.40 ^a	1.47 ± 0.07 ^b
	15	0.93 ± 0.40 ^a	0.98 ± 0.42 ^a	1.57 ± 0.07 ^{ab}

^{a,b} Means of the same treatment with different superscripts were significantly different ($p < 0.05$).

Steam blanching by autoclaving the rambutan peel (Table 1) also did not result in any significant difference in antioxidant capacity during 0 to 15 minutes of blanching. This result showed the same trend as a study by Jiratanan and Liu (2004) where antioxidant activity of canned beet did not change significantly after heating treatment which is retorting.

Table 2. Normalized degree of lightness (L), degree of redness (a) and chroma (c) values of rambutan peel extracts from different water blanching period

Attribute	Blanching period	Time (min)					
		0	2	4	6	8	10
L	0	1.000±0.00 ^a	0.997±0.00 ^a	0.989±0.01 ^a	0.987±0.01 ^a	0.987±0.01 ^a	0.987±0.01 ^a
	2.5	1.000±0.00 ^a	0.999±0.01 ^a	0.997±0.01 ^a	0.998±0.01 ^a	0.997±0.01 ^a	0.997±0.01 ^a
	5	1.000±0.00 ^a	1.000±0.00 ^a	1.000±0.00 ^a	1.000±0.02 ^a	1.000±0.01 ^a	1.000±0.02 ^a
a	0	1.000±0.00 ^a	0.965±0.02 ^a	0.890±0.10 ^a	0.865±0.11 ^a	0.865±0.10 ^a	0.860±0.10 ^a
	2.5	1.000±0.00 ^a	0.939±0.02 ^b	0.926±0.02 ^b	0.918±0.03 ^b	0.918±0.03 ^b	0.910±0.02 ^b
	5	1.000±0.00 ^a	0.915±0.10 ^a	0.908±0.10 ^a	0.906±0.10 ^a	0.898±0.10 ^a	0.892±0.10 ^a
c	0	1.000±0.00 ^a	0.994±0.00 ^a	0.962±0.05 ^a	0.946±0.04 ^a	0.950±0.04 ^a	0.946±0.04 ^a
	2.5	1.000±0.00 ^a	0.983±0.02 ^a	0.971±0.02 ^a	0.967±0.02 ^a	0.967±0.02 ^a	0.963±0.02 ^a
	5	1.000±0.00 ^a	1.008±0.06 ^a	1.001±0.06 ^a	1.003±0.05 ^a	0.997±0.06 ^a	0.995±0.05 ^a

^{a-b} Means of same attribute with different superscripts were significantly different ($p < 0.05$)

Table 3. Normalized degree of lightness (L), degree of redness (a) and chroma (c) values of rambutan peel extracts from different steam blanching period

Attribute	Blanching period	Time (min)					
		0	2	4	6	8	10
L	0	1.000±0.00 ^a	1.000±0.00 ^a	1.000±0.00 ^a	1.001±0.01 ^a	1.000±0.00 ^a	0.998±0.00 ^a
	5	1.000±0.00 ^a	0.997±0.00 ^a	0.998±0.00 ^a	0.996±0.00 ^a	0.998±0.00 ^a	0.998±0.01 ^a
	10	1.000±0.00 ^a	1.001±0.00 ^a	1.002±0.00 ^a	1.002±0.01 ^a	1.002±0.00 ^a	1.003±0.00 ^a
	15	1.000±0.00 ^a	0.995±0.00 ^a	0.997±0.01 ^a	0.996±0.01 ^a	0.996±0.01 ^a	0.994±0.00 ^a
a	0	1.000±0.00 ^a	0.995±0.02 ^a	0.997±0.03 ^a	0.987±0.02 ^a	0.987±0.02 ^a	0.983±0.01 ^a
	5	1.000±0.00 ^a	0.971±0.02 ^{a b}	0.967±0.01 ^{b c}	0.957±0.02 ^{b c}	0.949±0.02 ^{b c}	0.937±0.01 ^c
	10	1.000±0.00 ^a	0.909±0.10 ^a	0.901±0.10 ^a	0.893±0.10 ^a	0.895±0.10 ^a	0.883±0.10 ^a
	15	1.000±0.00 ^a	0.951±0.10 ^a	0.934±0.10 ^a	0.928±0.10 ^a	0.924±0.10 ^a	0.926±0.10 ^a
c	0	1.000±0.00 ^{a b}	0.997±0.01 ^{a b}	1.01±0.02 ^a	0.989±0.01 ^{a b}	0.986±0.02 ^{a b}	0.983±0.01 ^b
	5	1.000±0.00 ^a	0.981±0.01 ^{a b}	0.983±0.01 ^{a b}	0.976±0.01 ^{a b c}	0.966±0.02 ^{b c}	0.956±0.02 ^c
	10	1.000±0.00 ^a	0.928±0.06 ^a	0.923±0.06 ^a	0.916±0.06 ^a	0.925±0.06 ^a	0.912±0.06 ^a
	15	1.000±0.00 ^a	0.984±0.02 ^a	0.969±0.02 ^a	0.966±0.02 ^a	0.966±0.02 ^a	0.965±0.01 ^a

^{a-c} Means of same attribute with different superscripts were significantly different ($p < 0.05$)

Effect of thermal processing on antioxidant activity of vegetables has been investigated and the change of antioxidant activity varies with the food. A similar trend was observed for total polyphenol contents where there was no significant difference ($p < 0.05$) between the different blanching periods during steam blanching.

Total Anthocyanin Estimation

Based on Table 1, water blanched rambutan peel showed a significantly difference ($p < 0.05$) in total anthocyanin after 2.5 minutes of blanching using water. The higher anthocyanin was in agreement with the results reported by Rossi *et al.* (2003). Blanching of blueberry fruits was also reported to induce a higher anthocyanin retention (23% instead of 12%) which is twice compared to unblanched sample. The increased anthocyanin content after 2.5 min of water blanching could be due to the increase of rambutan peel permeability after being exposed to the hot blanching medium resulting in greater extraction

yield (Kalt *et al.*, 2000). Samples blanched using water for 5 minutes showed a significantly lower total anthocyanin compared to samples blanched for 2.5 mins. This could be due to the longer time the samples were exposed to the high temperature causing anthocyanin to leach into the blanching water.

As indicated in Table 1, steam blanching for 5 mins showed a significantly higher ($p < 0.05$) total anthocyanin compared to 0 min which is similar to water blanching. Increasing the blanching period to 10 mins caused a reduction in anthocyanin. Further increase of the blanching period to 15 mins did not show any significant difference on total anthocyanin. The changes for anthocyanin observed during steam blanching may also be due to the reactions as suggested for water blanching.

Peel extract colour

As extracts may be on the possible products of rambutan peel, the peel extract color of blanched samples was investigated. In general, the rambutan peel

extract colour changed from dark to horseradish and finally turned into brownish red. Table 2 summarizes normalized values of the color measurements performed on the rambutan peel extracts. For degree of lightness (L) and chroma (c), it was found that there was no significant difference ($p < 0.05$) during 10 minutes observation between all samples after water blanching for 2.5 and 5 mins. The degree of redness (a) also did not show any significant difference ($p < 0.05$) between unblanched and samples blanched for 5 minutes. However, samples blanched for 2.5 minutes showed a significant reduction ($p < 0.05$) in degree of redness after 2 minutes of observation and it remained low until 10 minutes. No significant differences were observed for degree of yellowness for all samples.

Based on Table 3, the degree of lightness (L) did not show any significant difference ($p < 0.05$) for all steam blanched samples during 10 minutes of observation. As for degree of redness (a), it also did not show any significant difference ($p < 0.05$) between unblanched samples and samples blanched for 10 and 15 minutes. Samples blanched for 5 minutes significantly decreased ($p > 0.05$) in degree of redness after 4 and 10 minutes observation. In steam blanching, degree of chroma (c) did not show any significant difference for all samples except for samples blanched for 5 minutes. Samples blanched for 5 mins significantly ($p < 0.05$) decreased after 10 minutes observation.

Conclusion

The present study demonstrated that water and steam blanching significantly reduced ($p < 0.05$) PPO and POD activities of rambutan peel. Both water and steam blanching period up to 5 and 15 minutes did not significantly affect the antioxidant activity as well as the total phenol content. However, colour loss was observed for both water and steam blanching. Total anthocyanin increased significantly ($p < 0.05$) in rambutan peel extracts when it was blanched for 2.5 minutes and decreased after 5 minutes blanching for both water and steam blanching.

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