

Original Research Article

Enzymatic Browning in *Artocarpus odoratissimus* (Terap) Fruits

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Abstract: Enzymatic browning, which intensifies in response to tissue stress, remains a major limitation in the food industry as it adversely affects the visual quality and consumer acceptance of products. *Artocarpus odoratissimus*, a climacteric fruit rich in polyphenols, possesses a shorter shelf-life and is highly perishable, undergoing significant changes in tissue softening and browning. The objective of the present study is to determine the degree of browning and the browning-related enzymes in *A. odoratissimus* fruit during storage. The mature fruits were harvested and stored at ambient temperature (25°C) before analyses. Based on observations, terap fruits stored at 25°C exhibited surface browning by day 4, progressing to dark browning and deterioration by day 8. The higher colour changes (ΔE) were obtained in fruit peel stored under 25°C, on day 4 at 23.70 ± 1.39 and were twice as high on day 8 at 44.12 ± 1.95 . Meanwhile, the flesh colour transitions from slightly yellow on Day 4 (28.26 ± 2.81) to entirely yellowish on Day 8 at 35.78 ± 0.36 due to oxidation. The degree of browning for the ripened fruit peel was 0.37 ± 0.01 and increased slightly from day 4 (0.41 ± 0.02) to day 8 (0.47 ± 0.01). Similarly, the flesh increased from 0.14 ± 0.01 to day 4 at 0.21 ± 0.01 and day 8 0.24 ± 0.01 . For the browning enzymes, the phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) significantly increased in response to the storage period for peel and flesh. The browning enzyme in the peel was highly correlated with the degree of browning and PAL ($R^2=0.859$) and PPO ($R^2=0.858$). As for the flesh, the degree of browning was highly correlated with PAL ($R^2=0.766$), respectively. This browning quantification can be used as a comprehensive insight to prevent browning in terap by treating them with anti-browning solutions, to enhance the fruits' quality, shelf life, and marketability.

Keywords: Enzymatic browning, shelf, terap, indigenous fruit, Sarawak Borneo

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1. Introduction

Enzymatic browning is the common limiting factor contributing to 50% quality deterioration of fresh fruits and vegetables (Moon *et al.*, 2020). It is an oxidation reaction that often occurs in fruits and vegetables, affecting the fresh fruits and vegetables' cosmetic looks, turning them brown. This phenomenon occurs due to polyphenol oxidase (PPO) and peroxidase (POD) enzyme activities (Zhu *et al.*, 2022), where it interacts with the release of phenolic substrates (Arnold & Gramza-Michalowska, 2022). The authors also highlight that the enzyme PPO facilitates the hydroxylation of monophenols in the presence of oxygen. At the same time, POD, an oxidoreductase capable of withstanding high temperatures, catalyses the oxidation of phenolic attributes via hydrogen peroxide (H_2O_2) to produce melanin, the ultimate dark-pigment product. In response to the surging demand for *A. odoratissimus*, also known as terap fruit, across the Borneo Islands, including Sarawak, Sabah, Brunei, and Kalimantan, recent studies indicate a consumption rate of 27% (Kasron *et al.*, 2020). Despite their nutritional richness, these fruits are underutilised due to their climacteric nature and high perishability, leading to flavour loss, tissue softening, and browning, thus limiting their shelf life. However, a notable gap remains in understanding the enzymatic browning processes associated with these fruits. Therefore, the present study aims to elucidate the degree of browning and the underlying enzymatic activities in *A. odoratissimus* fruit during storage. This study could provide insights into the mechanism of fruit browning in *A. odoratissimus* and guidance on developing appropriate post-harvest technology, particularly in evaluating anti-browning techniques to extend shelf-life and improve fruit quality and marketability.

2. Materials and Methods

2.1. Sample Collection and Treatment

The naturally ripened *A. odoratissimus* fruits were collected from Kampung Penan, Muslim Batu 10 in Bintulu, Sarawak. The fruits were brought directly to the laboratory for further analysis. Fruits free from pests and disease were selected and cleaned to remove the dirt from the fruit's surfaces. Then, the fruits were stored at ambient temperature under 25 °C, 70% relative humidity for 2 weeks. The fruit data were collected every 4-day interval to evaluate the browning incidence.

2.2. Visual Inspection

The colour of the fruit was evaluated based on its ripeness using a grading system that spans from 0 to 5 (Hulme, 1971). The fruit is classified on a scale of 0 to 5, where 0: light green, 1: green, 2: green with a yellowish hue, 3: green with brownish, 4: entirely brown, and 5: dark brown. Meanwhile, the pulp is assessed on a scale of 0 to 5, where 0: clear white, 1: slightly whitish-yellow, 2: equally white and yellow, 3: entirely yellowish, 4: somewhat brownish, and 5: altogether brown.

2.3. CIE Lab System Parameters

The study aimed to assess enzymatic browning by examining the peel and pulp of fruits on different storage days. A Hunter Lab colour meter (CHN SPEC Colour Meter Pro, China) was used to determine the surface colour of the fruits, and the colour coordinates were recorded. The total colour difference was then computed using the given equation:

$$\Delta E = \sqrt{(L^*_f - L^*_i)^2 + (a^*_f - a^*_i)^2 + (b^*_f - b^*_i)^2} \quad (2)$$

The variables L^* , a^* , and b^* express lightness-darkness, redness-greenness, and yellowness-blueness, respectively. The subscripts f and i represent the final and initial values, as indicated in a study by Nambi *et al.* (2015).

2.4. Degree of Browning Analysis

The degree of browning in *A. odoratissimus* fruits was determined using a modified protocol of Brandelli and Lopes (2005). Ten grams of the sample were homogenised in a beaker containing 20 mL of distilled water, followed by centrifugation at 800 x g for 10 minutes. After an hour of homogenization, 1.5 mL of ethanol was added to 1.0 mL of the supernatant in the centrifuged sample, and the resulting mixture was shaken and centrifuged again at 800 x g for 10 minutes. The degree of browning was determined by measuring the absorbance at 440 nm using a UV-VIS spectrophotometer. The experiment was performed in triplicate.

2.5. Sample Extractions

The extraction method outlined by Brandelli and Lopes (2005) was utilised with minor adjustments. Ten grams of peel and pulp were weighed and homogenised in an

ice-cold 0.2 M phosphate buffer at a pH of 7.0, adding 1 M NaCl. The resulting supernatants were centrifuged at 10,000 x g for 20 minutes at 4°C.

2.6. Phenylalanine Ammonia-Lyase (PAL) Analysis

The method developed by Peixoto *et al.* (1999) was used to determine phenylalanine ammonia-lyase activity (EC 4.3.1.5). The enzymatic reaction was initiated by incubating a mixture of 1.0 mL extract, 1.0 mL of 0.2 M borate buffer, and 1.0 mL of phenylalanine at 36°C for 60 minutes. The reaction was stopped by adding 0.1 mL of 6 N HCl to the mixture, and the absorbance was measured at 290 nm using a spectrophotometer. The results were expressed in unit moles min⁻¹.

2.7. Polyphenol Oxidase (PPO) Analysis

The PPO-EC 1.14.18.1 activity was determined using a method by Cano *et al.* (1997). McIlvaine buffer (0.1 M citrate-0.2 M phosphate) was mixed with the sample and homogenised for 3 minutes. The homogenate was then vacuum-filtered and centrifuged at 6522 g for 30 minutes at a temperature of 4°C. The reaction mixture contained 0.3 mL of crude extract, 1.75 mL of 0.05 M sodium phosphate buffer (pH 6), and 0.05 mL of 0.1 M catechol solution. The mixture was incubated for 30 minutes at 30°C. The enzymatic reaction was stopped by adding 0.7 mL of 5% sulfuric acid, and the absorbance was measured using a spectrophotometer at 395 nm. The results were expressed in µmoles of catechol transformed min⁻¹ g⁻¹.

2.8. Peroxidase (POD) Analysis

The POD-EC 1.11.1.9 method developed by Rossi *et al.* (1997) was used to determine peroxidase activity levels. The enzymatic extract was diluted in deionised water, and a mixture of 0.2 mL of the extract with 0.5 mL of 29% hydrogen peroxide solution and 0.5 mL of aminoantipyrine and phenol solution was used. The spectrophotometer was employed to obtain readings at 505 nm, and subsequently, the results were expressed in units of µmoles of decomposed H₂O₂ min⁻¹ g⁻¹.

2.9. Statistical Analysis

The data was analysed using SAS 9.4, employing single-factor analysis of variance (ANOVA) to assess mean comparisons. The post hoc Tukey's test ($p < 0.05$) was utilised to compare the degree of browning and enzymatic activity between the peel and pulp of *A.*

odoratissimus. The Pearson Correlation Coefficient was computed to model the relationship between the degree of browning and enzymatic browning activities.

3. Results and Discussion

3.1. Peel and Pulp Colour Change During Storage

Consumers' perception of quality and acceptance of food products is highly influenced by colour, which is a crucial parameter in determining the quality of the product. Various phytochemicals, including carotenoids, can alter the colour of fruits. These compounds impact the L^* , a^* , b^* , and ΔE values for colour change. After 2 weeks of storage, *A. odoratissimus* showed significant changes in the peel colour, as indicated in Table 1 ($p < 0.05$). The results have shown that the peel of the terap fruit, when stored for 4 days, had a full brown colour with an L^* value of 45.30 ± 0.84 , rapidly increasing to 64.70 ± 0.37 on day 8 to an intense dark brown. The a^* value of the peel also increased during the storage period. Similarly, the pulp also showed a similar trend, with the L^* value increasing from 56.70 ± 1.05 on day 0 to 53.06 ± 2.84 on day 4, then to 60.80 ± 0.40 on day 8. The a^* value of the pulp also increased slightly, while the higher b^* value indicated a more intense yellow colour. The fruit peel and pulp's total colour changes (ΔE) were further analysed. The fruit peel changed from green with slight brown to dark brown due to pigment loss (Ismail *et al.*, 2023) with a total change of 23.88 ± 0.69 on day 4, and 41.61 ± 0.93 on day 8. On the other hand, the pulp colour changed from clear white on day 4 (28.26 ± 2.81) to slightly brown (35.78 ± 0.36) in which the present study by Venkatachalam and Meenune (2012) has revealed that the activation of polyphenol oxidase (PPO) enzyme is facilitated by the oxidation of phenolic compounds in response to the air that is induced because of the surface damage in fruits. Besides, the decreased POD in the fruit peel during day 4 and pulp during day 8 was due to the peroxidase enzyme contributing to the development of adverse ripening traits in fruits, particularly by initiating browning through phenolic compound reduction. Not only that, previous research by Lavanya *et al.* (2019) found that ethylene plays a significant role in the development of fruits, affecting both the composition of the tissue and the degreening process. When the ripening process is triggered by ethylene from the surroundings, there is a quick alteration in colour, which can be seen in Table 1, and this change intensifies during the storage period. This finding showed that temperature reduces ethylene production and affects the fruit's colour, degrading and increasing the fruit's browning.

Table 1. Peel and pulp colour change in *A. odoratissimus* fruits during postharvest storage

Storage	Parameter	Natural ripen	Storage Days		
		Day 0	Day 4	Day 8	
Peel	Visual inspection				
	Code	3-Green with slightly brown	4-Full brown	5-Dark brown	
	L^*	53.36 ± 0.28^b (52.80-53.70)	42.10 ± 0.76^c (40.90-43.500)	64.70 ± 0.38^a (64.00-65.30)	
	a^*	10.90 ± 0.40^b (10.10-11.40)	14.00 ± 0.47^{ab} (13.10-14.70)	16.33 ± 1.21^a (14.70-18.70)	
	b^*	13.20 ± 0.61^a (12.10-14.20)	14.23 ± 0.58^a (13.20-15.20)	14.90 ± 0.47^a (13.20-15.20)	
	ΔE		23.68 ± 0.73^b (22.80-25.13)	41.32 ± 1.07^a (40.16-43.45)	
Pulp	Visual inspection				
	Code	2-White with slightly yellow	2-White with slightly yellow	4-Fully yellowish	
	L^*	53.03 ± 2.30^b (48.80-56.70)	53.07 ± 2.85^b (48.50-58.30)	60.80 ± 0.40^a (60.30-61.60)	
	a^*	1.20 ± 0.12^a (1.00-1.40)	1.23 ± 0.26^a (0.80-1.70)	1.27 ± 0.35^a (0.60-1.80)	
	b^*	25.53 ± 0.94^a (24.10-27.30)	23.13 ± 0.42^b (22.30-23.60)	25.73 ± 0.99^a (24.20-27.60)	
	ΔE	27.92 ± 2.55^b (24.90-33.00)	57.93 ± 2.61^a (53.96-62.86)	66.00 ± 0.20^a (66.20-65.61)	

Different superscript alphabets denote differences in the same row at a significance level of $p < 0.05$ (ANOVA, Tukey's test). Values are given as means $\pm$ standard error, and values in parentheses are the range.

3.2. Degree of Browning

The degree of browning in *A. odoratissimus* fruits was quantified to evaluate the extent of the Maillard reaction. The findings revealed a significant increase in browning after 8-day storage period, as illustrated in Figure 1. The degree of browning in the terap peel increased significantly from 0.37 ± 0.01 to day 4 (0.41 ± 0.02) and day 8 (0.47 ± 0.01) during storage. Likewise, the pulp also increases from 0.14 ± 0.01 to day 4 in T2 (0.21 ± 0.01) and day 8 in T3 (0.24 ± 0.01). Guangsen *et al.* (2022) reported that higher temperatures promote the degree of browning to occur. According to the author, the browning incidence

in *A. odoratissimus* fruits increased during storage due to the activities of polyphenol oxidase (PPO), which reacted to oxidation and resulted in browning, as illustrated in Figure 1. A recent study by Ismail *et al.* (2023) found that storing fruits under lower temperatures can delay the fruits' ripening, prevent them from turning brown, and enhance the fruit shelf life for more than 2 weeks.

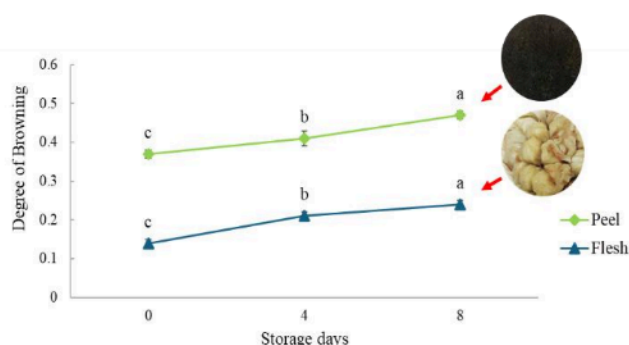


Figure 1. The enzymatic browning activities in *A. odoratissimus* fruits peel and pulp during postharvest storage

3.3. Enzymatic Activities Associated with Browning

The enzymatic activities in Figure 2 show that the *A. odoratissimus* fruits peel and pulp in PAL and PPO increase more during storage periods than POD. The phenylalanine ammonia-lyase EC 4.3.1.5 (PAL) activity (Figure 2a) in the fruit peels during the natural ripening was 368.66 ± 1.51 UE g⁻¹ min⁻¹ and increased rapidly on day 4 with 453.33 ± 1.25 UE g⁻¹ min⁻¹ to the maximum day 8 with 633.66 ± 5.17 UE g⁻¹ min⁻¹, respectively. Similarly, the pulp also increased from 291.66 ± 2.70 UE g⁻¹ min⁻¹ to day 4 (419.00 ± 1.39 UE g⁻¹ min⁻¹) and day 8 (614.33 ± 1.15 UE g⁻¹ min⁻¹). Increased phenylalanine ammonia-lyase (PAL) activity causes the release of phenolic compounds, which can act as reagents for PPO and POD enzymes (Ismail *et al.*, 2023). As for the PPO 1.14.18.1 activity (Figure 2b), the peel was observed where the values increased from 380.00 ± 3.00 UE g⁻¹ min⁻¹ to day 4 (530.00 ± 0.81 UE g⁻¹ min⁻¹) and day 8 (586.66 ± 2.33 UE g⁻¹ min⁻¹). Likewise, the pulp also recorded the same trend. According to Arnold and Gramza-Michalowska (2022), PPO catalyses the oxidation of polyphenol substances into diphenol, resulting in colour alteration and changes in the sensory and nutritional content. Meanwhile, the POD 1.11.1.9 activity (Figure 2c) in the peel for the natural ripening value was higher at 455.66 ± 0.88 but decreased on day 4 (310.33 ± 0.57 UE g⁻¹ min⁻¹); however, on day 8, the value increased slightly to 350.00 ± 2.16 UE g⁻¹ min⁻¹. While in the pulp, the POD activities value was 268.00 ± 2.00 UE g⁻¹ min⁻¹ and increased slightly on day 4 (275.66 ± 1.42) but then slightly decreased on day 8 (272.33 ± 1.74 UE g⁻¹ min⁻¹). According to research by Arnold and

Gramza-Michalowska (2022), the POD enzyme serves a crucial function as an antioxidant enzyme by effectively eliminating excess hydrogen peroxide. However, the POD activity decreased slightly in the peel, while in the pulp, the POD was increased after reaching a maximum after 8 days of storage. This change could be due to the increased lignification in the fruit tissue during the ripening process (Venkatachalam & Meenune, 2012).

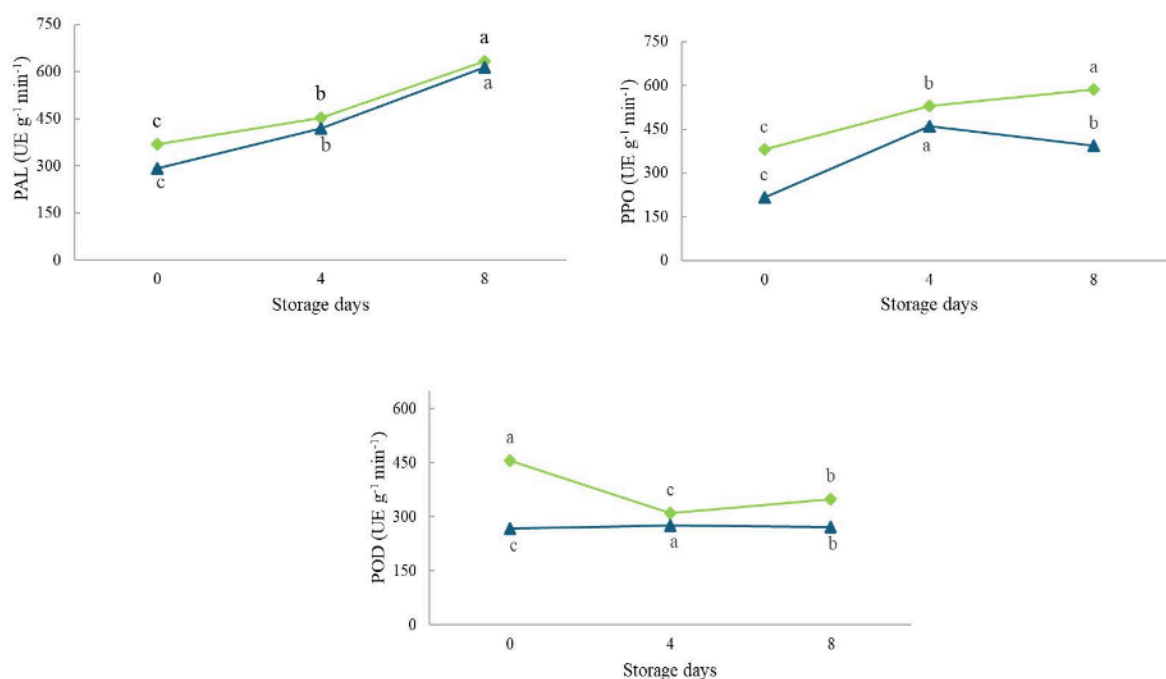


Figure 2. The enzymatic browning activities of (a) PAL, (b) PPO and (c) POD in *A. odoratissimus* fruit peel and pulp during storage

3.3. Browning Regression Associated with Enzyme Activities in the Peel

The regression analysis was carried out on the correlation attributes to determine the correlation between the degree of browning and enzymatic activities, as depicted in Figure 3. The degree of browning in the *A. odoratissimus* peels showed that there is a strong positive correlation with phenylalanine ammonia-lyase (PAL) ($R^2=0.859$) in Figure 3a and polyphenol oxidase (PPO) ($R^2=0.858$) in Figure 3b. Enzyme PAL significantly contributes to plant disease resistance by synthesising phenolic compounds that enhance plant structure and defense mechanisms (Zhang *et al.*, 2018). In this study, the increase in the PAL enzyme and the temperature and storage period cause the PPO to interact with phenolic oxidation, leading to browning incidence. This showed that PAL and PPO were the significant factors contributing to the browning appearance of *A. odoratissimus* fruits.

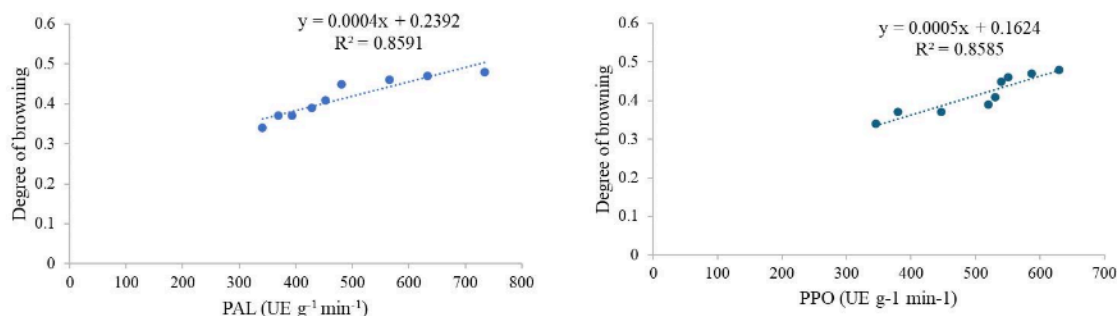


Figure 3. The regression correlation for (a) the degree of browning with PAL and (b) the degree of browning with PPO in *A. odoratissimus* fruit peel.

3.4. Browning Regression Associated with Enzyme Activities in the Pulp

The linear regression in Figure 4 shows that the *A. odoratissimus* pulp has a strong positive correlation with the degree of brown and PAL ($R^2=0.766$), respectively. This finding indicates that browning in the pulp was related to increased PAL activities due to the phenolic compound. Present research by Vitti *et al.* (2011) emphasises that PAL converts L-phenylalanine, an aromatic amino acid, into trans-cinnamic acid and free ammonia ions. This biochemical reaction is the initial step of a comprehensive range of responses that culminate in the biosynthesis of phenylpropanoids. As a result, higher PAL activity during storage was associated with the high preexisting latent level in the tissue of *A. odoratissimus* pulp, leading to browning due to the induction of the enzyme.

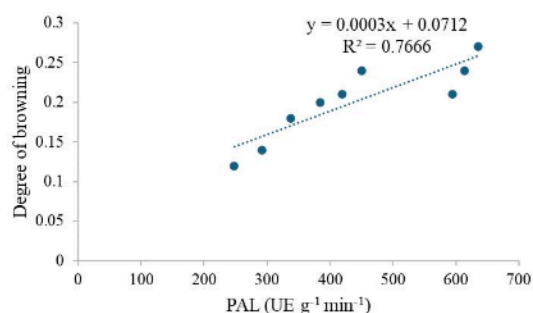


Figure 4. The regression correlation for degree of browning with PAL and PPO in *A. odoratissimus* fruit pulp

4. Conclusions

The finding revealed that information on the quantification of browning in *A. odoratissimus* fruits can be a valuable method to prevent browning. By applying anti-browning solutions to these products, their quality can be improved, their shelf life extended, and their marketability enhanced. This comprehensive approach can help to

prevent browning in terap and other produce, ultimately leading to better consumer satisfaction and increased profitability for growers and distributors.

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