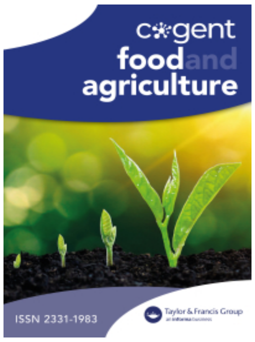

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Post-harvest storage effects on enzymatic browning, physicochemical and phytochemical characteristics of *Artocarpus odoratissimus* flesh

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ABSTRACT

The current study aimed to establish the chemical constituents, enzymatic browning, and physicochemical and phytochemical attributes of *Artocarpus odoratissimus* flesh during post-harvest ripening. This study employed a randomised complete block design (RCBD) with storage methods (with and without packaging) and temperature conditions (ambient at 25 °C and chilled at 10 °C) as the variables. After eight days of storage at 25 °C, the pH of the unpackaged fruits (control) significantly increased from 6.65 ± 0.02 in treatment 1 (T1) to 6.91 ± 0.01 by the eighth day in treatment 5 (T5). The fruit samples stored at room temperature also exhibited improved browning (0.25 ± 0.02) and phenylalanine ammonia-lyase (PAL) (667.66 ± 2.21 UE $g^{-1}min^{-1}$) and polyphenol oxidase (PPO) (527.00 ± 0.77 UE $g^{-1}min^{-1}$) enzymatic activities, particularly during days four to eight, with pronounced effects observed in packaged fruits. Meanwhile, storage at 10 °C delayed browning, documenting 0.18 ± 0.01 on the fourth day to 0.13 ± 0.01 on the 16th day. Enzymatic activities were also reduced, extending their flesh shelf life. The findings suggested that storage at 10 °C (low temperature) effectively prolonged the freshness of *Artocarpus odoratissimus* flesh. The method could be a practical approach to mitigating browning and enhancing shelf life and marketability.

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SUBJECTS
Agriculture; Food; Horticulture

1. Introduction

Artocarpus odoratissimus (*A. odoratissimus*) or terap is indigenous to Brunei, Kalimantan, Sabah, and Sarawak, collectively known as the Borneo Island (Bakar & Bakar, 2018; Karson et al., 2020). The climacteric fruit of the Moraceae family is renowned for its notably fruity smell and sweet, juicy flesh. In Sarawak, the terap fruit is typically in season from August to October and from December to January.

Given its surging demand and unique attributes, the Sarawak government aspires to capture the remarkable local and international commercial potential of *A. odoratissimus* (Ismail et al., 2023). The seasonal fruit has gained the interest of local fruit markets, contributing to the community's nutrition, food security, and income significantly (Goh et al., 2023). Terap offers health benefits to consumers, considering notable levels of phenolic compounds and excellent nutritional values (Alvarado, 2023). *A. odoratissimus* fruit flesh also possesses significant amounts of vitamins, including A, B1, B2, B3, and C, and essential minerals, such as potassium (Alvarado, 2023; Ismail et al., 2023). Furthermore, terap is potentially applicable in the functional food and nutraceutical industries as its flesh, kernel, and husk contain bioactive compounds, including phenolics, flavonoids, and antioxidants. Nevertheless, accelerated flesh browning and texture alterations limit the sweetness, juiciness, marketability, and economic value of *A. odoratissimus* fruit.

Artocarpus odoratissimus fruits are underutilised and not commercially classified (Ismail et al., 2023). The fruit is also significantly perishable and deteriorates rapidly due to the considerable moisture levels in its flesh. Moreover, post-harvest yields are limited by declining fruit quality, which results in undesirable flavour, tissue pulp softening, and surface browning. A predominant factor that restricts *A. odoratissimus* as a commodity is oxidative browning arising from flesh exposure (Ismail et al., 2023).

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Singh and Mirza (2018) revealed that enzymatic browning in fresh fruit spoilage occurs when oxygen reacts with phenolic compounds, forming dark o-quinones and polymerising into melanin. Enzymatic browning affects 50% of fresh fruit degradation, adversely impacting visual appearances due to brown pigment formations (Moon et al., 2020). The phenomenon also reduced the nutritional content and sensory qualities of fruits. Arnold and Gramza-Michałowska (2022) suggested that polyphenol oxidase (PPO) and peroxidase (POD) are vital enzymes influencing enzymatic browning. The report also indicated that phenylalanine ammonia-lyase (PAL) is the primary phenolic production-inducing enzyme via the phenylpropanoid pathway in response to stress during fruit cutting.

Although *A. odoratissimus* is gaining popularity, information on effective post-harvest storage and preservation techniques for the fruit is limited. Browning of the surfaces of the fruit surface negatively affects its appearance and reduces marketability. Furthermore, inadequate studies explore the relationship between packaging, temperature, and key enzymatic activities, such as PAL, PPO, and POD, which are known to influence browning, even though *A. odoratissimus* pulp is significantly valued. Consequently, this study aimed to evaluate the enzymatic browning activities and physicochemical changes in *A. odoratissimus* flesh under various storage conditions. This study specifically considered the effects of packaging and temperature on PAL, PPO, and POD activities.

The current study is among the first to offer comprehensive insights into the enzymatic browning processes and physicochemical alterations in the terap fruit flesh during storage. The data can also aid in extending the shelf life of the fruit and enhancing its quality and marketability by understanding the association between packaging, temperature, and enzymatic activities.

2. Materials and methods

2.1. Sample collection

The current study employed *A. odoratissimus* fruits of optimal maturity at stage 815 on the *Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie* (BBCH) scale (week 12 post-anthesis). The fruits were collected from an orchard in Sarawak, Malaysia, during peak season from December 2023 to January 2024. Defect-free fruits were immediately brought to the laboratory. Any dirt on the surfaces of the fruit samples was removed prior to further treatment.

2.2. Post-harvest treatments and observations

A total of 128 *A. odoratissimus* fruits were selected for the present study. The samples were subjected to post-harvest ripening treatments. The fruits were subjected to ambient temperatures of 23 °C–25 °C and a cool temperature of 10 °C and stored with and without packaging. The packaged fruit samples were packed in polyethylene (PE) bags with 5% poly-perforated bags with small holes. The procedures employed followed a randomised complete block design (RCBD). Each treatment consisted of 32 fruits and was evaluated over 4, 8, 12, and 16 days of storage.

2.3. Physicochemical characterisation

The current study prepared the fruit samples by opening the fruits, removing the flesh, and removing the seeds. A digital pH meter (Eutech pH 700, Germany) was employed to determine the pH of the fruit homogenate. Subsequently, the flesh was homogenised and filtered through a tea bag filter to obtain a clear juice sample to assess total soluble solids (TSS) and total titratable acidity (TTA). The TTS (°Brix) was quantified with a handheld refractometer (Juanjuan, China), while the TTA was evaluated following the AOAC 942.15 (2000) guidelines.

2.4. Enzymatic browning quantification

A visual evaluation system was employed during the colour characteristic analysis of the fruit pulp assessed in this study. A scale of 0 to 5 was applied during the assessment, where ‘0’ represented clear white flesh, ‘1’ was assigned to slightly whitish yellow samples, ‘2’ denoted equally white and yellow flesh, ‘3’ was attributed to completely yellowish samples, ‘4’ represented slightly brownish flesh, while ‘5’ was assigned to entirely brown samples. The present study also employed materials from fruits harvested under various ripening conditions during enzymatic browning evaluations. Colour coordinates on the crop surfaces were recorded with a colour reader. The procedure Nambi et al. (2015) outlined was adopted during the total colour difference (ΔE) determination.

2.5. Degree of browning

The degree of the flesh browning of the terap samples in this study was assessed per the report by Brandelli and Lopes (2005). An ultraviolet-visible (UV-Vis) spectrophotometer [UNICO, Fairfield, New Jersey (NJ), United States of America (USA)] was employed. The absorbances of the samples were evaluated at 440 nm.a

2.6. Enzymatic activities

Firstly, the pulp samples were weighed before homogenising in a cold 0.2 M phosphate buffer. The buffer was adjusted to pH 7.0 with 1 M sodium chloride (NaCl). Subsequently, the supernatant was centrifuged at 10,000 × g and 4 °C for 20 mins. After storing the supernatant at 4 °C, the samples were assessed for PAL, PPO, and POD levels.

The current study adopted the methodology described by Ismail et al. (2023) to determine the PAL (EC 4.3.1.5) levels of the fruit samples. Firstly, absorbances at 320 nm were established with a spectrophotometer, and outcomes in moles min^{-1} were recorded. Meanwhile, the guidelines reported by Cano et al. (1997) were employed to assess the PPO (EC 1.14.18.1) amounts in the terap samples. The PPO activities were established at 395 nm and the outcomes were recorded in µmol, which was converted to catechol min^{-1} g^{-1}. This study also applied the POD-EC 1.11.1.9 method developed by Rossi et al. (1997). The readings were determined at 505 nm and expressed in µmol of decomposed H_2O_2 min^{-1}.

2.7. Phytochemical analysis

For phytochemical assessments, the current study mixed 50 g of the flesh samples with 100 mL of 75% ethanol. The solution obtained was heated at approximately 80 °C for 5 mins. Another 100 mL of 75% ethanol was added to enhance the extraction of phytochemical compounds before the solution was heated for another 10 min. Subsequently, the extract volume was adjusted to 250 mL per the protocol established by Brandelli and Lopes (2005) and was employed during phytochemical quantification.

In this study, the indophenol titration approach (Method 974.29, AOAC, 2000) was adopted to establish the ascorbic acid contents of the samples. The total phenolic content (TPC) was quantified spectrophotometrically according to the Folin-Ciocalteu method (Ramaiya et al., 2021). Absorbances were documented at 740 nm in µg gallic acid equivalent (GAE) g^{-1}.

During chlorogenic acid (CA) level assessments, this study applied a standard curve (10–50 µg g^{-1}). The results were recorded in µg chlorogenic acid equivalent g^{-1} (Brandelli & Lopes, 2005). Spectrophotometric assessment was also employed for total flavonoid content determination (Ismail et al., 2023). A calibration curve was produced with standard quercetin and the total flavonoid concentration was recorded in µg QE g^{-1}.

2.8. Statistical analysis

The current study performed experiments in triplicates. Analytical evaluations were conducted with the SAS version 9.4 software. Means comparisons were also performed through a single-factor analysis of variance (ANOVA). Meanwhile, the degree of browning, enzymatic activities, and phytochemical content mean values of the *A. odoratissimus* flesh were established with post hoc Tukey's test ($p < 0.05$). The Pearson correlation coefficient was also calculated utilising the raw data, determining the association between browning activities and phytochemical levels. The relationship model was obtained with OriginLab Version 10.1.

3. Results and discussion

3.1. pH, TSS, and TTA

The results indicated that the pH, TTS, and TTA of the *A. odoratissimus* fruit flesh increased significantly when stored under different conditions (Table 1). The pH values of the control samples ($p < 0.05$) increased considerably, from 6.65 ± 0.02 on Day 4 (T1) to 6.91 ± 0.01 on Day 8 (T5). Similarly, the pH values of the packaged samples rose from 6.05 ± 0.02 to 6.45 ± 0.01 during the same period.

The rise in pH is associated with biochemical reactions that convert organic acids and complex compounds into sugars, serving as energy reserves for metabolic processes during ripening (Al-Dairi et al., 2021; Tolasa et al., 2021). The phenomenon might be accelerated by high temperatures and humidity in ambient conditions, elevating pH during storage. Nevertheless, the findings contrasted with the outcomes observed by Buthelezi and Mafeo (2024). The report noted a higher pH of avocados packed in LDPE during storage than the control.

The fruit samples stored under cooler temperatures (CT) documented diminished pH from 6.94 ± 0.02 to 6.03 ± 0.01 on Days 4 and 8. On Days 12 and 16, the pH levels slightly rose to 6.16 ± 0.01 and 6.65 ± 0.01, respectively. Meanwhile, packaged fruits exhibited only a marginal pH alteration, from 6.91 ± 0.01 on the fourth day to 6.90 ± 0.02 on the 16th day. The data suggested that storage at lower temperatures delays the pH increase rate, potentially

Table 1. Chemical compositional (pH, TSS-total soluble solid, and TTA- total titratable acidity) changes during different post-harvest treatments of *Artocarpus odoratissimus* fruits flesh.

Storage duration	Treatments		pH	TSS (°Brix)	TTA (%)
Freshly harvested	T0	Natural ripening	6.53 ± 0.01^{c} (6.52–6.55)	12.16 ± 0.72^{b} (11.00–13.50)	0.16 ± 0.03^{e} (0.10–0.20)
Day 4	T1	RT-Control	6.65 ± 0.02^{b} (6.60–6.69)	13.33 ± 1.20^{b} (11.00–14.00)	0.16 ± 0.03^{e} (0.10–0.20)
	T2	RT-Packaging	6.05 ± 0.02^{f} (6.01–6.09)	14.16 ± 0.44^{b} (13.50–15.00)	0.23 ± 0.03^{de} (0.20–0.30)
	T3	CT-Control	6.94 ± 0.02^{a} (6.91–6.96)	15.66 ± 2.58^{b} (10.50–18.50)	0.23 ± 0.03^{de} (0.20–0.30)
	T4	CT-Packaging	6.91 ± 0.01^{a} (6.89–6.93)	15.83 ± 0.16^{b} (15.50–16.00)	0.20 ± 0.05^{de} (0.10–0.30)
Day 8	T5	RT-Control	6.91 ± 0.01^{a} (6.90–6.93)	22.66 ± 0.66^{a} (22.00-23.50)	0.26 ± 0.03^{de} (0.20–0.30)
	T6	RT-Packaging	6.45 ± 0.01^{bc} (6.43–6.46)	22.67 ± 0.44^{a} (22.00–23.50)	0.40 ± 0.05^{bcd} (0.30–0.50)
	T7	CT-Control	6.03 ± 0.01^{f} (6.01–6.05)	23.33 ± 0.33^{a} (23.00–24.00)	0.36 ± 0.03^{cde} (0.30–0.40)
	T8	CT-Packaging	6.40 ± 0.01^{d} (6.38–6.44)	22.83 ± 0.44^{a} (22.00–23.50)	0.53 ± 0.06^{abc} (0.40–0.60)
Day 12	T9	CT-Control	6.16 ± 0.01^{e} (6.14–6.20)	23.00 ± 0.57^{a} (22.00–24.00)	0.60 ± 0.07^{ab} (0.50–0.70)
	T10	CT-Packaging	6.86 ± 0.01^{a} (6.84–6.89)	24.33 ± 0.33^{a} (24.00–25.00)	0.63 ± 0.06^{a} (0.60–0.70)
Day 16	T11	CT-Control	6.65 ± 0.01^{b} (6.62–6.68)	24.66 ± 0.88^{a} (23.00–26.00)	0.63 ± 0.03^{a} (0.60–0.70)
	T12	CT-Packaging	6.90 ± 0.02^{a} (6.87–6.94)	24.16 ± 0.60^{a} (23.00–25.00)	0.73 ± 0.03^{a} (0.70–0.80)

Different superscript alphabets indicate differences in the same column at $p < 0.05$ (ANOVA, Tukey's test). Values are given as means ± standard error and values in parenthesis are the range.
Control-without packaging, RT: room temperature, and CT: cool temperature.

due to the less acid produced from sugar breakdown (Al-Dairi et al., 2021; Buthelezi & Mafeo, 2024; Gupta & Jain, 2014).

In this study, the TSS demonstrated consistent increments across all treatments during storage. Nonetheless, the packaged samples stored at room temperature (RT) recorded the most substantial rise, from 14.16 ± 0.44°Brix to 22.66 ± 0.66°Brix during Days 4 to 8. The increase might be attributed to reduced oxygen exchange and moisture loss and the higher temperature during storage, accelerating metabolic activity. During the same period, the unpackaged control fruits documented a similar trend, rising from 13.33 ± 1.20°Brix to 22.66 ± 0.66°Brix. The outcome was consistent with the rapid sugar metabolism and breakdown at elevated temperatures.

The more rapid elevation of TSS in fruits stored at ambient temperatures than CT observed in this study aligns with the findings reported by Galvez et al. (2018). Biochemical reactions in jackfruit pulps are stimulated by higher storage temperatures. The living tissues continued respiration and consuming sugars, which increased TSS levels. Similar outcomes were also reported by studies employing mango (Gupta & Jain, 2014). Comparable TSS trends in the fruits stored at ambient and cool temperatures were observed.

The TSS of the fruit samples stored at CT increased from 15.66 ± 0.66°Brix on Day 4 to 24.66 ± 0.88°Brix on Day 16. During identical periods, the TSS levels of the packaged samples also documented a similar pattern, rising from 15.83 ± 0.16°Brix to 24.16 ± 0.60°Brix. In another study, sweet peppers had improved TSS throughout storage (Sattar et al., 2019). The findings suggested that CT delays the ripening process, resulting in sugar content preservation and slowed metabolic activities, supporting the hypothesis that biochemical reactions are reduced at lower temperatures during storage.

All terap samples evaluated exhibited notable increments during storage. The TTA levels of the packaged terap samples rose from 0.23 ± 0.03% to 0.40 ± 0.05% on the fourth to the eighth day, which was the most significant among the samples. Anaerobic respiration induced by limited oxygen exchange might explain the observations, which resulted in specific organic acid accumulation. The acids are produced in the form of sugars and metabolised, increasing the TTA levels (Bambalele et al., 2023; Kassim & Workneh, 2020).

The unpackaged terap fruit samples documented slower TTA increment rates than the packaged fruits. On Day 4 (T1), the samples recorded 0.16 ± 0.03% of TTA before elevating to 0.26 ± 0.03% on Day 8 (T5) when stored at 25 °C. The terap fruits stored at ambient temperature also had improved TTA amounts. The accumulation of organic acids due to accelerated respiration and ripening contributed to the findings. The TTA oxidation also accelerates during storage and increases pH (Hussain et al., 2017). In another study, Kassim and Workneh (2020) recorded rapid increments of TTA levels in Hass avocados stored at ambient temperatures.

From the fourth (T4) (0.20 ± 0.05%) to the 16th (0.73 ± 0.03%) (T12) day, significantly improved TTA was recorded by the packaged fruits stored at 10 °C. The unpackaged terap samples also followed a similar trend, recording TTA of 0.23 ± 0.03% and 0.63 ± 0.03%. The lower storage temperature might have delayed the metabolic processes influencing TTA activities throughout the storage period.

Pectin disintegration might trigger acidity enhancement during storage. The process stimulates cell walls to release galacturonic acid, improving TTA and TTS levels (Prathibha et al., 2019). During ripening, organic acids are frequently degraded and converted into sugars or utilised during respiration (Bambalele et al., 2023; Zheng et al., 2021). Higher storage temperatures accelerate the acid breakdown and elevate pH. Meanwhile, CT slows metabolic reactions, preserving TTA and TSS.

3.2. Flesh colour

Colour is a vital factor in customers assessing the acceptability of a fruit. Table 2 lists the effects of storage duration and packaging on total colour alterations (ΔE), chroma (C*), and hue angle (H°) values of the fruit samples evaluated in this study. The colour of the *A. odoratissimus* fruits stored at ambient temperature demonstrated significant quality diminishment. The samples completely deteriorated by the eighth day of storage ($p < 0.05$). The decline was attributed to the accelerated metabolic and enzymatic activities associated with higher temperatures, which commonly enhance enzymatic browning.

A higher total colour change (ΔE) was observed with the packaged fruit flesh stored at 25 °C on Day 4 (30.51 ± 2.04), whereas the unpackaged fruits documented a slight elevation on Day 8 (35.81 ± 2.82). The packaged *A. odoratissimus* fruit flesh stored under ambient temperature turned slightly yellow from opaque white before turning slightly brown throughout the storage period. Conversely, the unpackaged samples recorded a slower ΔE increase, ranging from 28.26 ± 2.81 to 35.78 ± 0.36.

Browning is attributable to enzymatic oxidative reactions. Sidhu et al. (2023) suggested that internal browning of fruit flesh during storage might be influenced by temperature-induced senescent breakdown, resulting in membrane integrity loss. Finally, the fruit flesh would exhibit altered fluidity, decompartmentalization, and cell wall fracturing.

The terap fruit samples stored at 10 °C had less colour change on Days 4 and 16 than at ambient temperature, recording 18.48 ± 0.36 and 20.81 ± 1.33, respectively. A similar trend was observed in the packaged fruits, which documented 18.98 ± 0.55 and 25.62 ± 2.19 on identical periods. The lower temperatures delayed the flesh browning due to slowed senescent breakdown throughout storage, indicating that CT can inhibit the browning of flesh, allowing superior shelf lives of fruits to be marketed.

The unpackaged and packaged fruits stored at 25 °C in this study recorded significant chroma (C*) values on Day 8. Nevertheless, the chroma value of the packaged samples was higher, 26.08 ± 0.47 (T6), than the control, which recorded 24.49 ± 0.37 (T5). The CT delayed the chroma alteration for both fruits during storage. The observations revealed that the ripening period and packaging affect flesh colour,

Table 2. Fruit colour changes of *Artocarpus odoratissimus* fruits flesh at different postharvest treatments.

	Room temperature (RT)			Cooling temperature (CT)		
Storage duration	Day 4	Day 8	Day 4	Day 8	Day 12	Day 16
Without packaging						
Scale	1-White with slightly yellow	3-Fully yellowish	1-White with slightly yellow	1-White with slightly yellow	1-White with slightly yellow	1-White with slightly yellow
						
Colour changes	28.26±2.81^{bc}	35.78±0.36^{a}	18.48±0.36^{f}	21.02±1.51^{def}	19.19±0.85^{f}	20.81±1.33^{ef}
(ΔE)	(23.48–33.22)	(35.21–36.46)	(17.92–19.18)	(18.68–23.85)	(17.82–20.77)	(18.62–23.22)
Chroma	23.06±0.38^{bc}	25.90±0.97^{a}	21.23±0.54^{c}	22.23±1.18^{bc}	22.50±0.57^{bc}	23.16±1.07^{bc}
(C*)	(22.33–23.66)	(24.27–27.64)	(20.30–22.20)	(20.20–24.30)	(21.50–23.50)	(21.41–25.11)
Hue	86.22±0.37^{e}	86.41±0.39^{de}	88.63±0.30^{bc}	88.89±0.18^{ab}	88.63±0.36^{bc}	88.90±0.17^{ab}
(H°)	(85.62–86.92)	(85.74–87.10)	(88.10–89.15)	(88.59–89.22)	(87.96–89.20)	(88.63–89.23)
With packaging						
Scale	2-Equal white and yellow	4-Slightly brown	1-White with slightly yellow	1-White with slightly yellow	1-White with slightly yellow	1-White with slightly yellow
						
Colour changes	30.51±2.04^{b}	35.81±2.82^{a}	18.98±0.55^{f}	18.78±0.75^{f}	23.95±2.19^{cde}	25.62±2.19^{cd}
(ΔE)	(26.65–33.63)	(30.67–40.42)	(18.08–19.98)	(17.60–20.20)	(20.86–28.20)	(22.70–29.28)
Chroma	24.49±0.37^{ab}	26.08±0.47^{a}	21.72±0.72^{c}	22.42±0.31^{bc}	23.39±1.13^{bc}	24.21±1.79^{ab}
(C*)	(23.87–25.18)	(25.33–26.95)	(20.50–23.00)	(21.91–23.00)	(21.31–25.20)	(21.21–27.42)
Hue	86.12±0.43^{e}	86.92±0.20^{d}	88.54±0.34^{bc}	88.62±0.38^{bc}	89.24±0.18^{a}	88.20±0.17^{c}
(H°)	(85.44–86.93)	(86.58–87.28)	(87.87–89.00)	(87.91–89.25)	(88.92–89.55)	(87.91–88.53)

Different superscript alphabets indicate differences in the same row (with packaging and without packaging) at $p < 0.05$ (ANOVA, Tukey's test). Data are displayed as means ± standard error and the ranges are in parentheses.

which degrades more rapidly at ambient temperatures than CT during senescence.

A higher hue angle increment was recorded by the packaged fruits (86.92 ± 0.20) than the control samples (86.41 ± 0.39) on the eighth day. The results revealed that senescence breakdown during storage delayed flesh colour changes, particularly at 10 °C, indirectly extending shelf life. Since *A. odoratissimus* is a climacteric fruit that continues to respire during storage, understanding the colour transition from clear white to browning in *A. odoratissimus* flesh can contribute to a better comprehension of post-harvest quality. The information might also offer potential strategies for managing oxidation to enhance quality and shelf life.

3.3. Browning via enzyme reaction associated with A. odoratissimus fruit flesh

Flesh browning is a primary challenge for exporting climacteric fruits, particularly when stored for long periods. The deterioration degree of physical properties regarding colour is determined by the accumulated brown pigments (Galvez et al., 2018). Figure 1 illustrates the influences of PAL, PPO, and POD on the browning of the fruit flesh samples.

The browning degree of the *A. odoratissimus* fruit flesh assessed was notably affected by packaging and storage temperature ($p < 0.05$) (Figure 1a). The degree of browning of the packaged fruit flesh samples stored under ambient temperature increased from 0.22 ± 0.01 (T1) to 0.24 ± 0.01 (T5) within eight days, whereas the control samples recorded 0.21 ± 0.01 to 0.24 ± 0.01. *A. odoratissimus* fruit flesh is considerably susceptible to browning due to oxidation upon contact with oxygen (Ismail et al., 2023; Bakar & Bakar, 2018). Storing at RT also causes physiological disorders leading to browning of the fruit pulps, altering its colour from opaque white to yellowish.

Higher storage temperatures might influence enzymatic activities associated with browning. Enzymatic mechanisms are more rapid under ambient temperatures than CT, arising from enhanced molecular movements, enhancing PPO reaction progression (El-Mesery et al., 2024). The control fruit flesh samples stored at CT recorded increased browning incidence, 0.18 ± 0.01 on Day 4. Nevertheless, the value reduced to 0.15 ± 0.01 and 0.11 ± 0.01 on Days eight and 12, respectively, before rising to 0.13 ± 0.01 on the 16th day. A similar declining trend was also documented by the packaged samples, decreasing from 0.20 ± 0.02 (T4) to 0.13 ± 0.01 (T12). Based on the results, storage under CT allowed the fruit flesh to remain opaque white after being cut open, limiting intense browning development. *A. odoratissimus* is more susceptible to browning at RT than CT.

In this study, the packaged and control terap fruit flesh samples stored at RT demonstrated superior

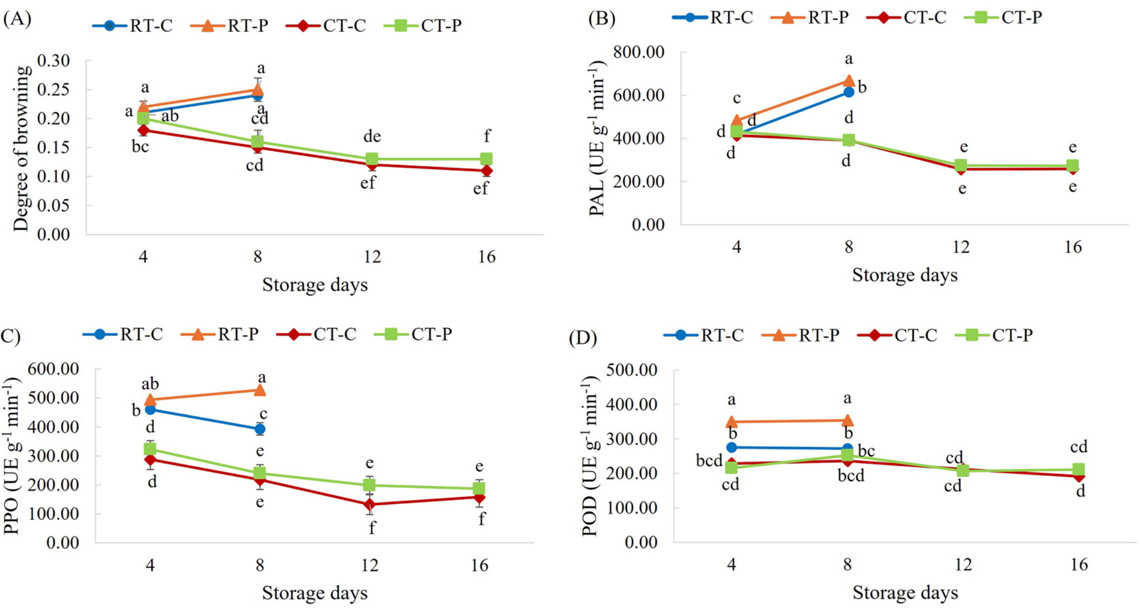


Figure 1. Enzymatic activities associated with flesh browning in *Artocarpus odoratissimus* at various postharvest treatments. (A) Degree of browning, (B) phenylalanine ammonia-lyase (PAL) (UE $g^{-1}min^{-1}$), (C) polyphenoloxidase (PPO) (UE $g^{-1}min^{-1}$), and (D) peroxidase (POD) (UE $g^{-1}min^{-1}$). Different superscript alphabets indicate differences at $p < 0.05$ (ANOVA, Tukey's test). RT-C (room temperature flesh without packaging/control), RT-P (room temperature flesh with packaging), CT-C (cooling temperature flesh without packaging/ control) and CT-P (cooling temperature flesh with packaging).

PAL activities than at CT (Figure 1b). The packaged *A. odoratissimus* fruit flesh stored at 25 °C also exhibited a rapid increase in PAL activity, from 483.00 ± 0.97 UE $g^{-1}min^{-1}$ (Day 4) to 667.66 ± 2.21 UE $g^{-1}min^{-1}$ (Day 8). Meanwhile, the control samples recorded a slower increasing rate, from 419.00 ± 1.39 UE $g^{-1}min^{-1}$ (T1) to 614.33 ± 1.15 UE $g^{-1}min^{-1}$ (T5), following storage.

The PAL is a crucial enzyme in developing phenolic compounds (Fukuoka et al., 2019; Venkatachalam et al., 2023). The PAL levels in the packaged and unpackaged *A. odoratissimus* fruits stored at ambient temperature were elevated, suggesting that PAL is triggered by various stress conditions. Upregulated PAL is typically linked to improved antioxidant activities. As the enzyme is essential for phenolic compound synthesis, it contributes to the antioxidant defence mechanisms of the fruit. Elevated PAL levels also led to the *A. odoratissimus* flesh samples browning from elevated phenolic production during oxidative stress. The pigmentation negatively affects appearance and customer perception, crucial components of fruit quality and marketability (Zhang et al., 2018; Chervin et al., 2004).

The fruit flesh samples stored at 10 °C had a rapidly declining PAL, from 432.66 ± 1.34 UE $g^{-1}min^{-1}$ to 391.00 ± 2.41 UE $g^{-1}min^{-1}$ on Days 4 and 8, respectively. The levels further decreased to 274.00 ± 0.28 UE $g^{-1}min^{-1}$ and 273.66 ± 0.31 UE $g^{-1}min^{-1}$ on Days 12 and 16, respectively. Similarly, the control samples documented diminished PAL levels, from 414.00 ± 0.78 UE $g^{-1}min^{-1}$ (T3) to 259.00 ± 0.62 UE $g^{-1}min^{-1}$ (T11). Conversely, storing the fruits at CT delayed PAL activities throughout storage by maintaining flesh colour for 16 days. Nonetheless, the PAL response varied among cultivars, indicating induction differences in the enzyme involved in phenolic metabolism (Arnold & Gramza-Michałowska, 2022).

Elevated PAL catalyses PPO oxidation, enhancing browning. Figure 1c demonstrates that packaging and storing the *A. odoratissimus* at 25 °C influenced PPO concentrations. The samples recorded 494.00 ± 2.20 UE $g^{-1}min^{-1}$ and 527.00 ± 0.77 UE $g^{-1}min^{-1}$ PPO levels on the fourth and eighth day, resulting in slightly brown pigments in the pulp. Meanwhile, the control samples had a rapidly declining PPO, from 459.66 ± 0.92 UE $g^{-1}min^{-1}$ (T1) to 393.33 ± 2.19 UE $g^{-1}min^{-1}$ (T5).

One of the primary causes of browning in fresh fruits and vegetables is phenolic substrate oxidation by PPO (Khan et al., 2021). The PPO increasing rates of the packaged and unpackaged samples stored at ambient temperature were significantly higher than at CT, revealing that PPO activities in fruit pulps stored at higher temperatures trigger pulp browning. The fruit pulps assessed also turned slightly brown from opaque white within eight days.

The packaged fruit flesh stored at CT had reduced PPO activities, recording 322.33 ± 1.14 UE $g^{-1}min^{-1}$ and 187.00 ± 0.36 UE $g^{-1}min^{-1}$ on the fourth and 16th day, respectively. A comparable trend was observed in the control samples, which had PPO reduction from 287.66 ± 0.77 UE $g^{-1}min^{-1}$ (T3) to 157.66 ± 1.45 UE $g^{-1}min^{-1}$ (T11). Nonetheless, the pulps remained opaque white with slight yellow under CT due to delayed PPO activities (up to 16 days of storage). Although the phenolic compounds in each fruit cultivar directly affect the extent of its browning, the results suggested CT is effective in preserving the *A. odoratissimus* fruit pulps.

The POD levels decreased in fruit flesh affected by browning. Based on Figure 1d, the POD activities of the control samples stored at 25 °C declined to 272.33 ± 1.74 UE $g^{-1}min^{-1}$ (T5) compared to the packaged fruits, which elevated to 354.00 ± 1.73 UE $g^{-1}min^{-1}$ on day 16. Meanwhile, the packaged samples recorded reduced POD levels of 191.33 ± 0.27 UE $g^{-1}min^{-1}$.

The unpackaged fruits assessed in this study had significantly higher POD activities on Day 4, which decreased at the end of the storage period compared to the packaged samples stored at ambient temperature. The observations were attributable to the POD catalysing the browning processes and resulting in fruit discolouration due to PPO (Singh & Mirza, 2018; Sommano et al., 2020). As such, POD enzymes accelerate the conversion of phenolic compounds into melanin with hydrogen peroxide and PPO, leading to browning during storage and at higher temperatures (Arnold & Gramza-Michałowska, 2022).

At 10 °C, the unpackaged fruit flesh recorded 211.33 ± 0.89 UE POD levels, indicating that storage at lower temperatures delayed POD increment rates by maintaining the freshness of the fruit flesh. Consequently, temperature significantly influences POD levels during *A. odoratissimus* fruit pulp storage. Loquat fruit pulps (Zhang & Shao, 2015) and potatoes (Vitti et al., 2011) also demonstrated similar PPO and POD trends. Elevated enzymatic levels at higher temperatures (RT) led to increased enzymatic reactions. Conversely, the freshness of the fruit flesh is maintained at CT. The findings also suggested that applying enzyme inhibitors can prevent browning in *A. odoratissimus* fruit flesh.

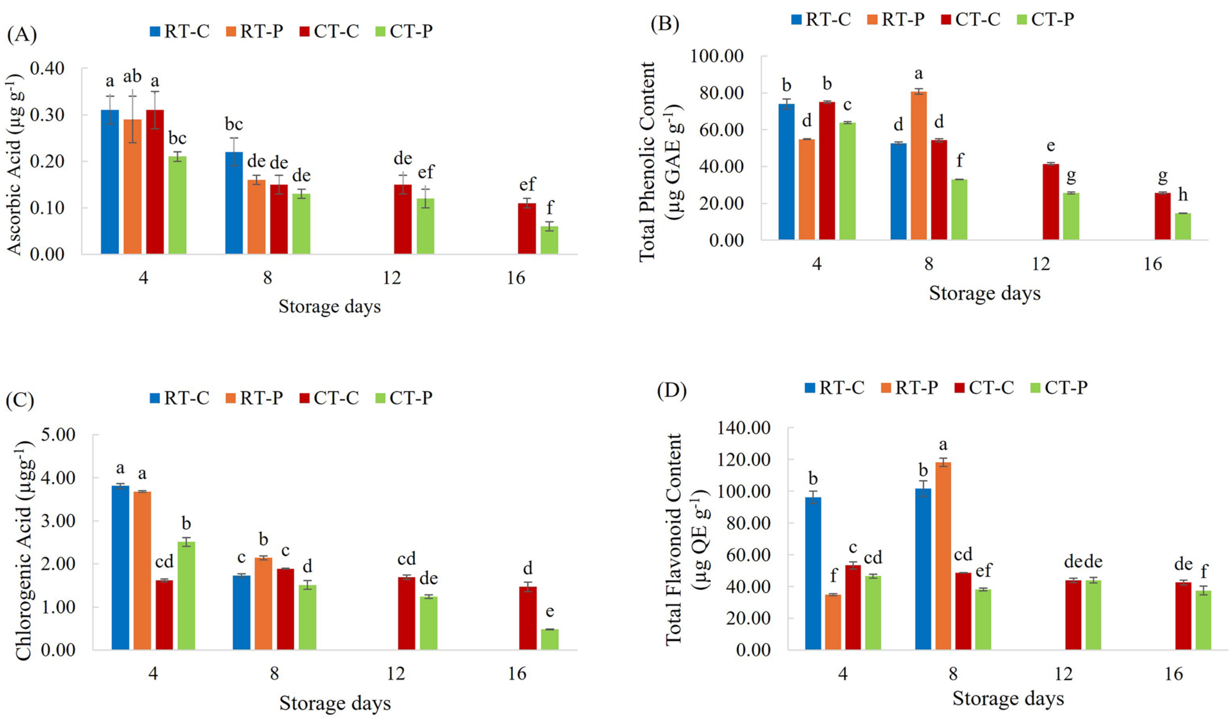


Figure 2. Phytochemical activities associated with flesh browning in *Artocarpus odoratissimus* at various postharvest treatments. (A) Ascorbic acid (µg g^{-1}), (B) TPC (µg GAE g^{-1}), (C) chlorogenic acid (µg g^{-1}), and (D) TFC (µg QE g^{-1}). Different superscript alphabets indicate differences) at $p < 0.05$ (ANOVA, Tukey's test). RT-C (room temperature flesh without packaging/ control), RT-P (room temperature flesh with packaging), CT-C (cooling temperature flesh without packaging/ control) and CT-P (cooling temperature flesh with packaging).

3.4. The effects of phytochemical compounds on A. odoratissimus flesh browning

The relationship between the chemical attributes of the fruit flesh and the influences of browning caused by post-harvest treatments was assessed with phytochemical compound. Ascorbic acid (AA), total phenolic (TPC), chlorogenic acid, total flavonoid (TFC), and DPPH contents of the *A. odoratissimus* fruit pulps are demonstrated in Figure 2.

According to Figure 2a, the AA levels in the *A. odoratissimus* pulp flesh documented a downward pattern during ripening storage. The unpackaged samples stored at RT had the most significant AA reduction from the fourth to the eighth day, 0.31 ± 0.03 µg g^{-1} to 0.22 0.03 µg g^{-1}, whereas the packaged fruits recorded 0.29 ± 0.05 µg g^{-1} (T2) to 0.16 ± 0.01 µg g^{-1} (T6), respectively. Temperature increments lead to the oxidative breakdown of AA, resulting in diminished levels during storage. The decline might also be influenced by oxygen. According to Lee and Kader (2000), AA concentration in fresh-cut produce degrades more rapidly when stored at room temperature than in refrigerated conditions.

The unpackaged fruit samples in the present study demonstrated a gradual AA decline from 0.31 ± 0.04 µg g^{-1} (Day 4) to 0.15 ± 0.02 µg g^{-1} (Day 8), which remained constant at 0.15 ± 0.02 µg g^{-1} (Day 12). Nevertheless, the levels decreased to 0.11 ± 0.01 µg g^{-1} (Day 6) at CT after 16 days of storage. The fruits stored at CT had a slow AA rate, which delayed degradation.

Fruit development and post-harvest preservation are regulated by AA (Zheng et al., 2022). The acid also manages fruit maturation and stress tolerance (Zheng et al., 2022). During handling and storage, water solubility, thermic decline, and enzymatic oxidation reduce AA levels (Galani et al., 2017). Enzymatic activities and gene expression in AA metabolic pathways might also be linked to fluctuations in its concentration during storage. The results were consistent with the report by Sattar et al. (2019), where AA levels in capsicum and sweet pepper declined during storage (Aryal et al., 2020).

The total phenolic content (TPC) in the *A. odoratissmus* fruit flesh assessed in this study was quantified with the The Folin-Ciocalteu oxidation-reduction reagent. At Day 4 (T2), the TPC of the packaged samples stored at RT was 54.83 ± 0.23 µg GAE g^{-1} before rapidly rising on Day 8 (T6) to 80.77 ± 1.49 µg GAE g^{-1} (Figure 2b). During similar periods, the control fruit flesh diminished from 74.01 ± 2.61 µg GAE g^{-1} to 52.70 ± 0.68 µg GAE g^{-1}. A diminished TPC could degrade phenolic content due to enzymatic activities, such as PPO and POD.

At CT, the packaged fruit samples exhibited decreasing phenolic levels, from 63.84 ± 0.49 µg GAE g^{-1} to 14.68 ± 0.11 µg GAE g^{-1} during storage. Similarly, the control samples recorded from 53.31 ± 2.29 µg GAE g^{-1} (T3) to 42.61 ± 1.50 µg GAE g^{-1} (T4) phenolic level reduction after 16 days of storage. The findings revealed that CT decelerated enzymatic activities by delaying TPC decline.

Several factors influence TPC, including genotype, harvesting period, growing location, and extraction techniques (Galani et al., 2017). Reduced TPC might also be attributed to inhibited polyphenolic production adhering to an altered enzyme activity sequence. Shah et al. (2017) also hypothesised that the direct contact of pro-oxidant enzymes with phenolics increased senescence subcellular compartment dysfunction.

This study observed superior phenolic contents on the packaged samples stored at RT than CT. The reduced phenolics were probably due to the loss of compartmentation. The phenomenon resulted in delayed *A. odoratissimus* fruit flesh browning for up to 16 days, whereas the samples stored at RT lasted eight days. The findings supported the observations noted by Attilio et al. (2023), where fresh figs recorded diminished TPC following storage under lower temperatures due to phenolic metabolism.

Higher temperatures can significantly influence the rapid degradation of chlorogenic acid in fruits, primarily due to oxidative degradation. Significant temperatures also might accelerate phenolic substance oxidation, including chlorogenic acid, by elevating enzymatic activities, such as PPO (Patthamakanokporn et al., 2008; Xi et al., 2016). The reaction can lead to undesirable flavour and colour alterations, negatively impacting the quality and marketability of the fruit. For instance, phenolic compound oxidation involving chlorogenic acid resulted in pear fruit flesh browning (Hou et al., 2022). The breakdown of the cellular compartments allowed the orthophenolic compounds to be accessible to PPO, catalysing the quinone oxidation.

According to Figure 2c the chlorogenic acid levels in the *A. odoratissimus* fruit flesh stored at RT were significantly higher on the fourth Day 4 (T1) (3.81 ± 0.06 µg g^{-1}) before decreasing rapidly on the eighth day (T5) (1.73 ± 0.04 µg g^{-1}). Similarly, the chlorogenic acid concentration of the packaged samples declined from 3.68 µg g^{-1} on Day 4 (T2) to 2.14 µg g^{-1} on Day 8 (T6). Conversely, the chlorogenic acid contents of the *A. odoratissimus* fruits stored at CT exhibited a less swift reduction from Day 4 to 16. The higher chlorogenic acid levels in packaged and unpackaged (control) fruit samples stored at RT than CT revealed that lower temperatures preserved the acid levels throughout the storage period. Chlorogenic acid is an essential component of physiologically active chemicals, prevalent in fruit flesh, and has notable antioxidant effects (Butkeviciute et al., 2022).

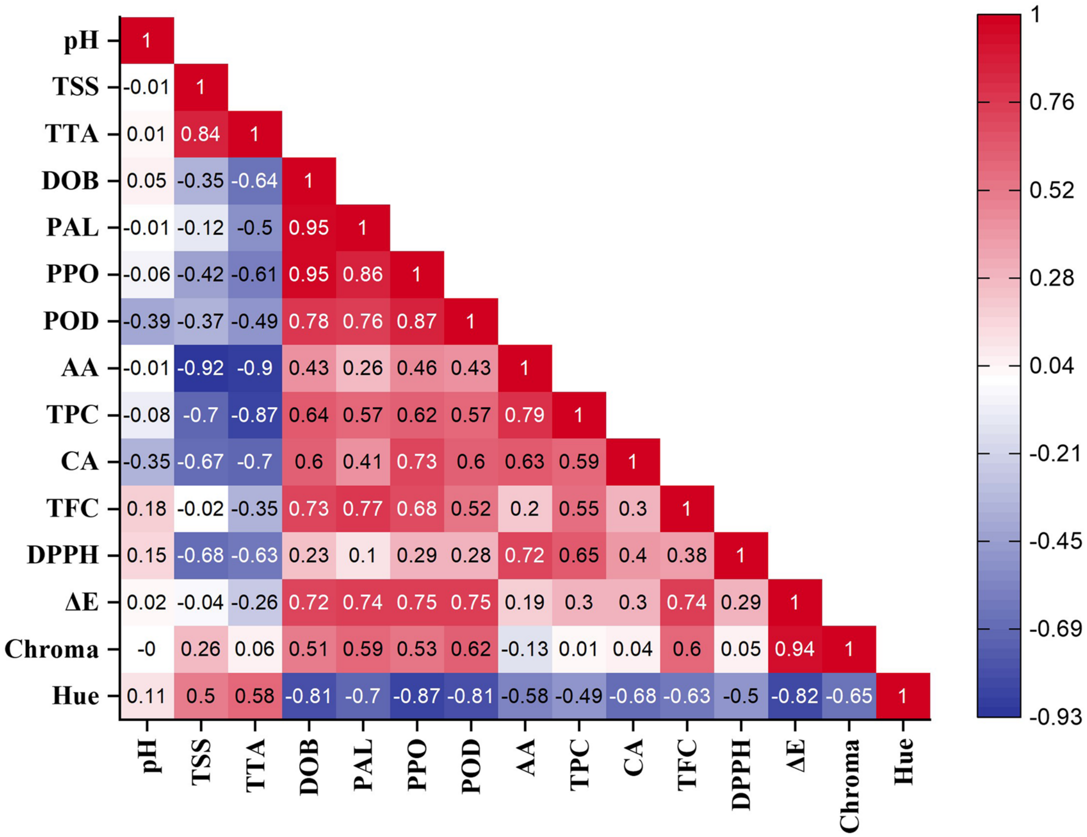
The TFC activities in the control *A. odoratissimus* fruit pulps stored at RT significantly decreased from T1 (day 4) (90.29 ± 3.88 µg QE g^{-1}) to T5 (53.31 ± 2.29 µg QE g^{-1}), as illustrated in Figure 2d. Meanwhile, the packaged samples had an increased TFC when assessed from days four (T2) to eight (T6), recording 35.00 ± 0.58 µg QE g^{-1} and 46.65 ± 1.26 µg QE g^{-1}, respectively. Conversely, a decreasing trend was observed with the control and packaged fruits stored at CT from Day 4 to 16.

Flavonoids are a group of polyphenolic compounds in various plant species (Hou et al., 2022). The chemicals act as antioxidants and are involved in redox reactions in plant cells (Hou et al., 2022). At ambient temperature, the reduction of TFC is higher, while CT diminishes the TFC degradation rates throughout storage. Huang et al. (2015) suggested that storing fruits for a long period could decrease their TFC due to phenolic chemicals undergoing oxidative breakdown and polymerising with proteins. Karpagavalli et al. (2014) also noted that polyphenols possess distinctive physicochemical associations with soluble solids, specifically with the cell walls of solid fruit pulp tissues. Overall, the findings demonstrated that AA, TPC, TFC, and CA degradations were influenced by oxidation. Storage temperature, specifically CT, also inhibited and preserved the flesh quality of *A. odoratissimus* for 16 days.

3.5. Pearson correlation matrix between chemical constituents of *A. odoratissimus* flesh

All 15 parameters assessed in the current study demonstrated a significant correlation ($p < 0.05$) with several variables pertaining to the chemical contents in *A. odoratissimus* flesh (Figure 3). Nonetheless, the degree of browning exhibited the most considerable association with all conditions.

Analytical data indicated a notably positive correlation ($r = 0.84$) between TTS and TTA. Accordingly, TTS and TTA levels increased when the *A. odoratissimus* fruit flesh was degraded and utilised for respiration. Meanwhile, the degree of browning was positively linked to PAL ($r = 0.95$). A strong



	pH	TSS	TTA	DOB	PAL	PPO	POD	AA	TPC	CA	TFC	DPPH	ΔE	Chroma	Hue
pH	1														
TSS	-0.01	1													
TTA	0.01	0.84	1												
DOB	0.05	-0.35	-0.64	1											
PAL	-0.01	-0.12	-0.5	0.95	1										
PPO	-0.06	-0.42	-0.61	0.95	0.86	1									
POD	-0.39	-0.37	-0.49	0.78	0.76	0.87	1								
AA	-0.01	-0.92	-0.9	0.43	0.26	0.46	0.43	1							
TPC	-0.08	-0.7	-0.87	0.64	0.57	0.62	0.57	0.79	1						
CA	-0.35	-0.67	-0.7	0.6	0.41	0.73	0.6	0.63	0.59	1					
TFC	0.18	-0.02	-0.35	0.73	0.77	0.68	0.52	0.2	0.55	0.3	1				
DPPH	0.15	-0.68	-0.63	0.23	0.1	0.29	0.28	0.72	0.65	0.4	0.38	1			
ΔE	0.02	-0.04	-0.26	0.72	0.74	0.75	0.75	0.19	0.3	0.3	0.74	0.29	1		
Chroma	-0	0.26	0.06	0.51	0.59	0.53	0.62	-0.13	0.01	0.04	0.6	0.05	0.94	1	
Hue	0.11	0.5	0.58	-0.81	-0.7	-0.87	-0.81	-0.58	-0.49	-0.68	-0.63	-0.5	-0.82	-0.65	1

Figure 3. Pearson correlation matrix between chemical constituents of *Artocarpus odoratissimus* fruit flesh Abbreviations: TSS, total soluble solid; TTA, total titratable acidity; DOB, degree of browning: PAL, phenylalanine ammonia-lyase; PPO, polyphenol oxidase; POD: peroxidase; AA: ascorbic acid; TPC: total phenolic content; CA: chlorogenic acid; TFC: total flavonoid content; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; ΔE: total colour changes.

positive correlation was also observed between the degree of browning and PPO (*r*=0.95) and POD) (*r*=0.78). Furthermore, the degree of browning indicated a significant correlation with TFC (*r*=0.73). Similar outcomes were noted by Min et al. (2017), where increased PPO and POD gene expression of lotus roots were associated with enzyme activities and browning degree under various storage temperatures.

Considerably positive associations were observed between PAL and PPO (*r*=0.86), PAL and POD (*r*=0.76), and PAL with TFC (*r*=0.77), indicating that the enzymatic activities and TFC increased *A. odoratissimus* fruit flesh browning during storage. The PPO levels also demonstrated superior positive correlations with POD (*r*=0.87) and chlorogenic acid (*r*=0.73). The enzyme also significantly negatively influenced the hue (*r*=−0.87) of the *A. odoratissimus* fruit flesh.

The PPO and total colour changes (ΔE) were notably positively correlated (*r*=0.75). Ercoli et al. (2017) observed similar associations in fresh-cut apples, revealing that PPO is the primary enzyme attributed to pulp browning. The phenomenon is attributable to PPO catalysing phenolic component oxidation (Arnold & Gramza-Michałowska, 2022; Moon et al., 2020). Conversely, no association was noted between TPC in the fruit flesh sample and the parameters assessed.

This study employed linear regression analyses to evaluate the regression equation for the degree of browning and enzyme activity. The PAL, PPO, and POD levels demonstrated a more positive correlation with the degree of browning, recording R^2 values of 0.7975, 0.7848, and 0.6382, respectively (Figures 4a–c). The outcomes indicated that browning is caused by enzymatic activities.

The results offered insights into the numerous parameters affecting the browning intensity of *A. odoratissimus* fruit flesh during post-harvest storage. Storage, particularly at ambient temperature, increased enzymatic levels. As such, the variable could be applied as an indicator. The findings might also provide potential solutions in determining anti-browning agents to enhance the durability and marketability of *A. odoratissimus* fruits.

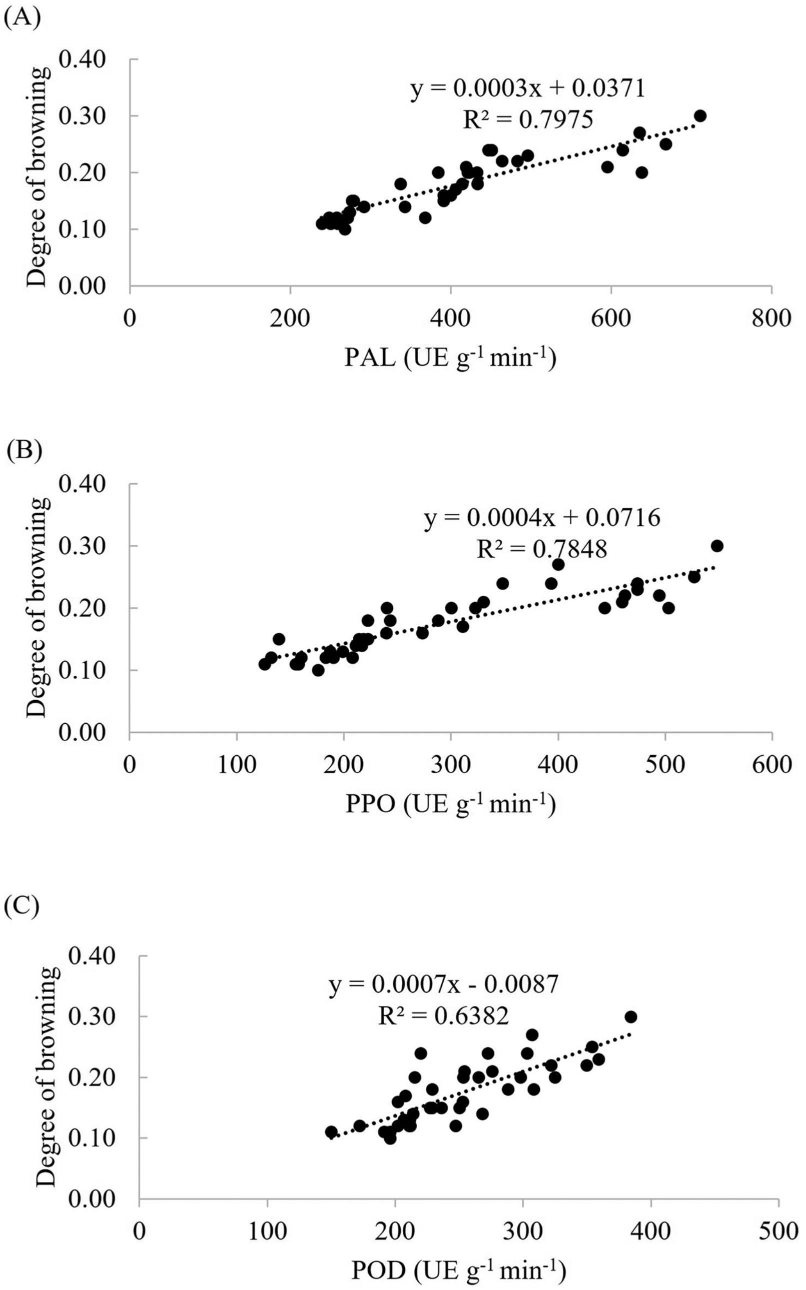


Figure 4. Linear regression between degree of browning and enzymatic activities of *Artocarpus odoratissimus* fruit flesh. (A) Degree of browning with phenylalanine ammonia-lyase (PAL), (B) degree of browning with polyphenoloxidase (PPO), and (C) degree of browning with peroxidase (POD).

4. Conclusions

The chemical constituents, enzymatic browning, and physicochemical and phytochemical attributes of *A. odoratissimus* flesh during post-harvest ripening are offered by this study. Storage temperature and duration are vital in extending the durability of terap fruits. Storing the *A. odoratissimus* samples at ambient temperature (25 °C) led to its significant deterioration after eight days. The samples also recorded elevated pH, TSS, and TTA. Moreover, the enhanced enzymatic activities resulted in notable colour alterations and browning of the fruits. Conversely, the browning and enzymatic reaction in the terap fruit samples were effectively diminished at 10 °C, preserving their freshness and quality for up to 16 days. The data underscored the importance of optimal storage conditions in preserving the sensory and nutritional values of *A. odoratissimus* fruits. Packaging also led to considerable preservation of the biochemical and physical integrity of the fruit samples. This study paves the way for excellent shelf-life management practices, which could ultimately benefit consumers and producers by identifying optimal preservation strategies. Nevertheless, future research should also focus on exploring anti-browning agents and evaluating the effectiveness of various packaging materials to improve the post-harvest management of *A. odoratissimus* fruits.

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Authors' contribution

I.R. and S.D.R. contributed to the conceptualisation, methodology, investigation, data analysis, writing, and review of the manuscript.

N.K. and H.K. validated, reviewed, and edited the manuscript.

All authors read and approved the final manuscript.

Disclosure statement

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Data availability statement

The data supporting the findings are available from the corresponding author, Shiamala Devi Ramaiya, upon reasonable request.

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