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Original Article

Inactivation of polyphenol oxidase and peroxidase activity in mangosteen pericarp via blanching: correlation between anthocyanins and enzyme activities

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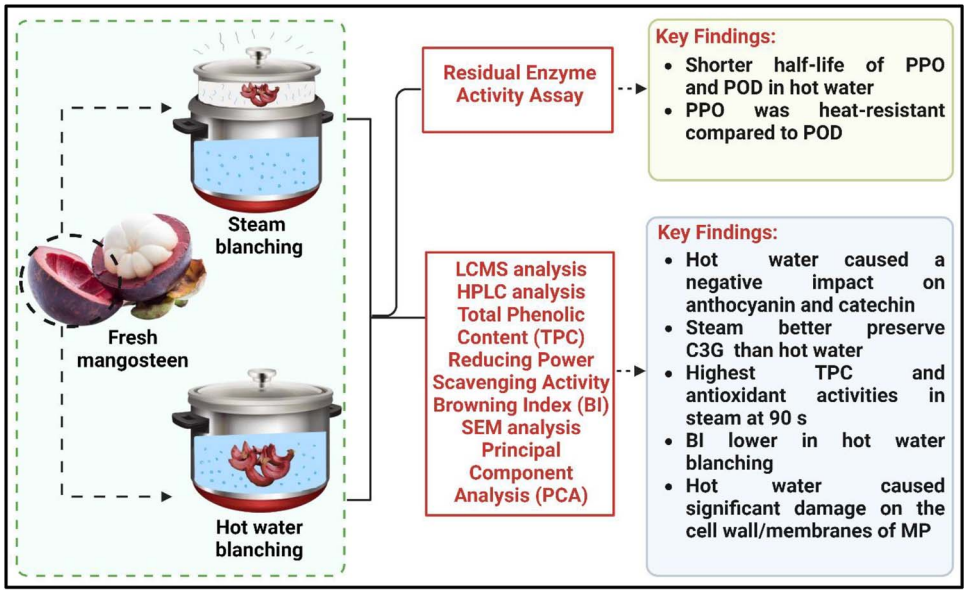
Abstract

Mangosteen pericarp (MP), rich in polyphenols and antioxidants, is often discarded as agro-waste. Its anthocyanins (ACNs), potential natural colourants, are compromised by high polyphenol oxidase (PPO) and peroxidase (POD) activity. This study examined the effects of hot water and steam blanching on PPO and POD inactivation in MP. Blanching for 0, 30, 60, 90, and 120 s at 100 °C. Hot water inactivated 89.57% PPO ($t_{1/2}$ = ~67 s) and 92.13% POD ($t_{1/2}$ = ~33.6 s), while steam inactivated 52.81% PPO ($t_{1/2}$ = ~113.7 s) and 97.07% POD ($t_{1/2}$ = ~61.8 s). Despite better enzyme inactivation with hot water, it adversely affected ACNs stability. Steam blanching for 90 s, as determined by principal component analysis, preserved the highest ACNs, phenolics, and antioxidant activities. Cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside were identified via liquid chromatography-mass spectrometry and high-performance liquid chromatography, offering insights for preserving ACNs as natural colourants.

Keywords: antioxidants, browning enzyme, cyanidin-3-*O*-sophoroside, enzyme residual activity, natural colourants, thermal blanching, waste valorisation

Graphical abstract



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Introduction

Mangosteen (*Garcinia mangostana* L.), a tropical fruit from Southeast Asia, is known for its deep purple pericarp and sweet, tangy white flesh, earning it the title "Queen of fruits." The mangosteen pericarp (MP) makes up about 70% of the mangosteen's total weight and is typically discarded as a by-product, contributing to environmental pollution (Cho et al., 2019). The MP has been reported to possess therapeutic and medicinal potential and has been explored and used traditionally for centuries. The abundance of polyphenolic compounds, such as xanthones. Catechins, rutin, ellagic acid, and anthocyanins (ACNs) are responsible for the medicinal potential of the MP (Albuquerque et al., 2023; Li et al., 2023; Nawawi et al., 2023).

ACNs are water-soluble pigments responsible for the bright red to purple, suitably used as a natural colourant (Ijod et al., 2022; Azman et al., 2022b). However, the stability of ACNs is compromised by various factors, such as pH, oxygen, temperature, light, and enzymes (Deylami et al., 2016; Azman et al., 2020a). The critical issue with MP is that it contains high levels of polyphenol oxidase (PPO) and peroxidase (POD) enzymatic activity, which rapidly cause browning to the pericarps. PPO is an endogenous copper enzyme with a nuclear copper core. Two separate reactions occur: the *o*-hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones, which are triggered by the aid of oxygen molecules. Quinones are highly reactive electrophilic molecules that can form covalent bonds with and degrade nucleophiles, such as ACNs, leading to the formation of brown pigments (melanin) (Deylami et al., 2016; Enaru et al., 2021). Therefore, blanching can be performed to address these issues and retain ACNs' natural colourant and biological activities.

Blanching is a process commonly used for vegetable and fruit pretreatment to inactivate enzymes, inhibit microbes' growth, soften tissue, and maintain texture and nutrients (Suksamran et al., 2022; Kaur et al., 2023). This method has inhibited PPO and POD activities in fruits and vegetables (Deylami et al., 2014). Steam and hot water blanching are the standard techniques employed owing to their cost-effective and straightforward setup and enabling the preservation of most water-soluble compounds (Kaur et al., 2023).

Although many studies have reported the inactivation of PPO and POD in MP using hot water and steam blanching methods, limited studies focus on the correlation between PPO and POD activity on individual ACNs in MP extracts. Therefore, this study investigated the effects of PPO and POD inactivation using hot water and steam blanching on the physicochemical properties of MP, including ACN composition, antioxidant activity, and total phenolic. In addition, Pearson correlation, general linear model (GLM), and principal component analysis (PCA) were also carried out to evaluate the correlations between the attributes.

Materials and methods

Chemicals

4,6-Tripryridyl-s-triazine (TPTZ), Folin–Ciocalteu reagent and gallic acid, and 2,2-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standard chemicals: cyanidin-3-*O*-glucoside (C3G), cyanidin-3-*O*-sophoroside (C3S), and pelargonidin-3-*O*-glucoside (P3G) were purchased from Systerm (Selangor, Malaysia). Other chemicals and solvents used were of analytical grade and bought from Merck (Darmstadt, Germany).

Sample preparation

The purple mangosteen (Index 6) was purchased from the local market in Serdang, Selangor, Malaysia. The fresh mangosteen was cleaned under running tap water, separated from the flesh, and cut into small pieces before blanching.

Blanching process: A cooker equipped with a steamer drawer (Panasonic, Haryana, India) was used for steam and hot water blanching. Distilled water (3 L) preheated at 100 ± 1 °C. Approximately 20 g MP was placed in the steam drawer or immersed in hot water for 0, 30, 60, 90, and 120 s, respectively. The blanched MP was immediately transferred to ice water to stop the blanching process.

Preparation of MP crude enzyme extract

The MP crude enzyme extract for residual enzyme activity (REA) analysis was prepared as described by Deylami et al. (2016) with a slight modification, where different volumes of the buffer-to-weight ratio of blanched MP were used. Five grams of blanched MP were ground in 50 ml of 0.1 M potassium phosphate buffer in pH 7.0 and 7.5 (1:10 v/w) using a hand blender (Pensonic, Penang, Malaysia). The buffer contained 4% (w/v) of polyvinyl pyrrolidone (PVPP) and 1% Triton X-100. The homogenate was centrifuged at 4 °C (7000 rpm, 15 min). The supernatant was collected as a crude enzyme extract.

PPO and POD residual activity

The PPO and POD residual activity were assayed according to Deylami et al. (2014, 2016) with slight modifications, where different concentrations of substrate and crude enzymes were used. The absorbance was monitored for 5 min at 1-min intervals at 420 and 470 nm using a spectrophotometer (Thermo BioMate 3, Waltham, USA).

The REA was calculated using equation 1:

$$\text{REA}\ (\%) = \frac{C_t}{C_0} \times 100 \tag{1}$$

where C_t and C_0 were the specific enzyme activities of blanched and unblanched samples, respectively.

Determination of thermal kinetic parameters

The rate constant (k) for zero (equation 2) and first-order (equation 3) of the kinetic reaction were determined according to Chisté et al. (2010) as follows:

$$C_t = C_0 - kt \tag{2}$$

$$\ln C_t = \ln C_0 - kt \tag{3}$$

where C_0 is the initial enzyme activity, C_t is the corresponding value at time t, k is the rate constant, and t is the blanching time (s). The half-life time ($t_{1/2}$) from zero and first-order was determined from the following equations 4 and 5:

$$t\frac{1}{2} = \frac{[C_0]}{2k}\ (\text{half} - \text{life from zero order reaction}) \tag{4}$$

$$t\frac{1}{2} = \frac{0.693}{k}\ (\text{half} - \text{life from first order reaction}) \tag{5}$$

where k is the rate constant of zero or first-order reaction.

Preparation of freeze-dried MP extract

The preparation of freeze-dried MP extract followed methods described by Azman et al. (2022a). Approximately 10 g of dried

MP was mixed with 100 ml 50% ethanol (1:10 w/v). The mixture was shaken at 50 °C for 2 h in a water bath (WITEG Labortechnik, Wertheim, Germany) at 180 rpm. The solution was vacuum-filtered using a Buchner funnel and Whatman No. 1 filter paper. The extracts were centrifuged using Kubota centrifuge (Model 4200, Tokyo, Japan) at 1,030 × *g* for 15 min to separate the clear extract from the precipitate and lyophilised using a freeze dryer (Labconco, Kansas City, MO, USA) at −45 ± 1 °C for 36 h. The dried MP was ground for 2 min to pass through a 0.50 mm (35 mesh) sieve and was stored at −20 °C for further analysis of liquid chromatography-mass spectrometry (LC–MS), ACNs, total phenolic content (TPC), antioxidant activity, and browning index (BI).

Identification of phenolic compounds by LC–MS

Dionex Ultimate 3000 Rapid Separation (RS) ultra-performance liquid chromatography (UPLC) system (Thermo Fisher Scientific Inc., USA) coupled to a Thermo Scientific Q Exactive Orbitrap Hybrid Tandem mass spectrometer and Heated-Electrospray Ionization II (H-ESI II) were used according to the method by Azman et al. (2020a). Chromatography was carried out on a Purospher STAR RP18 end-capped column (5.0 μm, 4.6 mm i.d. × 250 mm), and the column temperature was maintained at 30 °C. The mobile phases consisted of solvent A (0.2% formic acid in water) and solvent B (100% methanol) at a flow rate of 0.8 ml/min with an injection volume of 10 μl. The gradient elution programme was used as follows: 0–19 min, 15% B; 19–38 min, 35% B; 38–50 min, 60% B; 50–56 min, 80% B; 56 min, 15% B. Mass spectrometry (MS) spectra were operated in the positive and negative ion mode between *m*/z 100 and 1500 at a scan resolution of 70,000 (full MS scan) and 35,000 (ddMS2 scan). Qual Browser of Xcalibur software (Thermo Fisher Scientific Inc., USA) was used to analyse the acquired data for MS analysis.

Quantification of ACNs by high-performance liquid chromatography (HPLC)

HPLC analysis was carried out as Nawawi et al. (2023) described, and a fixed volume of 20 μl was injected into HPLC. Individual ACNs were quantified using external standard calibration curves: 0.01–0.1 mg/ml. The determination coefficient (R^2), limit of detection (LOD), and limit of quantification (LOQ) were calculated as

$$\text{LOD} = \frac{3Sa}{b} \text{ and LOQ} = \frac{10Sa}{b} \quad (6)$$

where *Sa* is the standard deviation of the response and *b* is the slope of the calibration curve.

Determination of TPC

The TPC assay followed Azman et al. (2020b) and was quantified by a spectrophotometer at 765 nm based on the Folin–Ciocalteu method using gallic acid (0–100 mg/L) as a standard. Results were expressed as milligrams of gallic acid equivalents per gram (mg GAE/g). The mean was calculated from triplicate measurements.

Determination of antioxidant activities

The antioxidant activities of fresh and blanched MPs were determined using DPPH free radical scavenging activity and ferric-reducing antioxidant power (FRAP) assays. DPPH radical scavenging activity was determined according to Ezzat et al. (2020) and Zhou et al. (2011), with modifications using different solvents, dilution factors, and extract volumes and measured at 517 nm spectrophotometrically. The mean was calculated from triplicate biological measurements.

The FRAP was analysed as described by Senevirathna et al. (2021) with minor modifications and observed spectrophotometrically at 593 nm. Trolox solution (0–2,000 μM) was used as the standard curve for quantifying scavenging activity and reducing power. The results are expressed in μmol of Trolox equivalent per gram of the sample (μmol TE/g). The mean was calculated from triplicate biological measurements.

Determination of BI

The BI of MP extracts was determined using the method described by Ding and Ling (2014). 1.0 g of MP powder was extracted with 20 ml of 65% (v/v) ethanol, incubated for 30 min at 20 °C, and filtered using Whatman No. 1 filter paper. BI was measured at 420 and 520 nm using a spectrophotometer, and the index was calculated as follows:

$$\text{BI} = \frac{520\ \text{nm}}{420\ \text{nm}}. \quad (7)$$

Morphological structure of MP

Scanning electron microscopy (SEM) studies were conducted to observe changes in the morphological structures of fresh and blanched MP. The samples were examined using a scanning electron microscope (JSM-IT 100, JEOL Ltd., Japan). They were mounted on a stub with clean double-sided tape and coated with gold particles before viewing at 5,000 × magnification at 10 kV.

Statistical analysis

All statistical analyses were conducted using one-way and two-way analysis of variance (ANOVA). Tukey's multiple range test was used with a probability of $p \leq .05$. The linear Pearson's correlation was also applied to evaluate the correlations between the analyses. Statistical analysis, correlogram plots, and GLM were conducted using Minitab V.21 (Minitab Inc., State College, PA, USA), and PCA was conducted in OriginPro 2024 (OriginLab, Northampton, MA, USA).

Results and discussion

REA: PPO and POD

As shown in Table 1, the highest REA values for PPO and POD were observed in fresh MP ($p \leq .05$). The residual activities of PPO and POD significantly decreased over time ($p \leq .05$). However, total inactivation of PPO and POD activity was not achieved after hot water and steam blanching. These findings agree with Deylami et al. (2014, 2016), who reported that only 86% and 60% of PPO and POD activities were inactivated after 12 min at 100 °C of hot water blanching.

In the present study, approximately 89.57% and 92.13% of PPO and POD were inactivated during hot-water blanching. The inactivation of POD activity was approximately 2.56% higher than that of PPO, suggesting the presence of heat-resistant PPO in MP, which required a longer time to achieve the same inactivation level of POD. Meanwhile, approximately 52.81% and 97.07% of the PPO and POD were inactivated during steam treatment. Both blanching techniques showed POD's thermolabile properties compared to PPO in MP. Similar results were obtained by Adetoro et al. (2020), who observed significantly higher inactivation of POD than PPO at 90 °C/30 s and 100 °C/60 s of pomegranate aril during hot water blanching. However, these results contradict those of Deylami et al. (2014, 2016), who reported higher thermostability of POD than PPO in MP. This contradiction could be influenced by several factors, such as the cultivated mangosteen's geographical

Table 1. REA and kinetic studies of PPO and POD in fresh, hot water, and steam MP.

Blanching time (s)	Hot water		Steam	
	PPO	POD	PPO	POD
0	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
30	92.93 ± 0.30^{b}	32.11 ± 0.57^{b}	79.34 ± 1.71^{b}	76.93 ± 0.86^{b}
60	80.98 ± 0.17^{c}	13.52 ± 0.23^{c}	78.95 ± 0.50^{b}	66.71 ± 0.42^{c}
90	18.40 ± 0.30^{d}	10.76 ± 0.94^{d}	60.98 ± 1.33^{c}	48.24 ± 1.47^{d}
120	10.43 ± 0.35^{e}	7.87 ± 0.17^{e}	47.19 ± 1.39^{d}	2.93 ± 0.88^{e}
Order	Zero	First	Zero	Zero
k	0.746	0.021	0.440	0.809
R^2	0.880	0.905	0.954	0.933
$t_{1/2}$ (s)	67.0 ± 0.27^{B}	33.6 ± 0.19^{D}	113.7 ± 2.97^{A}	61.8 ± 0.56^{C}

Note. Each value represents the mean ± SD ($n = 3$). Values with the same letter $^{a–e}$ in each row are not significantly different ($p > .05$). Values with the same letter $^{A–E}$ in each column are not significantly different ($p > .05$).

location and the presence of different types of PPO and POD enzymes, which affect the levels and stability of the enzymes (Matan et al., 2024).

In a kinetic study, the inactivation of PPO and POD in MP during hot-water blanching fitted zero- and first-order kinetics with $R^2 > 0.880$. The half-life ($t_{1/2}$) of PPO ($t_{1/2}$ = ~67.0 s) was significantly higher than that of POD ($t_{1/2}$ = ~33.6 s), indicating rapid inactivation of POD than PPO in hot water blanching. Similarly, the inactivation of PPO and POD in steam blanching fitted zero-order kinetics with $R^2 > 0.930$. The half-life of PPO ($t_{1/2}$ = ~113.7 s) is higher than that of POD ($t_{1/2}$ = ~61.8 s). A similar trend in enzyme thermostability was observed for both types of blanching. Despite the incomplete inactivation of PPO and POD, the drastic inhibition of these enzymes was highly affected by hot water rather than steam. This could be due to the direct contact of the blanching medium with MP, which causes a significant destructive effect on the secondary and tertiary structures of PPO and POD, thereby inhibiting their activity. In the case of steam, a less damaging impact was observed in the PPO, which could be associated with insufficient latent heat being transferred to the core of the MP owing to the short blanching time. Therefore, a longer time is required to inactivate PPO than POD in MP during steam blanching. Supplementary Figure SS1 shows the physical appearance of MP and freeze-dried MP powder in fresh and blanched samples.

LC–MS analysis

As shown in Supplementary Table SS1, the parent ions with m/z values ranging from 611.1612 to 611.1617 [M-H]+ were identified as C3S, as verified by the fragment ion at 287.0529–287.0539. Additionally, the parent ion at 449.1770–449.1780 [M-H]+ was tentatively identified as C3G, supported by the fragment ion at 287.1229–287.1241. Other bioactive compounds detected in the extract included xanthones, phenolic acids, and flavonoids. The identification of bioactive compounds in MP was based on the study by Salazar-Orbea et al. (2023), Li et al. (2023), Li et al. (2022), Razgonova et al. (2021), Araujo et al. (2020), Lv et al. (2015), and Zarena and Sankar (2012).

According to Zarena and Sankar (2012), three ACNs are found in MP: C3S, C3G, and P3G. However, P3G was not detected in the present study. These findings are consistent with those of Nawawi et al. (2023) and Li et al. (2023), who reported that only significant ACNs (C3S and C3G) were identified in MP. The aqueous ethanolic solvent used in this study could not extract a low concentration of ACNs, such as P3G, which explains the reason Zarena and Sankar (2012) used acidified ethanol [0.01% (v/v) HCl].

The percentage relative composition (%) of the polyphenol profile in fresh and blanched MP (Supplementary Table S2) was used to interpret the effect of blanching processes on the bioactive compounds in MP. The ACN trends were similar for both ionization modes. Hot water blanching negatively affected ACNs and catechins, drastically reducing C3S and catechins. Meanwhile, steam enriched the composition of these compounds. The reduction of glucose units in C3S was suspected during hot water blanching owing to their higher molecular weight than C3G, which caused some of the glucose units to degrade in the medium (Cakrawati et al., 2021).

Catechin reduction in hot water blanched MP is probably due to leaching or transformation into new compounds. Bioactive compounds, such as dihydroquercetin, quinic acid, xanthones (β-mangostin and α-mangostin), A-type proanthocyanidins, and procyanidin C1 degraded under both blanching treatments, with more significant degradation observed in hot water blanching except for dihydroquercetin. Heat-sensitive phenolic degradation in MP possibly results from oxidation and the Maillard reaction, leading to bioactive loss. The procyanidin trimer and procyanidin B1 were improved in both blanching treatments; meanwhile, an increase in (−)-epicatechin and procyanidin dimer was only observed after hot water treatment and significantly reduced in steam treatment. This could be due to the disruption of linked bonds in MP, which assisted the release of conjugated compounds during treatment due to enzymatic hydrolysis (Magangana et al., 2022).

Effect of blanching on ACNs

MP is characterized by their purple pericarp attributed to the ACNs content (Deylami et al., 2016; Zarena and Sankar, 2012). Figure 1A shows the individual ACNs of MP identified in fresh, steam, and hot water blanching, corresponding to C3S and C3G. The ACNs ranged from 2.09 to 3.03 mg/g in MP. Compared to fresh MP, steam (30, 90, and 120 s) and hot water (60, 90, and 120 s) are significantly high ($p \leq .05$). However, we observed no significant difference between the latter sample ($p \geq .05$), suggesting that both blanching methods can preserve ACNs up to 120 s. The ACNs content in MP was mainly C3S (~98%), followed by C3G (~2%) (Supplementary Figure S2). This finding agrees with that of Nawawi et al. (2023), who reported C3S (~95%) as the main ACNs in MP, followed by C3G (~4.8%).

The significantly lowest ACNs observed in fresh, steam (60 s), and hot water (30 s) were putatively due to several factors, such as the high activity of PPO and POD in fresh and steam MP (Deylami et al., 2014, 2016). This can be explained by the non-uniformity

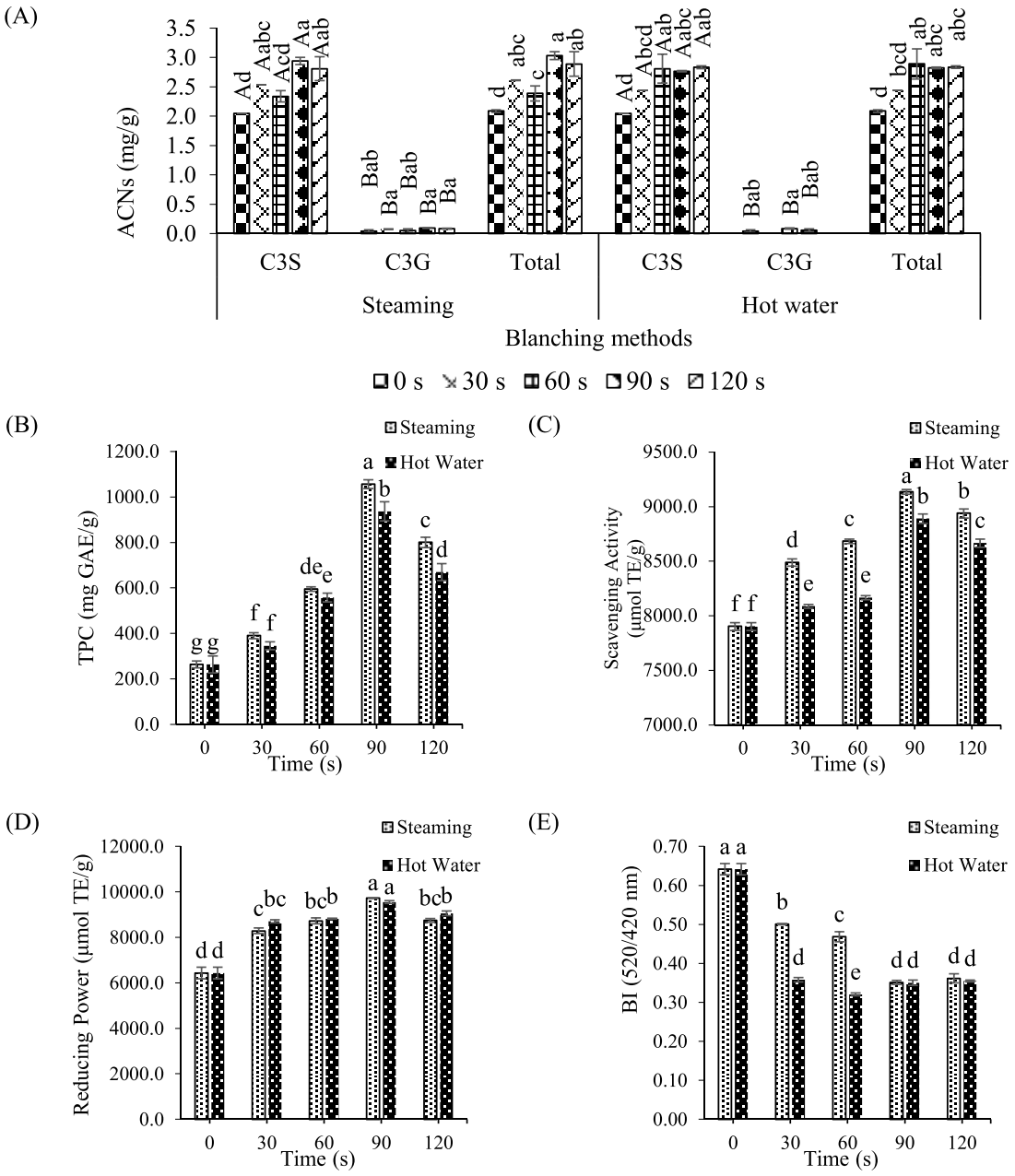

Figure 1. (A) ACNs composition in fresh, hot water and steam MP measured by HPLC. Values with the same letter $^{a-g}$ in each bar in the same group are not significantly different ($p > .05$), (B) TPC, (C) scavenging activity, (D) reducing power, and (E) BI of MP. Values with the same letter $^{A-D}$ between C3S and C3G within the same blanching techniques are not significantly different ($p > .05$).

of steam processes (Moscetti et al., 2019), as indicated by no significant differences ($p \geq .05$) in the inactivation of PPO during 30 and 60 s of steaming (Table 1). This was suspected because of heat-resistant PPO, which requires prolonged blanching for the denaturation of PPO's secondary and tertiary structures. Meanwhile, the low concentration of ACNs in hot water at 30 s was possibly due to the leaching of bound-free ACNs in the blanching medium, thereby triggering the degradation of ACNs. Prolonged blanching may increase the concentration of ACNs, as alteration of cell walls (lignin, cellulose, and hemicellulose) and membrane cells promotes the release of bound membrane ACNs, which facilitates solvent penetration into the matrix cells of MP, resulting in a high mass transfer of ACNs during extraction (Jiang et al., 2020).

Steaming was more effective in preserving C3G and had a less significant impact than hot water. These findings suggest that selecting a blanching method is critical when dealing with low concentrations of bioactive compounds, such as C3G in MP. Also, indirect contact with the blanching medium helps preserve water-soluble compounds, such as ACNs, by preventing them from leaching. In addition, a strong negative correlation between ACNs – PPO ($r = -0.646$, $p \leq .05$) and ACNs – POD ($r = -0.713$, $p \leq .05$) also suggests that the inactivation of PPO and POD enzymes ameliorates the ACNs content in MP.

Effect of blanching on TPC

Figure 1B shows the TPC in fresh, hot water and steam ranging from 263.36 to 1056.66 mg GAE/g. Significantly lowest TPC was observed in fresh MP and the highest in steam at 90 s, which was approximately 4.02-fold higher than fresh MP ($p \leq .05$). TPC values increased exponentially when prolonged blanching was applied, except at 120 s for hot water and steam. A significant reduction

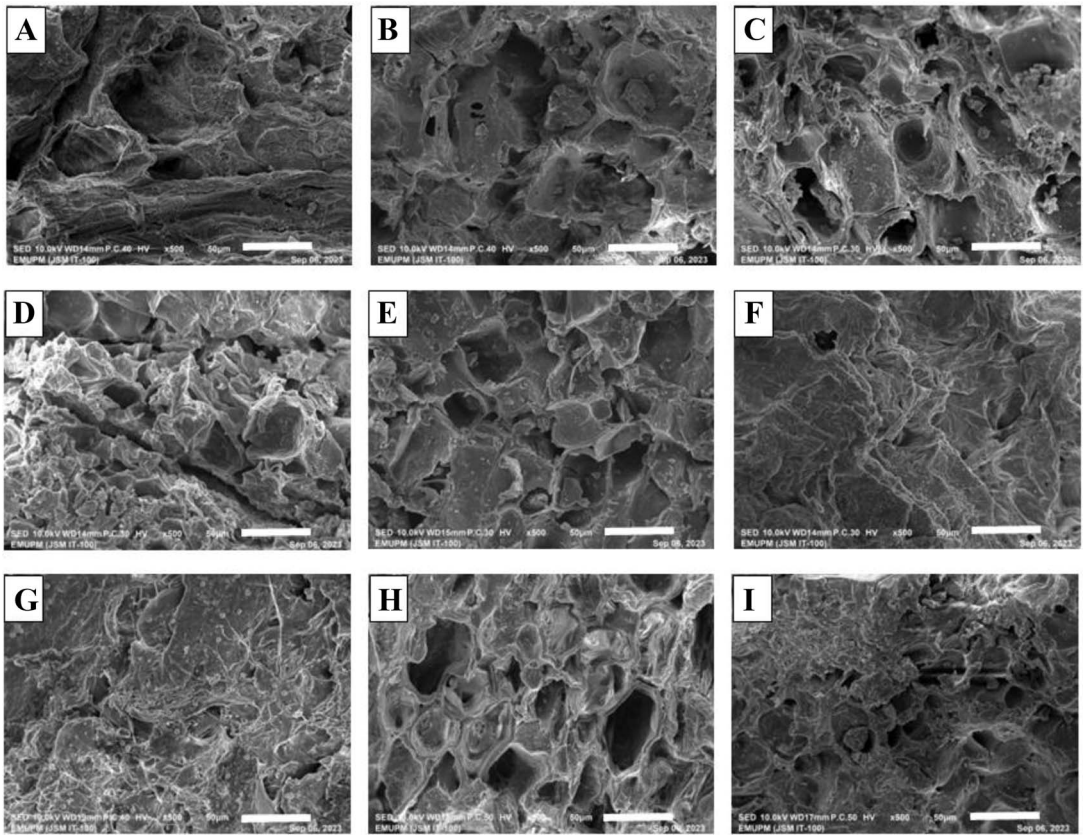

Figure 2. Morphological structure of MP in (A) fresh, hot water blanching at (B) 30 s, (C) 60 s, (D) 90 s, and (E) 120 s, and steam at (F) 30 s, (G) 60 s, (H) 90 s, and (I) 120 s.

of ~24.25% and ~28.52% in TPC at 120 s, compared to the highest TPC at 90 s in steam and hot water, respectively, was observed.

The improvement in the TPC over time can be attributed to several mechanisms during blanching. First, β-elimination degradation of pectin during hot water and steam softened MP cells, resulting in a porous cell membrane, destruction of cell wall components (pectin, hemicellulose, and cellulose), and increased release of bound phenolics due to bond alteration in MP (Wang et al., 2022). Second, the reduction in PPO and POD activity during blanching improved the phenolic content of MP. Although prolonged blanching could inactivate enzymes, it triggered the degradation of heat-sensitive phenolics, which explains the decline in TPC at 120 s in hot water and steam. It was also noted that the TPC value in steam was high for all blanching durations, suggesting that heat-sensitive phenolics were degraded during direct contact with hot water blanching.

The lowest TPC observed in fresh MP was associated with high levels of PPO and POD, which accelerated the degradation of phenolics in MP, as supported by a negative correlation between TPC, PPO, and POD ($r > -0.541$, $p \leq .05$). Thus, the negative correlation between phenolic content and PPO activity must be due to enzymatic browning. The correlation coefficients between TPC and ACNs ($r = 0.760$, $p \leq .05$) suggest that increasing ACNs is correlated with improving phenolic content in MP.

Effect of blanching on antioxidant activities

The scavenging activity and reducing power ranged from 7901.60 to 9135.60 μmol TE/g and 6439.00 to 9729.00 μmol TE/g, respectively (Figure 1C and D). Scavenging activity and reducing power were increased over time. A similar trend was observed, where significantly the highest values were observed at 90 s of steam and hot water before a significant reduction in antioxidant activities at 120 s ($p \leq .05$). However, steam treatment shows better improvement in the antioxidant activities of MP than hot water, suggesting the degradation of heat-sensitive compounds during hot water blanching.

The lowest scavenging activity and reducing power in fresh MP is associated with high levels of PPO, as confirmed by the negative correlation between PPO and scavenging activity ($r = -0.531$), reducing power ($r = -0.601$) at $p \leq .05$. PPO initiates their reactions in the presence of oxygen and phenolics by oxidizing phenolics into quinones and causing enzymatic browning (Zawawi et al., 2022). This reaction causes degradation of phenolics, resulting in a low antioxidant activity in the MP (Deylami et al., 2014, 2016). Both antioxidant activity gradually increased in steam and hot water blanching and decreased significantly after 90 s ($p \leq .05$). The scavenging activity in MP after steam outdid hot water blanching under the same blanching period, suggesting that heat-sensitive bound-free antioxidants possibly degraded in hot water (Deylami et al., 2016), and significantly increased in 90 s, indicating the release of bound-membrane bioactive compounds that were attributed to the scavenging activity.

The increase in scavenging activity in steam over fresh MP is due to softening and loosening of the cell wall, which affects the water- and non-soluble pectin of MP, thereby causing rapid de-esterification of pectin and enhancing tissue permeability (Wang et al., 2022). This enables the latent heat to diffuse into the cell and assists in releasing the antioxidant substances in MP. The improvement of antioxidant activities in MP suggests that an increase of TPC and ACNs ameliorates the scavenging activity

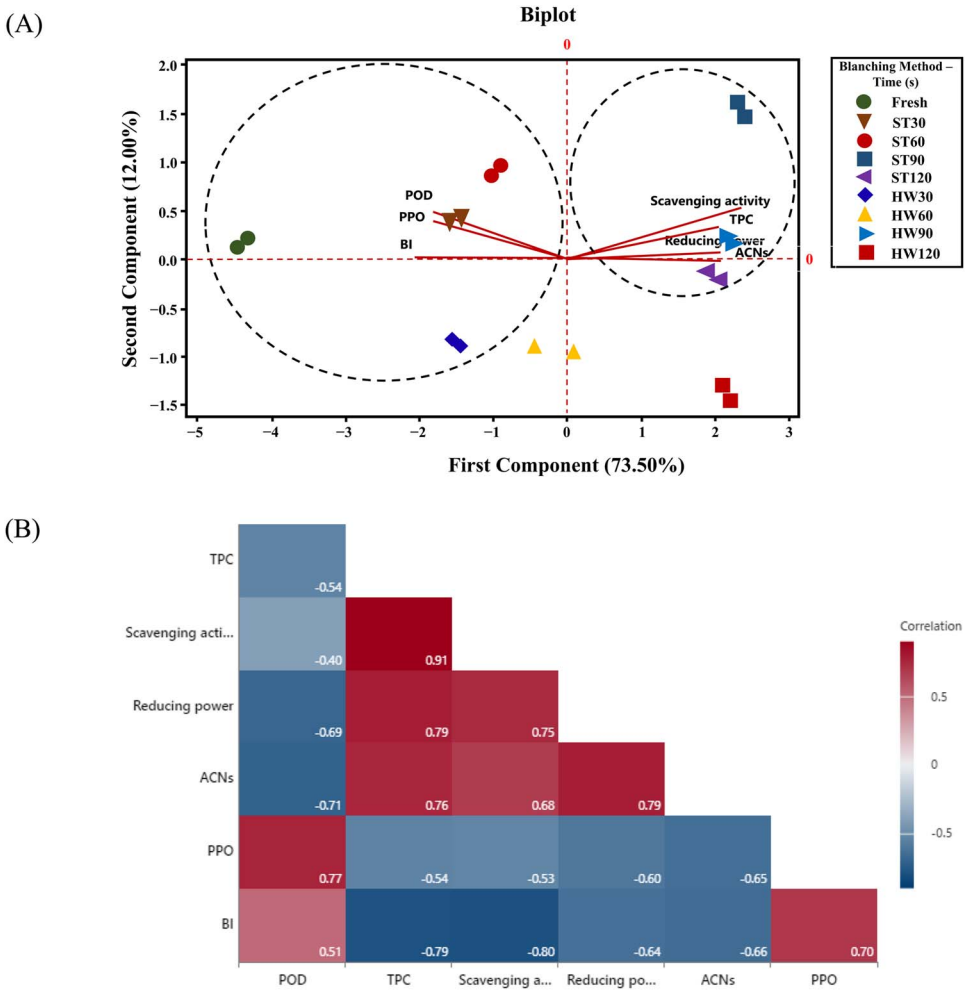

Figure 3. (A) Biplot PCA and (B) correlogram plot based on physicochemical properties, phenolic content, BI, and antioxidant activities of MP extracts at different blanching processes and times. BI = browning index; TPC = total phenolic content; ACNs = total anthocyanin content; PPO = polyphenol oxidase; POD = peroxidase; ST = steam blanching; HW = hot water; PC = principal component.

and reducing power of MP (Vo et al., 2023; Nawawi et al., 2023), as indicated by the correlation between scavenging activity and reducing power with TPC ($r = 0.906$, $r = 0.791$) and ACNs ($r = 0.677$, $r = 0.792$) at $p \leq .05$.

Effect of blanching on BI

The BI is a quantitative measure of the degree of browning caused by enzymatic or non-enzymatic reactions. Figure 1E shows that the highest BI was significantly observed in fresh MP, indicating high PPO and POD activities. Both hot water and steam reduced BI over time. At 30 and 60 s, hot water showed a drastic reduction of BI than steam. Direct contact of MP with hot water improved the reduction of BI, which could be the possible reason, as it enhanced the cell's heat transfer, increasing the rate of inactivation of PPO and POD.

Furthermore, there is no significant difference in BI value between hot water and steam after prolonged blanching to 90 and 120 s ($p > .05$), suggesting that inactivating PPO and POD reduces their biological activity. At this phase, the structure of PPO and POD is altered, slowing down the oxidizing process in MP. Therefore, less brown pigment formation (melanin) was observed. Overall, both blanching techniques can reduce the formation of melanin in MP. Hot water effectively reduces the BI in a shorter time than steam. However, it is comparable when prolonged blanching is applied, leading to the stabilization of BI.

Morphology structure of MP

The fresh MP cell structure was intact and less porous, suggesting a rigid cell wall and membrane cell of MP before the blanching process (see Figure 2A). The SEM images (Figure 2B–E) demonstrate that hot water blanching causes more rapid and extensive damage to the cellular structure of MPs. This is possibly due to the immersion of MP in the blanching medium, which accelerates the breakdown of cell wall components (cellulose, pectin, and hemicellulose) (Magangana et al., 2022).

The cell wall and membrane cells in MP were affected by steam blanching; however, the structural integrity of the cell was preserved over time, except at 90 and 120 s (Figure 2H and I), when large pores appeared in the cell due to deformation of the cell wall and membrane cell. This assisted the extractability of bioactive compounds in MP, explaining the high ACNs, TPC, and antioxidant activities in the steam-treated MP, especially at 90 s.

Steam blanching still causes damage, but at a slower rate, preserving more of the pericarp's structural integrity over time. These differences in morphological changes correlate with the blanching method's effectiveness in inactivating enzymes, improving bioactivity, and reducing the BI.

PCA and correlogram plot

Figure 3A illustrates the first (PC1) and second (PC2) components, explaining 73.50% and 12.00%, respectively. The total variance

of 85.50% in the biplot of PCA showed two clusters based on physicochemical properties: PPO and POD enzymes, ACNs, TPC, and antioxidant activities of fresh, hot water, and steam MP. The PC1 explains 73.50% of the total variability among the fresh and blanched MP, while the PC2 explains only 12.00%.

The observation indicates that MP blanched in steam and hot water at 90 was associated with high TPC, ACNs, and antioxidant activities along with a positive score in the PC1. Meanwhile, the positive scores along the PC2 could be related to high PPO, POD, and BI in fresh, steam, and hot water MP at 30 and 60 s, respectively.

Hot water and steam-treated samples at 90 s were positively distributed on the PC1 axis. Meanwhile, fresh, steam and hot water at 30 and 60 s were distributed on the positive side of the PC2 axis, suggesting that the PC1 is related to high phenolics and antioxidants in ST90 and HW90. Meanwhile, a positive score in PC2 presumably indicates enzyme-related indicators. The PCA results show that blanched and unblanched treatments for MP have significantly different physiochemical properties.

Conclusion

The blanching method and duration significantly influenced the MP's extract antioxidant properties and phenolic compounds. While blanching altered the membrane and cell wall morphology of MP, steaming proved more effective at preserving cell integrity. Hot water blanching was more efficient in inactivating PPO ($t_{1/2}$ = ~67.0 s) and POD ($t_{1/2}$ = ~33.6 s) activity than steam blanching (PPO: $t_{1/2}$ = ~113.7 s, POD: ~61.8 s), although both methods successfully inactivated >90% of POD. However, direct exposure to hot water caused substantial damage to heat-sensitive compounds in MP, particularly ACNs. As a result, steaming is crucial for preserving these heat-sensitive compounds in MP. Overall, steam blanching for 90 s was the most efficient process, retaining the highest levels of ACNs (3.03 ± 0.07 mg/g), TPC (1056.66 ± 19.08 mg GAE/g), and antioxidant activities (scavenging activity = 9135.60 ± 22.36 μmol TE/g and reducing power = 9729.00 ± 14.14 μmol TE/g). Additionally, steam blanching is more cost-effective than hot water blanching, which requires frequent replacement. These findings have potential applications in the food, cosmetic, and nutraceutical industries by enabling the use of MP ACNs as natural colourants.

Supplementary material

Supplementary material is available at *International Journal of Food Science and Technology* online.

Data availability

Research data are not shared.

Author contributions

Giroon Ijod (Formal analysis, Writing—original draft preparation, Software, Data curation, Visualization [lead]), Nur Izzati Mohamed Nawawi (Data curation, Software [equal]), Rabiha Sulaiman (Conceptualisation, Methodology, Writing—review & editing, Supervision [equal]), Nurul Izzah Khalid (Data curation, Writing—review & editing [equal]), Farooq Anwar (Writing—review & editing [equal]), Noranizan Mohd Adzahan (Conceptualisation, Methodology, Writing—review & editing, Supervision [equal]), and Ezzat Mohamad Azman (Conceptualisation, Methodology, Validation, Project administration, Funding acquisition [lead], Writing—review & editing [equal], Supervision [lead]). All authors reviewed the manuscript and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

Ethics approval was not required for this research.

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