

Effects of varied temperature on thermal inactivation of enzymes and phenolic composition in purple senggani (*Melastoma malabathricum*) flowers

Dea Tio Mareta¹, Widiastuti Setyaningsih^{1,2*}, Supriyadi Supriyadi^{1,2}, Umar Santoso¹

¹Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

²Center of Excellence for Indonesian Gastronomy, Jl. Flora No. 1 Bulaksumur, Yogyakarta 55281, Indonesia.

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ABSTRACT

Purple senggani (*Melastoma malabathricum*) is a tropical flowering plant valued for its antioxidant-rich phenolic compounds. Postharvest thermal pretreatments such as water blanching may inactivate enzymes but also affect phenolic stability. This study aimed to evaluate the effects of water blanching at (70, 80, and 90 °C) for 30–180 s on enzymatic activity and phenolic retention in purple senggani flowers. Polyphenol oxidase (PPO) and peroxidase (POD) activities were assessed spectrophotometrically. Ultrasound-assisted extraction followed by high-performance liquid chromatography coupled with a diode array detector analysis was used to identify and quantify key flavonoids. Total phenolic content (TPC) and total antioxidant capacity (TAC) were also measured. The results showed that PPO and POD activities decreased significantly with increasing blanching intensity, with maximum inactivation of 93% and 75%, respectively. However, blanching also led to substantial degradation of flavonoids such as rutin, quercetin 3-glucoside, trifolin, and astragaloside, with several compounds falling below detection levels. These reductions were reflected in sharp declines in TPC and TAC values. The study highlights a trade-off between enzyme inactivation and phytochemical preservation. Further comparative studies are needed to identify optimal processing conditions that maintain both enzyme control and antioxidant integrity in phenolic-rich plant materials.

1. INTRODUCTION

Melastoma malabathricum, commonly known as senggani, is a tropical flowering plant native to Southeast Asia [1]. Its flowers exhibit color variations ranging from white [2] to deep purple [3,4], with the purple variety particularly rich in phenolic compounds, including flavonoids such as rutin, quercetin 3-glucoside, and astragaloside. Figure 1 illustrates the morphological characteristics of the purple senggani flower, which displays vibrant purple petals and a distinct floral structure reflecting its ornamental and medicinal significance. This striking appearance corresponds to its high content of bioactive pigments and phenolic constituents. Conventionally, senggani has been widely used in folk medicine due to its therapeutic properties, including anti-inflammatory, antimicrobial, and wound-healing effects [5]. These bioactivities are mainly attributed to the presence of secondary metabolites – primarily phenolic compounds – known for their strong antioxidant potential [6].

Phenolic compounds play crucial roles in plant defense, contributing to color, taste, and stability in plant-derived products [7,8]. Among

them, flavonoids, phenolic acids, tannins, and lignins exhibit diverse structural complexity and biological activities [9]. However, phenolic compounds are susceptible to environmental and processing conditions. Thermal treatments, such as blanching, can induce degradation, oxidation, or polymerization of phenolics, potentially reducing their antioxidant efficacy [10]. While some flavonoids remain relatively heat-stable, others, such as anthocyanins, are particularly sensitive to thermal processing [11]. For instance, heating can cleave glycosidic bonds in flavonoids, generating aglycones with altered bioactivity [12]. The extent of degradation largely depends on temperature and exposure time, where moderate heating (e.g., blanching at 70°C for short durations) may preserve phenolics, whereas prolonged or high-temperature treatment (e.g., boiling at 100°C for extended periods) often leads to considerable losses [13].

Blanching is commonly applied as a pretreatment before drying to inactivate enzymes, shorten drying time, and suppress microbial activities [14]. In particular, polyphenol oxidase (PPO) and peroxidase (POD) are key enzymes responsible for enzymatic browning and phytochemical degradation in plant materials [15]. Their inactivation during blanching is essential for maintaining the quality and bioactivity of phenolic-rich plants. Interestingly, while enzyme inactivation may help preserve target compounds during storage, blanching itself may also cause phenolic loss through thermal degradation or leaching into the blanching medium.

*Corresponding Author:

Widiastuti Setyaningsih,

Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

E-mail: widiastuti.setyaningsih@ugm.ac.id



Figure 1: Purple flower of *Melastoma malabathricum* (senggani) collected from the special region of Yogyakarta, Indonesia.

Previous studies suggest that blanched plant materials may yield higher metabolite extraction due to enzyme inactivation [16]; however, there remains a knowledge gap regarding how blanching influences specific flavonoid compounds and antioxidant capacity in *M. malabathricum*. Therefore, this study aimed to evaluate the effects of water blanching at different temperatures and times on PPO and POD activity, individual phenolic profiles, total phenolic content (TPC), and total antioxidant capacity (TAC) in purple senggani flowers. The findings are expected to provide evidence-based recommendations for optimal thermal pretreatment to preserve the functional quality of this underutilized but bioactive-rich plant.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

High-performance liquid chromatography (HPLC)-grade methanol and acetic acid were purchased from Merck KGaA (Darmstadt, Germany). Water for injection was purchased from PT. Ikapharmindo Putramas Pharmaceutical Laboratories (Jakarta, Indonesia). Two flavonoid standards, quercetin 3-glucoside (CAS number: 482-35-9; purity: $\geq 98\%$) and rutin (CAS number: 207671-50-9; purity: $\geq 94\%$), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant Material and Sample Preparation

Purple *M. malabathricum* flowers were harvested from the Special Region of Yogyakarta, Indonesia (coordinates: 7°35'22.9"S 110°27'33.2"E). The flowers were picked in the morning and transported in sealed plastic bags for immediate processing. Water blanching was performed following the method described by Magangana *et al.* [16], with slight modifications, in a water bath (Mettler, Schwabach, Germany) at three temperatures: 70°C, 80°C, and 90°C for up to 3 min. Subsamples were taken every 30 s, rapidly cooled in ice water, and stored for further analysis. Unblanched flowers were used as the control.

All flower samples were frozen at -20°C for 2 days in a freezer (MDF-U55V-PE, Panasonic, Japan), followed by freeze-drying at -45°C for 2 days using a CoolSafe 4–15 L freeze dryer (LaboGene, Denmark). The freeze-dried flowers were ground into a fine powder using a laboratory grinder (Klaz, China) and stored at -20°C until further analysis.

All treatments were conducted using three independent biological replicates ($n = 3$), each consisting of flower samples collected on different days. For each biological replicate, all measurements (enzyme activity, HPLC analysis, TPC, and TAC) were performed in duplicate as technical replicates.

2.3. Determination of PPO Activity

PPO activity was determined according to the method described by Golan *et al.* [17], with slight modifications. A 15 g sample was blended with 60 mL deionized water for 30–60 s at 0°C , filtered, and kept in an ice bath. For analysis, 2.6 mL of 0.01 M acetate buffer (pH 5.0), 0.3 mL of 0.5 M pyrocatechol, and 0.1 mL enzyme extract were mixed, and the absorbance was monitored at 420 nm for 3 min. One unit of activity was defined as an absorbance change of 0.001/min/mL extract.

2.4. Determination of POD Activity

POD activity was analyzed using the spectrophotometric method described by Flick Jr. *et al.* [18], with minor modifications. Five grams of fresh flowers were blended with 0.1 M phosphate buffer (pH 6.5) and filtered. The filtrate was centrifuged at 3000 rpm for 5 min. Then, 10 mL of 0.05 M phosphate buffer, 1 mL of 0.5% guaiacol in 5% ethanol, and 1 mL of 0.3% hydrogen peroxide were added to the supernatant. Absorbance at 470 nm was recorded, and activity was expressed as absorbance change per minute.

2.5. Ultrasound-assisted Extraction (UAE)

Phenolic compounds were extracted using an ultrasonic probe system (UP200St, Hielscher, Germany; 26 kHz, 200 W, 7 mm probe) following Mareta *et al.* [4]. Extraction used 60% methanol in water, a 1:10 g/mL sample-to-solvent ratio, 60% pulse-duty cycle, for 15 min at 70°C using a recirculating bath (Frigitem-TFT-10, J.P. Selecta, Spain). Extracts were centrifuged at 4000 rpm for 15 min at 4°C (Sorvall ST-8R, Thermo Fisher, Germany), and the final volume adjusted to 25 mL with the extraction solvent.

2.6. HPLC-Diode Array Detector (DAD) Analysis of Phenolic Compounds

HPLC-DAD (Shimadzu Corp., Kyoto, Japan) system was used to analyze the senggani flower extract. The flavonoid compounds were isolated using a C18 reverse-phase column (5 μm , 4.6150 mm, Shimadzu Corp., Kyoto, Japan), maintained at a column oven temperature of 30°C . The method employed a mobile phase that included phase A (5% methanol, 2% acetic acid, and 93% water) and phase B (88% methanol, 2% acetic acid, and 10% water). The elution gradient (time, % solvent B) was programmed as follows: 0 min at 0%; 20 min at 100%; 25 min at 100%; and 27 min at 0%, with a flow rate of 1 mL/min [4]. To filter the extracts, a 0.45 μm nylon syringe filter was used. The chromatograms were analyzed by LabSolutions CS (Shimadzu Corp., Kyoto, Japan). DAD performed a complete scan for the spectrum (200–400 nm) to identify the flavonoid compounds. Identification was performed by comparing the spectrum and retention time of the compounds in the sample with those of standard compounds. The compounds (rutin and quercetin 3-glucoside) were quantified using a 260 nm wavelength. Only rutin and quercetin 3-glucoside were quantified using authentic standards. Other compounds (trifolin, astragal, and flavonoid derivative) were tentatively identified based on literature spectra and ultraviolet visible (UV-Vis) characteristics, and quantified as rutin equivalents [4,19].

2.7. Determination of TPC

TPC was measured using a modified Folin-Ciocalteu spectrophotometric method described by Bobková *et al.* [20]. A 1 mL aliquot of senggani flower extract, diluted 100-fold, was combined with 0.5 mL of 10% Folin-Ciocalteu reagent. This mixture was allowed to sit for 10 min before adding 5 mL of 2% sodium carbonate solution. Following an incubation period of 40–60 min, the absorbance was recorded at 765 nm using a UV-Vis Spectrophotometer (UV-2450, Shimadzu Corporation, Kyoto, Japan).

To prepare a standard solution for gallic acid, 0.1 g of gallic acid was dissolved in distilled water to achieve a final volume of 100 mL, resulting in a stock solution. From this stock, 1 mL was diluted to 200 mL with distilled water to create a working solution. A calibration curve was established, covering a range of 20–100 mg/L of gallic acid, with a coefficient of determination (R^2) of 0.996. Blanks were prepared using Folin-Ciocalteu reagent and distilled water without any standards or extracts. The TPC values were reported as milligrams of gallic acid equivalent (GAE) per gram of sample (mg GAE/g).

2.8. Determination of TAC

The TAC was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, based on the method described by Brand-Williams *et al.*, [21] with minor adjustments. A 0.1 mL sample of senggani extract, diluted 100-fold, was combined with a 0.1 mM DPPH solution. The mixture was allowed to stand for 40 min to reach equilibrium. The absorbance was measured at 517 nm with a UV-Vis Spectrophotometer (UV-2450, Shimadzu Corporation, Kyoto, Japan). The TAC was reported as % radical scavenging activity (% RSA), calculated using Equation 1.

$$\%RSA = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

The limits of detection (LOD) of the DPPH assay used in this study was approximately 0.25% RSA. Values below this threshold were considered undetectable and are reported as <LOD.

2.9. Replication and Statistical Analysis

All experiments were performed in triplicate using three independent biological replicates ($n = 3$). Each biological replicate represented a distinct batch of freshly harvested senggani flowers, individually processed for all blanching treatments and the control. Within each biological replicate, all analytical determinations – including PPO and POD enzymatic activities, HPLC-DAD quantification of individual phenolic compounds, TPC, and TAC – were conducted in technical triplicate. Results are expressed as mean \pm standard deviation (SD). Statistical significance among treatments was evaluated by one-way analysis of variance followed by Tukey's honestly significant difference *post hoc* test at a confidence level of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of Water Blanching on PPO and POD Activity

PPO and POD are key enzymes that contribute to the degradation of phytochemicals in plants, leading to enzymatic browning and reduced antioxidant activity [15]. PPO catalyzes the oxidation of phenolic compounds into quinones, which polymerize into brown pigments, while POD uses hydrogen peroxide to oxidize a broad range of substrates [22,23]. The suppression of both enzymes is critical to maintaining the quality of phenolic-rich plant materials during processing.

The results [Figure 2] showed that PPO activity was significantly reduced following blanching at all temperature-time combinations. At 70°C for 30 s, PPO activity decreased by 37%, and this reduction progressed with increased temperature and time, reaching up to 93% inactivation at 90°C for 180 s. This pattern is consistent with previous studies indicating that PPO is thermolabile, with optimal activity at 30–40°C and rapid deactivation at higher temperatures [24,25].

POD activity followed a similar but less dramatic trend. Blanching at 70°C for 30 s caused an 8% reduction in activity, which increased to 75% inactivation at 90°C for 180 s. The relatively greater thermal stability of POD compared to PPO is in line with earlier reports [26,27]. These findings suggest that water blanching is effective in reducing enzymatic browning potential in senggani flowers. However, while enzyme inactivation is desirable for preventing oxidative degradation, the subsequent sections reveal that blanching has notable consequences on the chemical composition and functionality of the flowers.

3.2. Changes in Individual Phenolic Compounds

The individual phenolic compounds were identified using HPLC-DAD by matching each compound's UV-Vis spectra and retention times with the standards. Five compounds were detected as rutin, quercetin 3-glucoside, trifolin, astragalins, and flavonoid derivative. Figure 3 shows the HPLC chromatogram of senggani extracts at 260 nm [Figure 3a] and the UV-Vis spectra of the five flavonoid compounds. UV-Vis spectra of rutin and quercetin 3-glucoside [Figure 3b and c] were obtained by matching the standards with the samples. Trifolin and astragalins [Figure 3d and 3e] were determined by matching the UV-Vis spectra obtained with the UV-Vis spectra in the study by Noh *et al.* [19]. This identification was corroborated by high-resolution

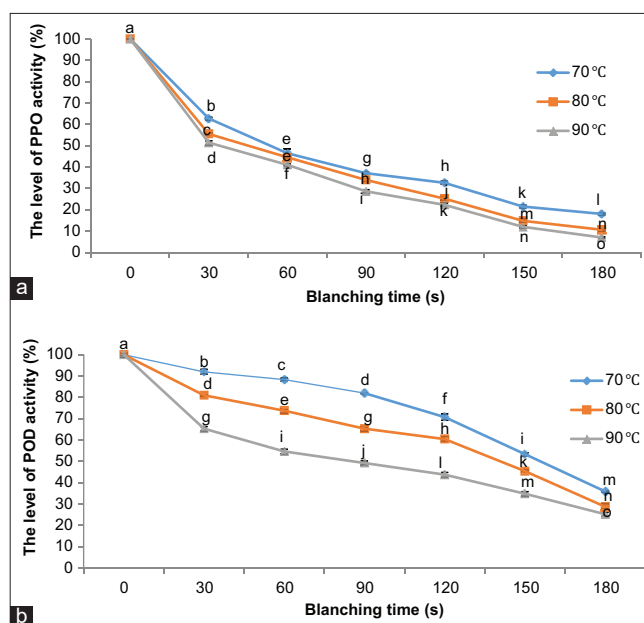


Figure 2: Changes in (a) polyphenol oxidase (PPO) and (b) peroxidase (POD) activity in *Melastoma malabathricum* (purple senggani) flowers after water blanching at different temperatures (70, 80, and 90 °C) and durations (30–180 s). PPO and POD activity decreased significantly with increased blanching temperature and time. Different lowercase letters (a–o) above the markers indicate statistically significant differences ($P < 0.05$) according to Tukey's HSD *post hoc* test. Error bars represent standard deviations.

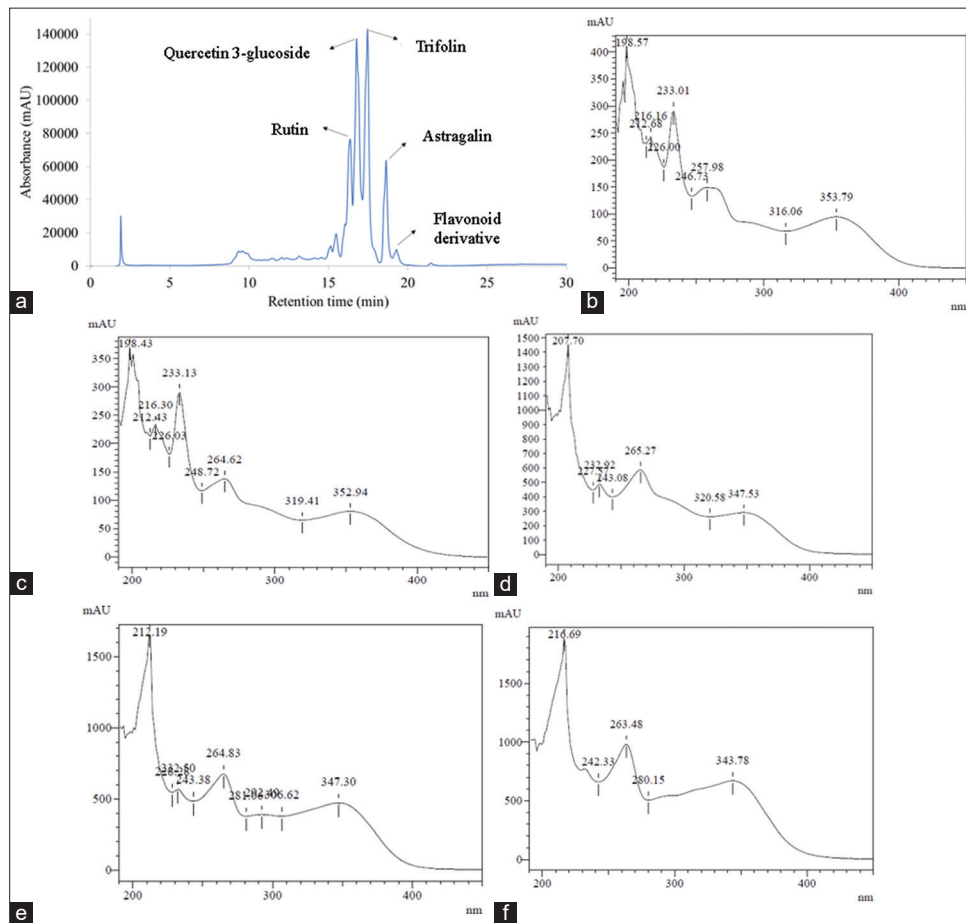


Figure 3: High-performance liquid chromatography (HPLC) chromatogram of senggani extracts at 260 nm (a) and ultraviolet visible (UV-Vis) spectra of individual phenolic compounds detected in *Melastoma malabathricum* (purple senggani) flower extract using HPLC-diode array detector: rutin (b), quercetin 3-glucoside (c), trifolin (d), astragalol (e), and a flavonoid derivative (f). The spectra indicate the characteristic absorbance features of flavonoid compounds in the UV region.

mass spectrometry profiling of *M. malabathricum* (senggani) flower extracts, as documented in our prior study [4]. The last compound exhibited a UV-Vis spectral pattern similar to that of typical flavonoid compounds; however, its exact structure has not yet been identified and was therefore designated as a tentatively identified flavonoid derivative [Figure 3f].

Rutin and quercetin 3-glucoside standard calibration curves with the range of 0.5–40 ppm were arranged to cover the concentration range of the sample. Table 1 shows the performance of the HPLC-DAD method for determining rutin and quercetin 3-glucoside. Regression analysis was employed to quantify the individual phenolic compounds in the sample and to assess the coefficient of determination (R^2) to demonstrate linearity within the examined range. The results showed high R^2 values for rutin (0.994) and quercetin 3-glucoside (0.999). The LOD and limit of quantification were calculated using the slope and the SD at the origin of the regression analysis of the calibration curve. Therefore, the chromatographic method utilized in this study proved reliable for identifying the compounds in the senggani flowers.

Blanching markedly decreased the concentration of these phenolics [Table 2]. Under control conditions (no blanching), rutin and quercetin 3-glucoside were the dominant flavonoids, with concentrations of 510.74 ± 8.96 and 686.43 ± 22.87 $\mu\text{g/g}$, respectively. However, as blanching time increased, the concentration of these flavonoids clearly

Table 1: Analytical performance of the HPLC-DAD method for the quantification of rutin and quercetin 3-glucoside.

Phenolic compounds	Range (0.5–40 ppm)		LOD (ppm)	LOQ (ppm)
	Linear equation	R^2		
Rutin	$y=26020x+4543.6$	0.994	1.20	3.63
Quercetin 3- glucoside	$y=31823x-5579.7$	0.999	0.73	2.22

HPLC-DAD: High-performance liquid chromatography-diode array detector, LOD: Limit of detection, LOQ: Limit of quantification

reduced, particularly at higher temperatures. At 70°C, after 30 s, the rutin concentration dropped significantly to 119.90 ± 6.65 $\mu\text{g/g}$ and quercetin 3-glucoside decreased to 121.88 ± 5.76 $\mu\text{g/g}$. Longer blanching durations and higher temperatures led to further losses, with most flavonoids falling below the LOD beyond 120 s of blanching.

At 80°C, a similar trend was observed. The rutin level fell to 112.57 ± 5.05 $\mu\text{g/g}$ after 30 s of blanching, while quercetin 3-glucoside decreased to 105.05 ± 0.92 $\mu\text{g/g}$. Again, longer blanching times led to levels below LOD. At the higher temperature (90°C), the degradation of phenolic compounds was even more pronounced. After 30 s, the rutin concentration was reduced to 93.50 ± 6.94 $\mu\text{g/g}$, and quercetin 3-glucoside dropped to 46.01 ± 3.13 $\mu\text{g/g}$. Prolonging the time to 120 s or more resulted in undetectable levels of these compounds.

Table 2: Concentration of individual phenolic compounds in purple senggani flowers after water blanching at various temperatures and durations.

Blanching condition	Phenolic compounds ($\mu\text{g/g}$)				
	Rutin	Quercetin 3-glucoside	Trifolin**	Astragalín**	Flavonoid derivative**
Control	510.74 \pm 8.96 ^f	686.43 \pm 22.87 ^e	569.34 \pm 13.49 ^d	590.35 \pm 11.53 ^d	414.26 \pm 30.06 ^a
70°C, 30 s	119.90 \pm 6.65 ^e	121.88 \pm 5.76 ^f	54.75 \pm 6.43 ^{b*}	48.72 \pm 8.37 ^{bc*}	<LOD
70°C, 60 s	105.40 \pm 1.97 ^d	91.30 \pm 0.15 ^{cd}	87.15 \pm 5.30 ^{e*}	43.84 \pm 5.99 ^{ab*}	<LOD
70°C, 90 s	67.59 \pm 3.98 ^{b*}	42.23 \pm 2.22 ^{b*}	<LOD	<LOD	<LOD
70°C, 120 s	<LOD	<LOD	<LOD	<LOD	<LOD
70°C, 150 s	<LOD	<LOD	<LOD	<LOD	<LOD
70°C, 180 s	<LOD	<LOD	<LOD	<LOD	<LOD
80°C, 30 s	112.57 \pm 5.05 ^{de}	105.05 \pm 0.92 ^e	53.27 \pm 3.78 ^{b*}	57.77 \pm 2.79 ^{c*}	<LOD
80°C, 60 s	108.47 \pm 8.59 ^d	101.91 \pm 4.12 ^{de}	47.88 \pm 7.47 ^{b*}	50.04 \pm 8.26 ^{bc*}	<LOD
80°C, 90 s	90.82 \pm 9.08 ^c	87.22 \pm 6.82 ^c	37.43 \pm 3.44 ^{a*}	36.57 \pm 7.60 ^{a*}	<LOD
80°C, 120 s	<LOD	<LOD	<LOD	<LOD	<LOD
80°C, 150 s	<LOD	<LOD	<LOD	<LOD	<LOD
80°C, 180 s	<LOD	<LOD	<LOD	<LOD	<LOD
90°C, 30 s	93.50 \pm 6.9 ^c	46.01 \pm 3.13 ^b	<LOD	<LOD	<LOD
90°C, 60 s	85.08 \pm 2.66 ^c	37.23 \pm 2.30 ^{ab*}	<LOD	<LOD	<LOD
90°C, 90 s	48.36 \pm 4.75 ^{a*}	28.89 \pm 1.25 ^{a*}	<LOD	<LOD	<LOD
90°C, 120 s	<LOD	<LOD	<LOD	<LOD	<LOD
90°C, 150 s	<LOD	<LOD	<LOD	<LOD	<LOD
90°C, 180 s	<LOD	<LOD	<LOD	<LOD	<LOD

Values are presented as mean \pm standard deviation. Different lowercase letters within the same column indicate statistically significant differences ($P<0.05$) based on Tukey's honestly significant difference *post hoc* test. LOD: Limit of detection, LOQ: Limit of quantification. Values between LOD and LOQ are marked with an asterisk (*); compound concentration expressed as rutin equivalents are marked with two asterisks (**). LOD of rutin=24.00 $\mu\text{g/g}$; LOD of quercetin 3-glucoside=14.60 $\mu\text{g/g}$. Trifolin, astragalín, and the flavonoid derivative were reported as rutin equivalents and share the same detection threshold

Flavonoid compounds such as rutin, quercetin 3-glucoside, trifolin, and astragalín are known for their antioxidant properties and contribute significantly to the health benefits of plant extracts [19]. However, these compounds are susceptible to thermal treatment [28], as demonstrated in this study, which analyzed the effects of blanching at various temperatures and durations on the phenolic content in senggani flowers.

This rapid degradation can be attributed to both thermal decomposition and leaching into the blanching water. Although flavonoids are generally poorly soluble in water, heat increases their solubility by weakening hydrogen bonding and enhancing molecular motion [29-31]. Consequently, flavonoids may dissolve more readily into the blanching medium. Previous studies on other plant matrices have reported similar trends, where prolonged boiling or steaming results in the loss of water-soluble antioxidants [32].

Furthermore, heat may cleave glycosidic bonds in flavonoid glycosides such as quercetin 3-glucoside and rutin, potentially forming aglycones with reduced solubility or reactivity [12]. Similar declines in flavonoid content following thermal treatment have been documented in other phenolic-rich plants [28]. These results affirm that while blanching suppresses enzymatic degradation, it concurrently accelerates the loss of valuable phenolic compounds.

In this study, trifolin, astragalín, and flavonoid derivatives were quantified using a standard calibration curve for rutin (equivalent to rutin). Although their relative changes are meaningful, absolute quantification should be interpreted cautiously. Future work needs to include Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis and standard-based quantification to increase confidence in compound determination.

3.3. Impact on TPC and Antioxidant Capacity

Phenolic compounds, particularly flavonoids, contribute substantially to the antioxidant properties of plant extracts [7,33,34]. While some plants have shown increased phenolic levels after blanching due to enhanced release of bound phenolics [35,36], others experienced a decrease [37] including purple senggani that consistently exhibited reductions [Figure 4]. This may be due to the predominance of free flavonoids in senggani, which are more prone to degradation or solubilization, rather than thermal release from conjugated forms.

The TPC in unblanched samples was 441.12 \pm 5.95 mg GAE/g, but dropped to 92.52 \pm 1.98 mg GAE/g after blanching at 70°C for 30 s. At the highest treatment (90°C for 180 s), TPC declined sharply to 4.21 \pm 0.00 mg GAE/g. A similar trend was observed for TAC, which dropped from 55.60 \pm 1.53% in unblanched flowers to below the detection limit at the most intense blanching condition (90°C for 150 s).

The decrease in the TPC and TAC values of senggani flowers due to the blanching treatment results from the reduction in the value of individual phenolic compounds, which are damaged due to high temperatures and dissolve in water during the blanching treatment. The phenolic compounds in senggani flowers, most of which are flavonoids (rutin, quercetin 3-glucosides, trifolin, astragalín), are polar, so they easily dissolve in polar solvents such as water [38]. These results aligned with our previous research, which stated that the highest TPC value was produced when senggani flowers were extracted using water as a solvent, compared to methanol, ethanol, and hexane [4].

The reduction in TPC and TAC values may also result from the degradation of other phenolic components, such as anthocyanins and

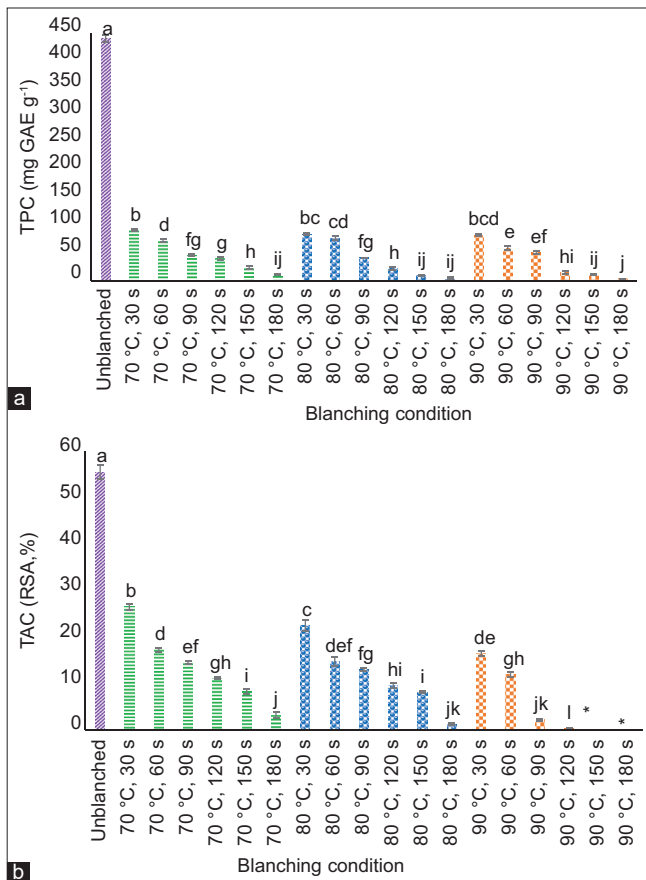


Figure 4: Changes in (a) total phenolic content (TPC) and (b) total antioxidant capacity (TAC) of *Melastoma malabathricum* (purple senggani) flowers after water blanching at 70, 80, and 90°C for 30–180 s. Different lowercase letters (a-l) above the bars indicate statistically significant differences ($P < 0.05$) based on Tukey's honestly significant difference *post hoc* test. Error bars represent standard deviations. Values below the limit of detection (LOD) are marked with an asterisk (*).

tannins present in senggani flowers [3,39]. These compounds are highly water-soluble and thermally labile, rendering them susceptible to loss during blanching processes [40-43]. However, given the focus of this study on flavonoid compounds, future investigations should examine the stability of anthocyanin and tannin pigments in senggani flowers.

3.4. Interpreting the Trade-off Between Enzyme Inactivation and Phenolic Loss

The dual role of blanching – suppressing enzyme activity while risking phytochemical degradation – poses a significant challenge in optimizing postharvest treatment. Although enzyme inactivation is beneficial for preventing browning and prolonging shelf life, excessive thermal exposure it also reduces antioxidant capacity and phenolic content, which are important indicators of phytochemical preservation. The results of this study affirm that higher blanching temperatures and longer durations should be avoided for phenolic-rich materials like senggani flowers.

Although this study did not compare different processing methods, previous studies have reported better phenolic retention under milder or non-thermal treatments. Future research is recommended to investigate techniques such as steam blanching, freeze-drying, or cold

storage to evaluate their potential in enhancing flavonoid retention while minimizing thermal degradation, as observed in other botanical matrices [10,12,32]. This study did not include blanching-only (non-immersion heat) or blanching-water analysis controls, which limits our ability to distinguish between thermal degradation and leaching as mechanisms of flavonoid loss. Future studies should address this gap to clarify compound fate during blanching.

Taken together, this study offers new insights into the processing sensitivity of *M. malabathricum* flowers. By systematically evaluating enzyme activity and phytochemical composition under various blanching conditions, we provide practical recommendations for preserving their nutritional and functional properties in future applications.

4. CONCLUSION

Water blanching effectively inactivated PPO and POD enzymes in purple *M. malabathricum* (senggani) flowers, with up to 93% and 75% reduction in activity, respectively. However, this thermal treatment also led to a significant decline in individual flavonoid compounds – such as rutin, quercetin 3-glucoside, trifolin, and astragalin – as well as in TPC and TAC. Most flavonoids fell below detection limits after blanching at higher temperatures and longer durations, indicating their thermal sensitivity and solubility in hot water.

These findings highlighted a critical trade-off between enzyme inactivation and phytochemical preservation. Although water blanching was effective in reducing enzyme activity, it also led to considerable losses in phenolic compounds and antioxidant capacity. Thus, blanching as a pretreatment should be applied cautiously, and future comparative studies are needed to evaluate alternative techniques such as freeze-drying or cold storage for optimal preservation of phenolic-rich plant materials. The results of this study provide evidence-based guidance for optimizing postharvest processing strategies for phenolic-rich plant materials.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data are available from the authors and will be provided upon reasonable request.

10. PUBLISHER'S NOTE

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The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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