

## EFFECTS OF PROCESSING PARAMETERS ON BIOACTIVE COMPOUNDS AND FUNCTIONAL PROPERTIES OF GUAVA (*PSIDIUM GUAJAVA* L.) LEAF TEA BAGS

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### ABSTRACT

**Background.** Guava (*Psidium guajava* L.) leaves are rich in polyphenols and flavonoids with established antioxidant and antidiabetic properties. However, the quality and functionality of guava leaf tea are influenced by processing parameters, including leaf maturity, drying temperature, grinding size, and brewing conditions. This study aimed to determine the optimal processing conditions for enhancing the bioactive compound content and functional properties of guava leaf tea bags.

**Materials and methods.** Leaves at three maturity stages (young, mature, and old) were dried under different temperatures (40–70°C and cooling drying at 45°C), ground into four particle size ranges, and packed into tea bags. The teas were brewed at water temperatures between 70°C and 100°C. Total polyphenol content (TPC) and total flavonoid content (TFC) were quantified spectrophotometrically. Antioxidant capacity was assessed by DPPH radical scavenging, while  $\alpha$ -glucosidase inhibitory activity was measured to evaluate antidiabetic potential. Sensory attributes were evaluated by a trained panel.

**Results.** Drying at 50°C retained the highest TPC (176.19 mg GAE/g dw) and TFC (9.72 mg QE/g dw), while grinding to 0.5–1 mm enhanced compound extraction without compromising sensory quality. Brewing at 100°C yielded the greatest concentrations of bioactive compounds; however, infusions prepared at 90°C received the highest flavor and aroma scores. The optimized tea infusion also exhibited strong antioxidant activity (35.92% DPPH inhibition) and potent  $\alpha$ -glucosidase inhibitory activity ( $IC_{50} = 6.97 \mu\text{g/mL}$ ), approximately 17 times more effective than acarbose.

**Conclusion.** These findings indicate that guava leaf tea, when processed under optimal conditions, has the potential to serve as a functional beverage with significant health-promoting properties, particularly through its antioxidant activity and blood sugar-regulating effects.

**Keywords:** guava leaf tea, polyphenols, flavonoids, antioxidant activity,  $\alpha$ -Glucosidase inhibition

### INTRODUCTION

Tea is among the most widely consumed beverages worldwide and occupies a prominent place in daily life across many cultures. Beyond traditional varieties such as green and black tea, the development of herbal and functional teas has attracted growing attention,

driven by increasing consumer demand for health-promoting beverages. Among various product formats, tea bags have become particularly popular due to their convenience and ease of preparation.

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To expand product diversity, manufacturers are increasingly incorporating a broad range of plant-based ingredients, including grains (e.g., barley, brown rice, black beans) and herbs. These natural materials are rich in bioactive compounds associated with diverse health benefits, such as antioxidant, anti-inflammatory, and antidiabetic effects. Numerous studies have reported the antioxidant activities of various plant extracts used in herbal tea production, including *Coleus aromaticus* (Kumaran and Karunakaran, 2006), green tea (Gramza-Michałowska et al., 2007), guava (*Psidium guajava* L.) leaves (Melo et al., 2020), tropical almond leaves (Nga et al., 2024), and purple rice (Loan et al., 2025). Previous research has also demonstrated their antioxidant (Luo et al., 2019), antidiabetic (Oh et al., 2005; Luo et al., 2019), antibacterial (Jaiarj et al., 1999), anticough, and antispasmodic (Lozoya et al., 2002) properties.

These functional effects are primarily attributed to bioactive constituents such as quercetin, other flavonoids, and phenolic acids (e.g., gallic, ferulic, and caffeic acids) (Díaz-de-Cerio et al., 2017; Kumar et al., 2021). As a result, guava leaf extract has been widely investigated as a promising source for functional food and nutraceutical applications. However, the concentrations and bioactivity of its compounds in tea beverages are influenced by several processing factors. These include leaf maturity at harvest, drying temperature and duration, grinding particle size, and brewing conditions—all of which can significantly affect the antioxidant capacity and sensory quality of the final product. For instance, variations in harvest age have been shown to alter polyphenol and flavonoid levels in leaf extracts (Nantitanon et al., 2010; Çetin-kaya and Kulak, 2016; Anwar et al., 2017), while high drying temperatures can degrade phenolic compounds (Nguyen et al., 2022). Similarly, water temperature and brewing time influence the extraction efficiency of bioactive compounds in green and black teas (Chong and Nyam, 2022; Del Caro et al., 2022; Winiarska-Mieczan and Baranowska-Wójcik, 2024), although studies focusing on herbal teas remain limited. Despite the well-documented antioxidant and antidiabetic potential of guava leaf extracts, research on the processing factors that influence the quality and functionality of guava leaf tea remains scarce. In particular, limited attention has been given to the production of guava

leaf tea bags – a convenient format that aligns with modern consumer preferences.

To address this gap, the present study investigated the effects of key technological parameters – specifically drying temperature, grinding particle size, and brewing water temperature – on the levels of bioactive compounds and sensory quality of guava leaf tea bags. Additionally, the study assessed antioxidant activity and  $\alpha$ -glucosidase inhibitory potential to provide deeper insight into the nutritional and functional value of the final product.

## MATERIALS AND METHODS

### Plant materials

Fresh guava leaves (*Psidium guajava*) were collected from Ha Bac Commune, Hai Phong Province, Vietnam – an area recognized for guava cultivation. Leaves were harvested between 8:00 and 10:00 AM and 3:00–4:00 PM under dry weather conditions. These collection times correspond to local agricultural practices, during which guava leaves are routinely pruned to promote tree growth and fruit development.

### Chemicals and reagents

Folin-Ciocalteu phenol reagent, gallic acid, sodium carbonate, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox),  $\alpha$ -glucosidase enzyme, phosphate-buffered saline (PBS), and *p*-nitrophenyl- $\alpha$ -D-glucopyranoside were purchased from Sigma-Aldrich (St. Louis). Ethanol (EtOH), methanol (MeOH), acetic acid, aluminum chloride ( $\text{AlCl}_3$ ), and potassium acetate ( $\text{CH}_3\text{COOK}$ ) were obtained from Xilong Scientific Co., Ltd (Guangdong Province, Shantou City, China). All other chemicals and reagents were of analytical grade.

### Sample preparation

Guava leaves at three maturity stages (young, mature, and old) were collected and transported to the laboratory. Leaf maturity was classified as follows: young leaves were newly emerged and light green; mature leaves were fully expanded and dark green; old leaves were thick, leathery, and exhibited visible signs of senescence (Nantitanon et al., 2010). The leaves were rinsed with tap water, air-dried, and subsequently

dried in a hot air oven at 50°C until the moisture content was reduced to below 8%, in accordance with herbal tea processing standards. The dried leaves were then cut into 2–3 mm pieces, packed into 2 g tea bags, and infused with 100 mL of boiling water (100°C) for 10 min. The resulting infusions were analyzed for total polyphenol content (TPC) and total flavonoid content (TFC) to determine the optimal leaf maturity for tea production.

For subsequent experiments, guava leaves were dried at various temperatures (40°C, 50°C, 60°C, and 70°C using a Memmert oven or by cold drying at 45°C. After drying, the leaves were cut into 2–3 mm pieces and packed into 2 g tea bags. The influence of particle size was evaluated using ground leaves of different sizes: fine powder (< 0.5 mm), 0.5–1 mm, 2–3 mm, and 4–5 mm. The tea bags were then brewed with water at different temperatures (70°C, 80°C, 90°C, and 100°C) to identify conditions yielding maximal extraction of bioactive compounds. The effects of leaf maturity, drying temperature, particle size, and brewing temperature on TPC, TFC, and sensory quality were assessed to establish optimal processing parameters. Additionally, guava leaf tea samples were analyzed for antioxidant activity and  $\alpha$ -glucosidase inhibitory potential to evaluate their prospective anti-diabetic properties.

#### **Determination of total polyphenol content (TPC)**

The total polyphenol content was determined using the Folin-Ciocalteu method as described by Singleton and Rossi (1965). A 0.5 mL aliquot of diluted tea extract was mixed with 0.25 mL of 1 N Folin-Ciocalteu reagent. After 5 min of reaction at room temperature (25°C), 1.25 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added. The mixture was then incubated for 30 min, and absorbance was measured at 755 nm using a UV-1800 UV-Vis spectrophotometer (Shimadzu, Japan). Gallic acid (0–100 µg/mL) was used to generate the standard calibration curve ( $y = 0.0068x - 0.025$ ,  $R^2 = 0.9927$ ). Results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dw).

#### **Determination of total flavonoid content (TFC)**

TFC was determined using the aluminum chloride colorimetric method described by Chang et al. (2002), with quercetin as the reference standard. A quercetin

stock solution (10 mg/mL in methanol) was prepared and diluted to obtain standard concentrations ranging from 0 to 100 µg/mL. One mL of tea extract or quercetin solution was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% AlCl<sub>3</sub>, 0.1 mL of 1 M CH<sub>3</sub>COOK, and 2.8 mL of distilled water. After incubation at room temperature for 30 min, absorbance was measured at 415 nm using a UV-1800 spectrophotometer. TFC was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g dw), based on the calibration curve:  $y = 0.0071x - 0.0121$  ( $R^2 = 0.9846$ ).

#### **Sensory evaluation**

Sensory quality was evaluated using a 0–5 point scoring scale, with weighting coefficients of 0.8, 0.8, 1.2, and 1.2 assigned to appearance, color, aroma, and taste, respectively, in accordance with the Vietnamese national standard TCVN 3215-79. Panelists were trained and provided with reference samples to calibrate their evaluations of appearance, color, aroma, and taste attributes. The evaluation was conducted under controlled laboratory conditions (quiet, well-ventilated, clean, and well-lit). A panel of seven trained and healthy individuals participated in the assessment throughout the study. During evaluation, tea cups were positioned uniformly under adequate lighting. Panelists assessed appearance and color visually, aroma by smelling the open tea cup, and taste by sipping the infusion.

#### **Determination of antioxidant capacity**

Antioxidant capacity was assessed using the DPPH radical scavenging assay as described by Tabart et al. (2009). Upon reaction with antioxidants, the purple DPPH solution turns yellow-orange, accompanied by a decrease in absorbance at 517 nm. The radical scavenging activity was calculated using the following equation:  $AA\% = [(A_0 - A_s)/A_0] \times 100$ , where AA% is the antioxidant activity, A<sub>0</sub> is the absorbance of the blank, and A<sub>s</sub> is the absorbance of the sample. Trolox was used as a standard, and results were expressed as micromoles of Trolox equivalents per gram of dry weight (µmol TE/g dw).

#### **Determination of $\alpha$ -glucosidase inhibition activity**

Guava leaf tea bags (2 g) were brewed in 100 mL of boiling water for 10 min. The infusion was concentrated

using a rotary evaporator (Buchi R-210, Switzerland) at 50°C and 175 mbar, then freeze-dried at –50°C and 0.45 mbar (Thermo Scientific ModulyoD, USA) to obtain powder extracts. The extract powder was dissolved in 100% DMSO to a final concentration of 20 mg/mL and subsequently diluted in 10 mM phosphate buffer (pH 6.8). A reaction mixture consisting of 130 µL phosphate buffer (100 mM, pH 6.8), 20 µL of  $\alpha$ -glucosidase enzyme (0.5 U/mL), and 50 µL of the extract was incubated at 37°C for 15 min to yield final extract concentrations of 4, 20, 100, and 500 µg/mL. Subsequently, 40 µL of 5 mM *p*-nitrophenyl- $\alpha$ -D-glucopyranoside was added, and the mixture was incubated again at 37°C for 60 min. The reaction was terminated by adding 80 µL of 0.2 M Na<sub>2</sub>CO<sub>3</sub>, and absorbance was measured at 405 nm using a microplate reader (Biotek, USA). Acarbose was used as the positive control. The inhibitory activity was calculated using the following equation: % inhibition =  $[1 - (A_s/A_c)] \times 100$ , where  $A_s$  is the absorbance of the sample and  $A_c$  is the absorbance of the control. The IC<sub>50</sub> value was determined using TableCurve 2D v4 software.

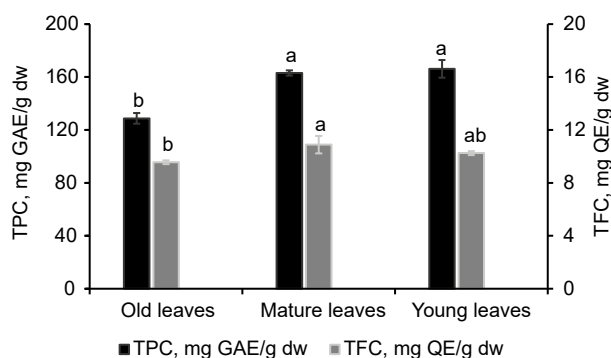
### Statistical analyses

All experiments were conducted in triplicate, and data are presented as the mean  $\pm$  standard deviation (SD). Statistical analyses were performed using Minitab 16 (Minitab, LLC, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine significant differences among treatments. Differences were considered statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Effect of guava leaf harvesting age (young, mature, and old leaves) on total polyphenol and flavonoid contents

Guava leaves were harvested at different maturity stages, resulting in variations in total polyphenol content (TPC) and total flavonoid content (TFC) (Nantitanon et al., 2010; Chang et al., 2013; Çetinkaya and Kulak, 2016; Anwar et al., 2017). Leaf age is considered a key factor influencing the quality of the final product. As shown in Fig. 1, the TPC values of young and mature leaves were 166.12 mg GAE/g dw and 162.99 mg GAE/g dw, respectively, with no



Values are means  $\pm$  standard deviation. Within the same parameter, values marked by different letters indicate significant differences ( $P < 0.05$ ).

**Fig. 1.** Polyphenol and flavonoid contents in guava leaves at different maturity stages

statistically significant difference between these stages. Likewise, the TFC values of young and mature leaves were 10.25 mg QE/g dw and 10.89 mg QE/g dw, respectively, with no significant difference observed. Both young and mature leaves exhibited significantly higher TPC and TFC levels than old leaves, indicating that the harvest stage substantially influences the accumulation of these bioactive compounds.

Photosynthetic activity has been reported to play an important role in the biosynthesis of phenolic compounds across different growth stages (Çetinkaya and Kulak, 2016; Anwar et al., 2017). Aziz and Ghulamahdi (2011) observed that harvesting 50% of the leaves from tertiary branches (comprising young and mature leaves) during the generative phase resulted in the highest TFC levels in red guava leaves. Similarly, Nantitanon et al. (2010) demonstrated that both extraction parameters and leaf maturity affect the antioxidant capacity and phenolic content of guava leaf extracts, with young leaves exhibiting the highest activity due to their elevated concentrations of phenolic compounds.

Previous research has also confirmed that guava leaf extracts display antioxidant activity through both radical scavenging and reducing mechanisms, primarily attributed to their high TPC and TFC levels (Chen and Yen, 2007; Díaz-de-Cerio et al., 2017; Zhu et al., 2020; Kumar et al., 2021). In the present study, the significantly higher TPC and TFC values in young and mature

**Table 1.** Impact of drying temperature on polyphenol and flavonoid contents in guava leaf tea bags

Drying temperature °C	Drying time h	Moisture content %	TPC mg GAE/g dw	TFC mg QE/g dw
40	12	7.28	167.15 <sup>ab</sup> ± 11.49	7.98 <sup>bc</sup> ± 0.22
50	6	7.40	176.19 <sup>a</sup> ± 2.92	9.72 <sup>a</sup> ± 1.02
60	4.5	6.95	165.23 <sup>ab</sup> ± 14.06	6.86 <sup>c</sup> ± 0.36
70	3	5.12	145.88 <sup>b</sup> ± 5.20	6.71 <sup>c</sup> ± 0.52
Cooling drying (45°C)	48	7.14	166.93 <sup>ab</sup> ± 4.63	8.77 <sup>ab</sup> ± 0.35

Values are means ± standard deviation. Within the same column, followed by different letters indicate significant differences ( $P < 0.05$ ).

leaves compared with old leaves suggest that these two maturity stages are optimal for producing guava leaf tea products, with enhanced antioxidant potential.

#### Effect of drying temperature on total polyphenol and flavonoid contents in guava leaf tea bags

Beyond leaf maturity, drying temperature significantly influences the levels of bioactive compounds in plant-based extracts. In tea production, drying is a critical step for reducing the moisture content of fresh plant materials. This process helps prevent biochemical degradation, inhibits microbial activity, preserves phytochemicals, extends shelf life, and reduces packaging and transportation costs. Importantly, the chosen drying temperature plays a key role in maintaining the sensory quality of the final product. As shown in Table 1, the highest total polyphenol content (TPC) and total flavonoid content (TFC) were observed in samples dried at 50°C, reaching 176.19 mg GAE/g dw and 9.72 mg QE/g dw, respectively. These were followed by samples dried at 40°C, 60°C, cooling-dried at 45°C, and 70°C. The total soluble solids (TSS) of tea brewed from leaves dried at 50°C (2 g/100 mL) were also higher than those from samples dried at 60°C, 70°C, or by cooling-drying, although not significantly different from those dried at 40°C. Several studies have reported similar trends, indicating that high drying temperatures can cause degradation of phenolic compounds in medicinal plants (Nantitanon et al., 2010; Nguyen et al., 2022). Nguyen et al. (2022) demonstrated that guava leaves dried at 50°C retained significantly higher TPC and TFC than those dried at 60°C–80°C. Likewise, Nantitanon et al. (2010)

observed that drying guava leaves at 50°C yielded the highest phenolic content, particularly when combined with sonication-assisted extraction, achieving up to 136.02 mg GAE/g dw. Díaz-de-Cerio et al. (2016) also reported the highest TPC using an EtOH/H<sub>2</sub>O 80:20 (v/v) solvent system, with values of 113.34 mg GAE/g dw and 86.12 mg GAE/g dw – both substantially lower than the 176.19 mg GAE/g dw obtained in the present study from hot-water extraction at 100°C using guava leaves dried at 50°C. This difference likely reflects variations in solvent polarity and extraction method between ethanol–water extracts and the hot-water infusions used in our research. Variations in reported TPC and TFC values among studies may also arise from differences in guava leaf variety, agroclimatic conditions, and drying parameters, including final moisture content and drying duration (Nantitanon et al., 2010; Aziz and Ghulamahdi, 2011; Nguyen et al., 2022).

In the present study, guava leaves dried at 50°C for 6 hours exhibited the greatest retention of both TPC and TFC. A significant reduction in TPC was observed at 70°C, while no significant differences were found among samples dried at 40°C, 60°C, or by cooling drying at 45°C (Table 1). TFC followed a similar pattern, peaking at 50°C and decreasing thereafter, except in the cooling-dried sample. Nguyen et al. (2022) reported that employing a moderate drying temperature (50°C) and shorter drying duration helps preserve phenolic compounds. In contrast, lower temperatures coupled with prolonged drying times (e.g., air-drying or sun-drying) expose samples to heat, oxygen, and light for extended periods, thereby promoting oxidative

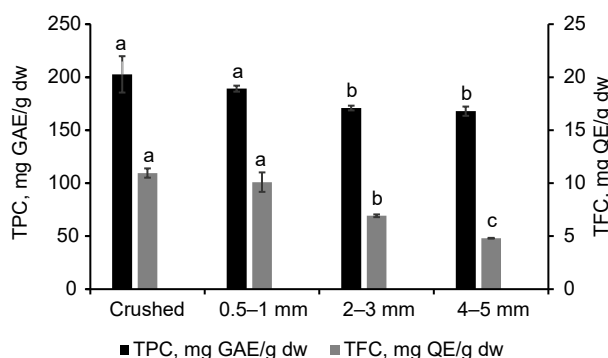
degradation. Conversely, excessively high temperatures can induce thermal degradation of heat-sensitive constituents (Gąsecka et al., 2020; Nguyen et al., 2022; ElGamal et al., 2023).

In summary, our findings, consistent with those of Nantitanon et al. (2010) and Nguyen et al. (2022), indicate that drying guava leaves at 50°C for 6 hours is the most effective condition for maximizing the retention of bioactive compounds in guava leaf tea products.

### Effect of guava leaf particle size on total polyphenol and flavonoid contents in guava leaf tea bags

The particle size of ground guava leaves exerts a substantial influence on the extraction efficiency of bioactive compounds during infusion. Finer particle sizes provide a larger contact surface area between the plant material and the solvent, thereby facilitating greater release of total polyphenols (TPC) and total flavonoids (TFC). However, excessive grinding can adversely affect the sensory characteristics of the tea, as very small particles may pass through the filter bag, producing a cloudy infusion with diminished visual appeal. In contrast, overly coarse particles may restrict solvent penetration and diffusion, resulting in reduced extraction of bioactive constituents and, consequently, lower functional quality. Following the identification of 50°C as the optimal drying temperature, the present study investigated the effect of particle size on TPC and TFC content in guava leaf tea infusions. Dried guava leaves were ground into four particle sizes: fine powder (< 0.5 mm), 0.5–1 mm, 2–3 mm, and 4–5 mm, then packed into 2 g tea bags and brewed in boiling water for 10 min. As illustrated in Fig. 2, an inverse relationship was observed between particle size and the concentration of extracted bioactive compounds. Specifically, TPC values were 202.64, 189.21, 170.93, and 167.91 mg GAE/g dw, while TFC values were 10.95, 10.09, 6.92, and 4.80 mg QE/g dw, for fine powder, 0.5–1 mm, 2–3 mm, and 4–5 mm particle sizes, respectively.

Previous studies have highlighted guava's promising antioxidant activity, with some researchers examining factors that influence the extractability of its bioactive compounds. Nantitanon et al. (2010) investigated how extraction parameters, including solvent polarity, affect the antioxidant capacity and TPC of guava leaves. Among hot water, ethyl acetate, and



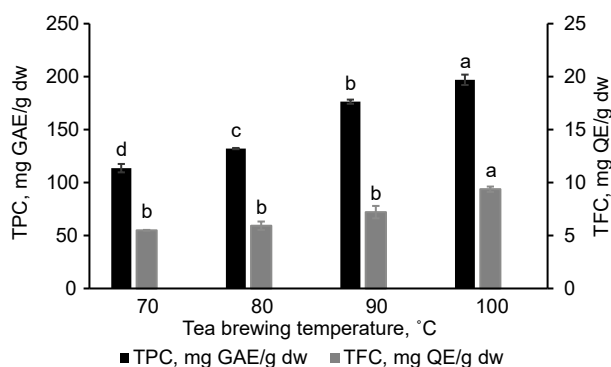
Values are means ± standard deviation. Within the same parameter, values marked by different letters indicate significant differences ( $P < 0.05$ ).

**Fig. 2.** Influence of guava leaf particle size on total polyphenol and flavonoid contents in guava leaf tea bags

ethanol, hot water proved to be the most effective solvent. In the present study, guava leaves with particle sizes of 0.5–1 mm yielded statistically higher TPC and TFC values compared with larger particle sizes (2–3 mm and 4–5 mm). However, no significant difference ( $P > 0.05$ ) was observed between the finely ground powder and the 0.5–1 mm fraction, indicating that further particle size reduction below 0.5 mm does not markedly enhance extraction efficiency. Although fine grinding enhances extraction by increasing surface area, it can also lead to turbidity as small particles escape the tea bags. Moreover, excessive extraction efficiency may release high levels of tannins and catechins, intensifying bitterness and astringency and thereby compromising sensory acceptability. Therefore, grinding guava leaves to a particle size of 0.5–1 mm represents the most effective compromise, achieving high extraction efficiency while maintaining desirable sensory quality. This particle size range may also reduce time and energy consumption during grinding, thereby improving production efficiency.

### Effect of tea brewing temperature on total bioactive compound contents and sensory quality of guava leaf tea bags

Brewing temperature is a key factor influencing both the extraction efficiency and sensory quality of tea. During infusion, water-soluble bioactive compounds, including polyphenols, flavonoids, tannins, amino



Values are means  $\pm$  standard deviation. Within the same parameter, values marked by different letters indicate significant differences ( $P < 0.05$ ).

**Fig. 3.** Effect of tea brewing temperature on total polyphenol and flavonoid contents in guava leaf tea bags

acids, and other phytochemicals, are released from the plant matrix into the brew (Pal et al., 2013; Pérez-Burillo et al., 2018; Winiarska-Mieczan and Baranowska-Wójcik, 2024). Elevated water temperatures promote cell wall disruption and increase membrane permeability, thereby enhancing the release of these compounds into the infusion. As shown in Fig. 3, increasing the brewing temperature improved the extraction of total polyphenols and flavonoids, with the highest values recorded at 100°C. At this temperature, total polyphenol content reached 176.19 mg GAE/g dw, while flavonoid content reached 9.37 mg QE/g dw. These findings are consistent with previous reports demonstrating the strong influence of brewing temperature on TPC and TFC in tea infusions (Pérez-Burillo et al.,

2018; Saklar et al., 2015). For example, Pérez-Burillo et al. (2018) observed that concentrations of gallic acid, epigallocatechin gallate (EGCG), and caffeine increased significantly when tea was brewed above 80°C for more than 10 min. Similarly, extraction times of 7 min or more yielded significantly higher bioactive compound levels, particularly at temperatures near 98°C. Lin et al. (2014) and Saklar et al. (2015) reported that catechin concentrations tend to plateau after 10–15 min of brewing, whereas Braud et al. (2015) found no significant difference in catechin content between 5- and 15-minute brewing durations. Chong and Nyam (2022) likewise noted that kenaf leaf tea brewed at 80°C and 100°C for 10 min exhibited the highest antioxidant capacity and phenolic content.

In the present study, the brewing time was fixed at 10 min based on prior evidence indicating that this duration yields optimal extraction of bioactive compounds. The results confirmed that temperature is a key determinant of both TPC and TFC in guava leaf tea. A subsequent sensory evaluation was conducted after determining suitable processing parameters. As shown in Table 2, although brewing at 100°C produced the highest TPC and TFC values, the most favorable overall sensory quality – including aroma, taste, and color – was obtained when the tea was brewed at 90 °C. Tea brewed at 100°C exhibited an intense aroma due to the rapid vapor release, but was perceived as more bitter, likely due to the degradation of certain compounds at elevated temperatures. In contrast, teas brewed at 70–80°C received lower sensory scores owing to their pale color, weak aroma, and lack of characteristic guava flavor. Overall, these findings demonstrate that

**Table 2.** Effect of tea-brewing temperature on sensory scores of the guava leaf tea bags

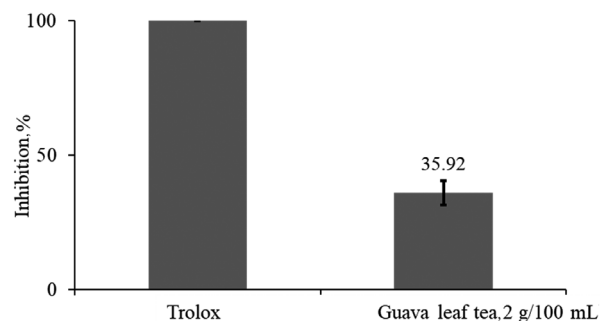
Tea brewing temperature, °C	Score of the indicators				Total quality score	Classification
	appearance	color	aroma	taste		
70	5.00 <sup>a</sup>	2.75 <sup>c</sup>	3.42 <sup>b</sup>	3.75 <sup>ab</sup>	14.80	Average
80	5.00 <sup>a</sup>	3.75 <sup>b</sup>	4.00 <sup>ab</sup>	4.33 <sup>ab</sup>	17.00	Fair
90	5.00 <sup>a</sup>	4.67 <sup>a</sup>	4.67 <sup>a</sup>	4.42 <sup>a</sup>	18.63	Good
100	5.00 <sup>a</sup>	3.67 <sup>b</sup>	4.42 <sup>a</sup>	3.58 <sup>b</sup>	16.53	Fair

Values are means  $\pm$  standard deviation. Within the same column, values followed by different letters indicate significant differences ( $P < 0.05$ ).

while brewing at 100°C maximizes the extraction of bioactive compounds, brewing at 90°C offers the optimal balance between functional and sensory attributes. This temperature is therefore recommended for guava leaf tea preparation, providing both high nutritional value and desirable consumer acceptability. The pronounced bitterness observed at 100°C is attributed to excessive extraction of tannins and catechins, known contributors to astringency, whereas the lower sensory scores at 70–80°C may result from the incomplete extraction of pigments and volatile compounds, producing paler infusion and muted aroma.

#### Evaluation of antioxidant activity and $\alpha$ -glucosidase inhibitory effect of guava leaf tea infusion

Extensive research has demonstrated the ethnopharmacological potential of *Psidium guajava* L. leaves in the treatment of various ailments, largely attributed to their abundance of bioactive constituents (Díaz-de-Cerio et al., 2017; Zhu et al., 2020; Melo et al., 2020; Kumar et al., 2021). The therapeutic properties of guava leaves have been linked to key phytochemicals such as quercetin, catechin, vescalagin, gallic acid, peltatoside, hyperoside, isoquercitrin, and guaijaverin. As shown in Fig. 4, the guava leaf tea infusion exhibited a DPPH radical scavenging activity of 35.92%, benchmarked against the standard antioxidant Trolox. Previous studies have reported a strong correlation between antioxidant capacity and total polyphenol content in guava leaf extracts (Nantitanon et al., 2010; Pal et al., 2013; Nguyen et al., 2022). Nantitanon et al. (2010) demonstrated a significant



**Fig. 4.** Evaluation of the antioxidant capacity of guava leaf tea, 2 g/100 mL

positive relationship between antioxidant activity and polyphenol concentration, suggesting that phenolic compounds are the primary contributors to the observed antioxidant properties. Similarly, Pal et al. (2013) found a comparable association between TPC and antioxidant activity in black tea infusions. Under the optimized extraction conditions – drying at 50°C and a leaf particle size of 0.5–1 mm – the guava leaf tea infusion in the present study exhibited a TPC of 176.19 mg GAE/g dw and a TFC of 9.72 mg QE/g dw, corresponding to a DPPH inhibition of 35.92% (Fig. 4). These findings further substantiate the link between polyphenolic and flavonoid concentrations and antioxidant potential.

Beyond antioxidant properties, guava leaves have been shown to possess  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities, offering potential benefits for managing postprandial blood glucose levels (Oh et al., 2005; Cheng et al., 2009; Wang et al., 2010; Guo et al., 2013). Oh et al. (2005) reported that the butanol-soluble fraction of guava leaves exhibited antidiabetic activity, particularly by inhibiting  $\alpha$ -glucosidase. Cheng et al. (2009) found that quercetin in aqueous guava leaf extracts enhanced glucose uptake in hepatic cells, while Wang et al. (2010) evaluated different extraction solvents and observed the following order of  $\alpha$ -glucosidase inhibitory activity: n-BuOH > EtOAc > 75% ethanol > residue > CH<sub>2</sub>Cl<sub>2</sub>.

Despite these insights, few studies have directly assessed the  $\alpha$ -glucosidase inhibition potential of guava leaf tea in consumer-ready-to-use formats. In the present work, the  $\alpha$ -glucosidase inhibitory activity of guava leaf tea infusion was compared with that of acarbose, a standard pharmaceutical control. As shown in Table 3, inhibition was concentration-dependent but nonlinear. At 500  $\mu$ g/mL, acarbose inhibited 78.01% of  $\alpha$ -glucosidase activity, whereas the guava leaf tea infusion achieved significantly higher inhibition at 98.96%. The IC<sub>50</sub> values were 119.17  $\mu$ g/mL for acarbose and 6.97  $\mu$ g/mL for the guava leaf tea infusion, indicating approximately 17-fold greater  $\alpha$ -glucosidase inhibitory potency in the latter. The pronounced inhibition observed may result from synergistic interactions among multiple flavonoids, including quercetin, catechin, and guaijaverin, as well as from the presence of phenolic glycosides unique to guava leaves. These compounds are known to inhibit

**Table 3.** Evaluation of the  $\alpha$ -glucosidase enzyme inhibitory ability of the guava leaf tea bag infusion

Concentration, $\mu\text{g/mL}$	Guava leaf tea bag infusion		Control (Acarbose)	
	Inhibitory ability, %	SD	Inhibitory ability, %	SD
500	98.96	1.95	78.01	1.96
100	97.03	1.31	51.98	1.47
20	87.45	1.14	18.66	0.93
4	8.10	0.34	8.80	0.28
IC <sub>50</sub>	6.97 $\pm$ 0.20		119.17 $\pm$ 9.62	

carbohydrate-hydrolyzing enzymes, contributing to antidiabetic effects (Cheng et al., 2009; Guo et al., 2013; Oh et al., 2005; Wang et al., 2010). Collectively, these findings highlight the potential of guava leaf tea as a natural functional beverage that may aid in managing hyperglycemia through partial  $\alpha$ -glucosidase inhibition. The product represents a promising complementary approach for individuals with type 2 diabetes, offering potential advantages in safety, cost, and accessibility compared with conventional pharmaceutical inhibitors.

## CONCLUSION

This study confirms that processing conditions play a crucial role in determining the functional quality of guava leaf tea. Careful optimization of leaf selection, drying temperature, particle size, and brewing parameters markedly enhances the extraction of health-promoting compounds. The resulting infusion exhibits pronounced antioxidant and antidiabetic properties, underscoring its potential as a natural functional beverage. Overall, these findings advance the development of value-added herbal products and promote the incorporation of traditional plant ingredients into modern dietary formulations.

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## DECLARATIONS

### Data statement

All data supporting this study has been included in this manuscript.

### Ethical Approval

Not applicable.

## Competing Interests

The authors declare that they have no conflicts of interest.

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