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Effect of Extraction Conditions on Total Anthocyanin Content from *Perilla frutescens* (L.) Britton and Its Application in Coloring Butter Biscuit

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Abstract

The consumer trend toward safe and healthy food has spurred research and application of natural colors in traditional biscuit products to improve quality and sensory value. *Perilla frutescens* (L.) Britton is a member of the Lamiaceae family and is widely found in East Asian regions, where it is used as both a spice in cooking and in traditional medicine. It is rich in anthocyanins, a class of polyphenolic compounds that act as natural colorants and possess strong antioxidant properties. This study aimed to evaluate the extraction process of perilla leaf for enriching the total anthocyanin content, which is applied in coloring butter biscuits. The investigated factors included the sample-to-water ratio for extraction (1:10, 1:15, 1:20, and 1:25 g:mL), extraction temperatures (60°C, 70°C, 80°C, and 90°C), and extraction times (20, 25, 30, and 35 minutes). The obtained extract was analyzed for anthocyanin content using the pH differential method. The findings suggested that a sample-to-water ratio of 1:20 g:mL, an extraction temperature of 90°C, and an extraction duration of 30 minutes were the most suitable extraction conditions to achieve a high total anthocyanin content (1.70 $mg.g^{-1}$ DW). The addition of perilla leaf extract (PLE) significantly affected the physical properties of the biscuits (diameter, yield, brightness, and hardness of the biscuits); the higher the amount of PLE added, the darker and crispier the biscuits became. Sensory analysis showed that biscuits supplemented with PLE were rated higher than the control in terms of texture, color, and flavor. The results showed that the biscuits that received the highest scores across all parameters were supplemented with PLE at a rate of 7.5%. Overall, the analysis suggests that it is possible to produce butter biscuits with a characteristic color and improved nutritional profile by adding PLE.

Keywords: Anthocyanin, Butter biscuit, Extraction, Lamiaceae, *Perilla frutescens*

Introduction

Customers find food more attractive and appealing when it is presented in a visually appealing color. Due to their low cost, color stability during storage, synthetic colorants are commonly used in food. Aromatic rings and azo functional groups are included in the majority of synthetic colorants, which can lead to cancer, allergic responses, hyperactivity, or asthma episodes (Singh, Pandey, Dash, Zanwar, & Singh, 2023). Finding natural and safe colorants with biological activity is the current focus of research (Vega, Ciudad-Mulero, Fernández-Ruiz, Barros, & Morales, 2023). The environment contains the most representative substances with the ability to produce color, including anthocyanins (red, blue, and purple), carotenoids (orange and yellow), or chlorophyll (green) (Singh *et al.*, 2023).

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Anthocyanins are polyphenolic compounds responsible for the shades of pink, red, purple, and blue found in flowers, vegetables, and fruits. Plant security systems, physiology, and propagation largely rely on anthocyanins (Alappat & Alappat, 2020). Anthocyanins are essential for food coloring, drugs, functional foods, flavoring, and preservation in human life (Alappat & Alappat, 2020; Patra, Makhal, Jaryal, More, & Kaki, 2022). The anthocyanin content of perilla leaves is quite high. According to Meng, Lozano, Bombarda, Gaydou, & Li, (2009), the total anthocyanin content of two *Perilla* varieties (*frutescens* var. *frutescens*) from different regions of China was recorded as 1.6 $mg.g^{-1}$ (dry matter) and 1.8 $mg.g^{-1}$ (dry matter), respectively, while the total anthocyanin content of two varieties (*frutescens* var. *crispa*) from China and Japan was 0.7 $mg.g^{-1}$ (dry matter) and 1.6 $mg.g^{-1}$ (dry matter), respectively.

A genus of the Lamiaceae family, *Perilla frutescens* (L.) Britton, commonly known as perilla, is found through East Asian regions including China, Japan, South Korea, Vietnam, and India. It is grown as a vegetable and has been used in traditional medicine since antiquity (Ahmed, 2019; Hou *et al*., 2022). Currently, perilla has attracted more attention from several scientists due to its therapeutic properties and phytochemicals, including essential oils, triterpenoids, polyphenols, anthocyanins, omega-3 fatty acids, vitamins, and minerals (Adam *et al*., 2023; Hoang, Nguyen, Nguyen, Tran, & Le, 2024). Numerous studies have demonstrated that perilla leaves contain a range of polyphenolic compounds with potent antioxidant properties. Tea made from perilla leaves is very popular in East Asian countries due to its high antioxidant capacity, and has been used to treat crab and fish poisoning in these nations (Hou *et al*., 2022). Perilla is easy to grow and suitable for Vietnam's climate; developing this application will create additional income for farmers (Cuong, Tung, Duc, & Quynh, 2021).

Although anthocyanins have been extensively studied, research on extracting them from perilla leaves and utilizing them as a natural coloring in baked products remains limited. Therefore, " Study on optimal conditions for extracting anthocyanins from perilla leaves (*P. frutescens* (L.) Britton) and its application in coloring butter biscuit" was conducted to obtain anthocyanin-rich extracts and evaluate their application in biscuits. This study aims to introduce more types of naturally colored biscuits with enhanced nutritional value and antioxidant properties, contributing to the trend of using natural and functional foods in food science.

Materials and Methods

Materials, Chemicals, and Instruments

Perilla (*P. frutescens* (L.) Britton) leaves (Fig. 1 A) bought in Binh Diem market, Phu Loi town, Ho Chi Minh city, were transported to Thu Dau Mot University laboratory, washed thoroughly with water to remove dust. The fresh leaves were separated and dried in the sunlight until they reached a consistent weight. After that, they were ground and sieved through a 1 mm mesh and stored in sealed glass jars at room temperature before being used in experiments (Fig. 1 B).

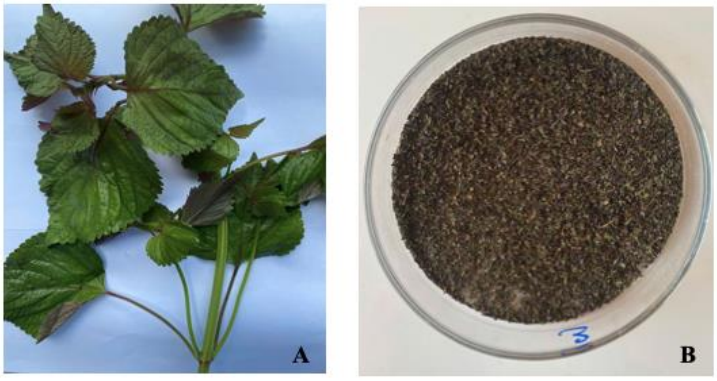

Fig. 1. *P. frutescens* (L.) Britton. A. whole plant, B. perilla leaf powder

The ingredients for the butter biscuits include flour, unsalted butter, sugar, eggs, salt, baking soda, and vanilla, which were purchased from Co-opmart Binh Duong supermarket in Thu Dau Mot Ward, Ho Chi Minh City, Vietnam.

Phytochemical Screening for Flavonoids and Polyphenols in Perilla Leaf Extract

Flavonoids and polyphenols in perilla leaf extract were qualitatively analyzed according to the method described by Apoorva, Pooja, & Vidyasagar, (2021).

Briefly, perilla leaf powder (1 g) was added to 20 mL of water in a 100 mL Erlenmeyer flask, then heated in a reciprocal shaker bath (Firstek B601D, Taiwan) at 70°C for 30 minutes. The mixture was filtered through NewStar filter paper (Ø11 cm) and used for qualitative analysis. The tests were conducted as shown in Table 1. The flavonoid test produced a yellow precipitate, whereas the polyphenol test yielded a bluish-green coloration. These observations confirm the presence of both flavonoids and polyphenols in the extract.

Table 1- Phytochemical screening for flavonoids and polyphenols in perilla leaf extract (Apoorva *et al.*, 2021)

Bioactive compounds	Qualitative chemical reaction	Signs of recognition
Flavonoids	0.5 mL extract + 0.5 mL $Pb(CH_3COO)_2$ 10%	Yellow precipitate
Polyphenols	0.5 mL extract + 3-4 drops $FeCl_3$ 5%	Bluish-green appearance

Anthocyanin Extraction

Effect of sample-to-water ratio on total anthocyanin content in perilla leaf

The perilla leaves powder (1 g) was extracted with water at 70 C for 30 minutes in a reciprocal shaker bath. The varying sample-to-water ratios were studied at the following concentrations: 1:10, 1:15, 1:20, and 1:25 (g:mL), which were selected based on previous reports demonstrating their effectiveness for anthocyanin extraction (Dam & Lam, 2013; Nhon, Tran, Phuc, Linh, & Anh, 2025). After extracting, the mixtures were passed through filter paper and used directly to analyze total anthocyanin concent (TAC).

Effect of Extraction Temperature on Total Anthocyanin Content in Perilla Leaf

In order to determine the optimum temperature , the extract temperatures were adjusted at 60°C, 70°C, 80°C, and 90°C. After 30 minutes, the extracts were filtered to analyze TAC.

Effect of Extraction Time on Total Anthocyanin Content in Perilla Leaf

The perilla leaf powder was extracted with water at an appropriate ratio in a reciprocal shaker bath at a proper extract temperature (determined in the previous experiment). The extraction times were set for 20, 25, 30, and 35 minutes. Then, the mixtures were filtered to determine TAC.

Anthocyanin Quantification Method

Total anthocyanin content (TAC) is determined based on the principle that monomeric anthocyanin pigments change color reversibly when pH changes (Lee, 2005; Hoang *et al*., 2024).

The perilla extract was diluted with buffer solution (KCl 0.025M, pH 1.0) and (CH_3COONa 0.4M, pH 4.5), let stands for 30 minutesThe absorbance was then measured using a spectrophotometer (Biochrom WPA Biowave II, UK) at 520 and 700 nm. The diluted test samples were compared to the control tube containing distilled water. TAC in the extract was calculated as follows:

$$TAC\ (mg.g^{-1}) = \left[\frac{A.M.K.V.10^3}{\varepsilon.L.m}\right] \quad (1)$$

In which:

A: absorption of anthocyanin with A = (A_{520} - A_{700}) pH=1) - (A_{520} - A_{700}) pH= 4.5)

M: molecular weight of cyanidin 3-glucoside (449.2 $g.mol^{-1}$)

L: length of cuvette (1 cm)

K: dilution factor

ε: molar absorption coefficient (26900 $L.mol^{-1}.cm^{-1}$)

V: volume of diluted samples (L)

m: weight of the sample (g)

Application of Perilla Leaf Extract in Coloring Butter Biscuits

Biscuits Preparation

The butter biscuits were prepared based on the formulation described by Phuc, Ngoc, & Thu, (2022) with minor adjustments (Fig. 2). The initial step of making butter biscuits was to mix wheat flour with other ingredients (sugar (56%), butter (60%), eggs yolk (17%), baking soda (1%), vanilla (1%), and salt (0.5%)), based on the weight of the wheat flour. Perilla leaf extract (PLE) was added to the butter biscuits according to Moczkowska‐Wyrwisz, Jastrzębska, & Wyrwisz, (2022) at different quantities of 5%, 7%, and 9% with 0.1% citric acid relative to the total mass of the dough after the ingredients had been well blended. After resting for twenty minutes, the dough was divided into small parts (10 g), shaped into the round shapes, and baked for 25 -30 minutes at 150°C in a preheated oven. The butter biscuits were cooled for 5 minutes at room temperature before being wrapped in a bag, sealed for further analysis.

Physical Analysis

Dimensions of the Biscuits after Baking

The biscuit's diameter and weight after baking were measured using a caliper and an analytical balance, respectively. The average values were calculated from 10 samples. The biscuit yield was determined according to Moczkowska‐Wyrwisz *et al*. (2022) and calculated as follows:

$$BY = \left[\frac{W_b}{W_a}\right] \times \mathbf{100} \quad (2)$$

In which:

BY: cooking yield (%)

Wb: weight of sample before baking (g)

Wa: weight of sample after baking and cooling (g)

Color Analysis

The color of PLE biscuit samples was determined using a colorimeter (CR-410, Konica Minolta, Japan) based on the L^*, a^*, b^* color system, where L^* represents lightness (0 = black, 100= white), a^* indicates the green–red component, and b^* represents the blue–yellow component (Azuan, Mohd Zin, Hasmadi, Rusli, & Zainol, 2020). The hue angle (h◦) was calculated according to formula: $h^\circ = \tan^{-1}(b^*/a^*)$ (3) (Biswas *et al*., 2025). Three biscuits were analyzed for each formulation.

Texture Analysis

The hardness of the biscuit samples was determined using a texture analyzer (BROOKFIELD CT3, USA). The pre-test and test speeds were 2 and 1 $mm.s^{-1}$, respectively. Hardness, defined as the maximum peak force, was measured in triplicate and expressed in kilograms (Najjar, Alkaabi, Alketbi, Stathopoulos, & Ranasinghe, 2022).

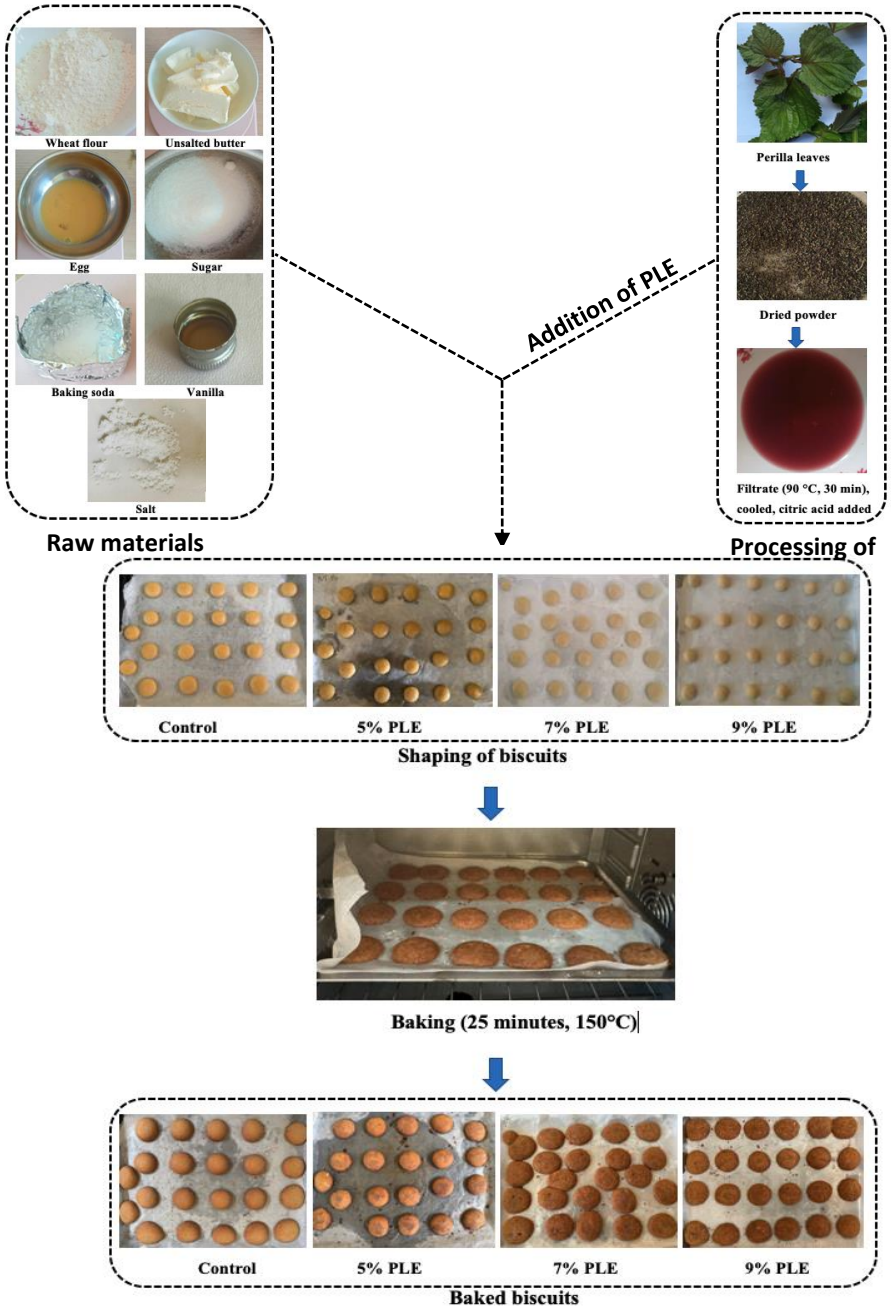

Fig. 2. Processing steps of PLE biscuits

Sensory Evaluation

The evaluation of final product with added PLE was conducted using QDA (Quantitative Descriptive Analysis) based on a quality scale of 1-5 for texture, color, and flavor (Quyen, 2024). The evaluation team (10 panelists) were lecturers and students from the Biotechnology and Food Technology Department of the Institute of Green and Sustainable Technology, Thu Dau Mot University, Vietnam.

Statistical Analyses

Statistical analyses were conducted by Minitab 16 software. One-way ANOVA was used to test for differences among treatments, and Tukey's post-hoc test was used to identify significant pairwise differences at a 5%

significance level ($p < 0.05$). The mean ± SD, with three replications, is displayed in all experimental data.

Results and Discussion

Phytochemical Screening for Flavonoids and Polyphenols in Perilla Leaf Extract

Polyphenols are phytochemicals, the primary source of antioxidants in the human diet. They are classified into four most essential subgroups: flavonoids, phenolic acids, stilbenes, and lignans (Ciupei, Colişar, Leopold, Stănilă, & Diaconeasa, 2024). In particular, anthocyanins are polyphenolic compounds with potent antioxidant and anti-inflammatory properties, which can help prevent various chronic diseases, including cardiovascular disease, neurodegeneration, and diabetes (Zhang & Zhu, 2023). To improve the efficiency of anthocyanin extraction, a preliminary investigation was conducted to determine the presence and content of flavonoids and polyphenols in perilla leaf extract.

After extraction, the mixture was filtered (tube 1). Then, reagents were added, yielding a yellow precipitate (tube 2) and a bluish-green appearance (tube 3) (Fig. 3), indicating the presence of flavonoids and polyphenols in perilla leaf extract (Table 2).

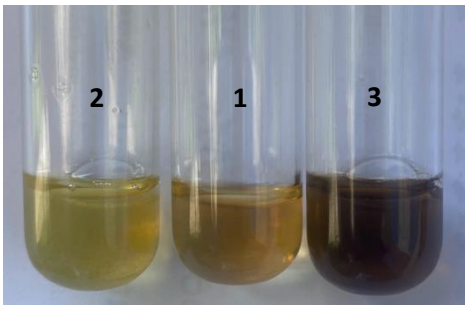

Fig. 3. Qualitative analysis of perilla leaf. (1) Sample before reaction, (2) qualitative analysis of flavonoids, and (3) qualitative analysis of polyphenols

Many studies reported that ferilla contained many polyphenols and flavonoids (Skowyra, Falguera, Azman, Segovia, & Almajano, 2014; Nguyen, Truong, & Nguyen, 2024; Lia & Baron, 2025). Perilla leaves contain various active constituents, including carotenoids, polyphenols, α-tocopherol, phytosterols, and anthocyanins. According to Zhang *et al*. (2022), anthocyanin was present in perilla at a concentration of 8.37 $mg.g^{-1}$.

Table 2- The presence of flavonoids and polyphenols in perilla leaf extract

Bioactive compounds	Test	Perilla leaf extract
Flavonoids	Lead acetate 10%	+
Polyphenols	Ferric (III) chloride 5%	+

(+) presence

Effect of Sample-to-water Ratio on Total Anthocyanin Content in Perilla Leaf

The sample-to-solvent ratio is a crucial factor influencing the efficiency of the extraction process. The amount of water used must be sufficient to ensure that the material is completely submerged, allowing the water to contact the sample and maximize extraction efficiency. In this study, the sample-to-water ratios treatments were 1:10, 1:15, 1:20, and 1:25 (g:mL). After extraction, the TAC was measured for each treatment (ratio) with three replications. The results of these experiments are presented in Table 3.

Table 3- Effect of sample-to-water ratio on TAC in perilla leaf

Sample/water (g:mL)	TAC (mg.g^{-1} DW)
1:10	$0.15^{c} \pm 0.03$
1:15	$0.13^{c} \pm 0.01$
1:20	$0.47^{a} \pm 0.03$
1:25	$0.28^{b} \pm 0.01$

*The means with different letters indicate a significant difference at the 5% level, according to the Tukey's test

The results in Table 3 show that the TAC obtained increased gradually as the amount of water increased from 10 to 20 mL, and the highest TAC (0.47 mg.g^{-1} DW) was obtained in the treatment with a sample-to-water ratio of 1:20 (g:mL). However, the TAC decreased (0.28 mg.g^{-1} DW) when the amount of water increased to 25 mL. When the water volume was only 10 - 15 mL, the solvent was insufficient to fully extract anthocyanins from the plant cells, resulting in lower TAC. However, increasing , the amount of water (25 mL) did not increase the amount of anthocyanin in the extract because the anthocyanin content in the sample was fixed (Pham & Pham, 2025).

The findings of the statistical analysis showed that the sample-to-water ratio significantly affected the TAC content ($p < 0.05$) (Table 3). As a result, 1:20 (g:mL) was the proper sample-to-water ratio for this experiment.

Effect of Extraction Temperature on Total Anthocyanin Content in Perilla Leaf

Anthocyanin is a standard water-soluble pigment in plants, responsible for the characteristic colors of red, orange, purple, and blue. The stability of anthocyanins is influenced by various factors, including pH, temperature, light, atmospheric oxygen, ascorbic acid, and enzymes (Enaru, Drețcanu, Pop, Stănilă, & Diaconeasa, 2021). During the extraction process, temperature is an essential factor because as the temperature increases, the viscosity of the solution decreases, thereby increasing the diffusion rate. This promotes the transport of soluble substances, including anthocyanin, from the raw material into the solvent, allowing the process to proceed more quickly (Nhon *et al.*, 2025). However, excessively high temperatures can cause anthocyanins to degrade or decompose (*Nhut et al.*, 2019; Thuy, Han, Minh, & Tai, 2022). Therefore, determining the optimal extraction temperature is necessary to maximize extraction efficiency and achieve the highest anthocyanin content in the extract.

The results of the temperature effect on anthocyanin content in perilla leaf are presented in Table 4.

Table 4- Effect of extraction temperature on TAC in perilla leaf

Temperature (°C)	TAC (mg.g^{-1} DW)
60	$0.15^{d} \pm 0.03$
70	$0.34^{c} \pm 0.02$
80	$0.55^{b} \pm 0.00$
90	$0.63^{a} \pm 0.05$

*The means with different letters indicate a significant difference at the 5% level, according to the Tukey's test

TAC extracted from perilla leaf powder showed a statistically significant difference ($p < 0.05$) when different extraction temperatures (60°C, 70°C, 80°C, and 90°C) were applied. The results showed that the application of 90°C yielded the highest TAC, reaching 0.63 mg.g^{1} DW. Yu, Shiau, Pan, & Yang, (2024) reported that temperature is an essential factor affecting the extraction efficiency of bioactive compounds. Increasing the extraction temperature not only reduces the viscosity of the solvent but also promotes the diffusion, mass transfer, and osmosis of the solvent into plant tissue. At the same time, high temperature

can break the structural bonds of the cell wall, creating favorable conditions for the solvent to contact and dissolve intracellular compounds, including anthocyanin. According to Thuy *et al*. (2022), TAC in Peristrophe bivalvis L. Merr leaf extract increased with increasing extraction temperature from 80°C to 90°C, but TAC content decreased when extracted at 100°C. Similiarly, Yu *et al.* (2024) also reported that the highest TAC was obtained when butterfly pea flower and grape skin powders were extracted with water at a solid-to-solvent ratio of 2% (w/v). The optimal extraction conditions were 90°C for 30 - 90 minutes for butterfly pea flower and 90°C for 30 minutes for grape skin. Under these conditions, TACs were 1.75 and 2.79 mg.g^{-1} DW, respectively. Conversely, Nhon *et al.* (2025) showed that perilla leaf powder (1 g) extracted with 20 mL of 72% ethanol at 63°C for 90 minutes yielded an optimal anthocyanin content of 3.373 mg.g^{-1} DW. Differences in results may be due to variations in extraction conditions such as: source of raw material, extraction temperature and time, extraction method, sample particle size, solids-to-solvent ratio, stirring conditions, and analytical methods used to analyze TAC (Yu *et al*., 2024).

Based on experimental results, the optimal extraction temperature for anthocyanin recovery from perilla leaf powder was determined to be 90°C.

Effect of Extraction Time on Total Anthocyanin Content in Perilla Leaf

A perilla powder-to-water ratio of 1:20 (g:mL) and a temperature of 90°C were selected for the extraction process. The extraction of anthocyanins also depends on the extraction time. If the extraction time is too short, the solvent may not have sufficient time to dissolve all the anthocyanins present in the raw material. Conversely, if the extraction time is too long, the quality of the extract may be affected due to the dissolution of undesirable compounds. Additionally, prolonged exposure to high temperatures may lead to the degradation of anthocyanins (Thuy *et al*., 2022).

In this study, extraction times of 20, 25, 30, and 35 minutes were applied to determine the optimal duration for obtaining the highest anthocyanin content. The results corresponding to each extraction time are presented in Table 5.

Table 5- Effect of extract time on TAC in perilla leaf

Time (minute)	TAC (mg.g^{-1} DW)
20	$0.55^{c} \pm 0.00$
25	$1.13^{b} \pm 0.09$
30	$1.70^{a} \pm 0.29$
35	$1.16^{b} \pm 0.03$

*The means with different letters indicate a significant difference at the 5% level, according to the Tukey's test

According to the results presented in Table 5, the TAC at 20 minutes of extraction was 0.55 mg.g^{-1} DW. TAC increased to 1.13 mg.g^{-1} DW at 25 minutes. The highest TAC was observed at 30 minutes, reaching 1.70 mg.g^{-1} DW. However, at 35 minutes, the anthocyanin content decreased to 1.16 mg.g^{-1} DW. This indicates that extraction time significantly affects the anthocyanin yield. Extraction times of 20 and 25 minutes seem insufficient for the complete dissolution of anthocyanins into the solvent. At 30 minutes, optimal extraction conditions allow maximal solute diffusion into the solution. Nevertheless, extending extraction time beyond equilibrium does not enhance extraction efficiency. Additionally, anthocyanins are susceptible to oxidation by heat and atmospheric oxygen during prolonged extraction (Thuy *et al*., 2022), which likely causes the decrease observed at 35 minutes.

Similarly, according to Nhut *et al*. (2019), the highest anthocyanin recovery from *Hibiscus sabdariffa* L. (Roselle) flowers was 180.821 mg.L^{-1} at an extraction temperature of 60°C with a 50% ethanol and an extraction period of 30 minutes at a liquid-solid ratio of 8:1.

Thus, in this experiment, 30 minutes was chosen to extract anthocyanin from perilla leaf powder.

Application of Perilla Leaf Extract (PLE) in Coloring Butter Biscuit

The dimensions of the butter biscuit produced by adding different amounts of PLE are shown in Table 6. The diameter of the sample increased progressively, and biscuit yield reduced as the amount of PLE added to the biscuits increased. This may be due to increasing the water content originated from PLE, which made the dough softer, allowing the dough to spread and rise more quickly during the initial stage of baking. The extra water also helped to form more air bubbles, resulting in a lighter texture that reduced the biscuit's weight and led to a slightly larger diameter (Miller, Hoseney, & Morris, 1997; Mandala, Ioannou, & Kostaropoulos, 2006).

Table 6- Different dimensions of the PLE biscuits prepared by different formulations

Sample added PLE	Diameter (cm)	Biscuit yield
Control	$3.99^{b} \pm 0.12$	$85.97^{a} \pm 3.71$
5%	$3.99^{b} \pm 0,10$	$86.24^{a} \pm 1.21$
7%	$4.55^{a} \pm 0.33$	$85.00^{ab} \pm 0.67$
9%	$4.75^{a} \pm 0.23$	$82.62^{b} \pm 0.76$

*The means with different letters indicate a significant difference at the 5% level, according to the Tukey's test

The proportion of PLE added to the dough mixture significantly affected the color parameters L*, a*, b*, and h◦ of the product ($p < 0.05$) (Table 7).

Table 7- Color analysis of the PLE biscuits prepared by different formulations

Sample added PLE	L*	a*	b*	h°
Control	$69.26^{a} \pm 1.47$	$5.35^{b} \pm 1.59$	$32.61^{a} \pm 1.13$	$80.74^{a} \pm 2.40$
5%	$55.30^{b} \pm 0.63$	$10.69^{a} \pm 0.87$	$27.22^{b} \pm 2.13$	$68.57^{c} \pm 0.08$
7%	$49.90^{c} \pm 1.43$	$10.77^{a} \pm 0.47$	$29.44^{ab} \pm 1.32$	$69.88^{bc} \pm 1.36$
9%	$47.31^{c} \pm 2.69$	$12.62^{a} \pm 0.85$	$28.82^{ab} \pm 2.13$	$66.28^{c} \pm 2.19$

*The means with different letters indicate a significant difference at the 5% level, according to the Tukey's test

The L* value of the butter biscuits gradually decreased with increasing PLE content, indicating a reduction in product brightness. At PLE levels of 7% and 9%, the biscuits exhibited the lowest L* values (49.90 and 47.31, respectively) and a deeper red coloration. In addition, the h◦ value also decreased as the PLE concentration increased, indicating a color shift from light yellow to red–brown tones. This trend is consistent with previous studies on biscuits and functional food products incorporating pigment-rich plant ingredients. Choi, Oh, & Lee, (2009) reported that cookies supplemented with 1% and 3% perilla powder showed reduced L* values (54.38 and 43.22, respectively) and improved functionality. Similarly, Najjar *et al.* (2022) observed that cookies containing Khalas date powder exhibited darker coloration, with the 7% formulation being the most preferred (L* = 40.06). More recently, Biswas *et al.* (2025) found that increasing red dragon fruit peel powder from 0 to 9% resulted in decreases in both L* and h◦ values, with the 6% sample being the most preferred (L* = 42.57; h◦ = 59.65).

The results of evaluating the hardness of butter biscuits supplemented with PLE are presented in Table 8. The hardness of the butter biscuits tended to decrease as the PLE content in the dough increased. This suggests that adding high amounts of PLE may increase the formation of air pockets during baking, resulting in a more porous texture compared to the control; therefore, the hardness of the butter biscuits decreased with increasing PLE. The hardness values from 3.31 to 3.94 kg showed no significant difference ($p > 0.05$), which is consistent with the study of Biswas *et al.* (2025), who observed that replacing red dragon fruit peel powder (0 - 9%) did not significantly

affect the hardness of the biscuits (hardness from 3.33 to 3.59 kg).

The results of sensory evaluation of finished butter biscuits supplemented with PLE (0 - 9%) (Fig. 4) on texture, color, and tatse are presented in Table 9.

Table 8- Texture analysis of the PLE biscuits prepared by different formulations

Sample added PLE	Hardness (kg)
Control	3.94[a] ± 0.65
5%	3.65[a] ± 0.30
7%	3.35[a] ± 0.55
9%	3.31[a] ± 0.52

*The means with different letters indicate a significant difference at the 5% level, according to the Tukey's test

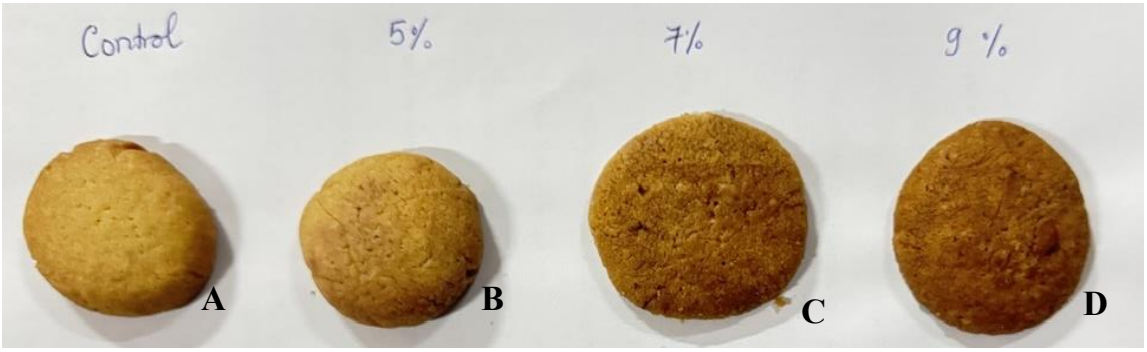

Fig. 4. Butter biscuit with added perilla leaf extract. A. 5% extract; B. 7% extract; C. 9% extract

In terms of texture, the sensory scores were higher in samples with added PLE, particularly in the sample with a 9% addition (4.70 points). This may be due to the porous, crisp texture of the biscuit samples, which have a high extract ratio. According to hardness measurements, biscuits containing PLE had lower hardness compared to the control sample. Hardness is a texture characteristic that plays a vital role in evaluating baked goods. The lower this parameter, the more desirable the product is for biscuits (Najjar *et al*., 2022).

Color is considered a fundamental physical characteristic of food. It significantly impacts the evaluation of a product's visual quality. When evaluating the perceived color of a product, panelists are not only interested in brightly colored products but also in the product's characteristic color. In this experiment, butter biscuits with added PLE, which had a deep red color, were scored higher than the control sample, especially the sample containing 9% PLE (4.4 points). Similarly, Najjar *et al*. (2022) also reported that the cookie sample with a high percentage of added date powder (7.5%) was preferred over the sample with a low percentage of added date powder or the control.

Taste scores were also higher for biscuits containing 9% PLE, with an average score of 4.30 compared to 2.30 for the control sample ($p < 0.05$). The addition of PLE may incorporate to formation a distinctive flavor.

Based on the analyzed sensory criteria, the 9% PLE sample was scored the highest in terms of texture, color, and taste. Therefore, the 9% PLE ratio was chosen as the optimal level to be included in the butter biscuit formulation (Fig. 5). The addition of this natural ingredient not only enhances the sensory value but also has the potential to improve the nutritional and functional value of the product.

Table 9- Sensory evaluation of the PLE biscuits prepared by different formulations

Sample	Sensory score		
	Texture	Color	Flavor
Control	$3.40^{c} \pm 0.70$	$2.00^{c} \pm 0.67$	$2.30^{c} \pm 0.95$
5%	$3.60^{bc} \pm 0.70$	$2.90^{b} \pm 0.74$	$3.20^{bc} \pm 0.92$
7%	$4.30^{ab} \pm 0.95$	$3.70^{ab} \pm 0.67$	$4.00^{ab} \pm 0.47$
9%	$4.70^{a} \pm 0.48$	$4.40^{a} \pm 0.84$	$4.30^{a} \pm 0.67$

*The means with different letters indicate a significant difference at the 5% level, according to the Tukey's test

Fig. 5. Butter biscuits with added 9% PLE and product packaging

Conclusion

The highest anthocyanin content was obtained using a perilla powder-to-water ratio of 1:20 g:mL, extraction at 90°C for 30 minutes (1.70 $mg.g^{-1}$ DW). The study also demonstrated the success of using PLE (9%) to create the characteristic color of Biscuit butter , while simultaneously enhancing their nutritional and functional properties. These results showed that using natural colorants in food improves both the vision and nutritional quality of baked goods, which aligns well with what consumers want nowadays safer, healthier food options. Further work should focus on optimizing extraction, testing compound stability in foods, and evaluating consumer response. This study highlights the potential of PLE for both food and medicinal purposes.

Author Contributions

Pham Thi My Tram designed the experiments. **Nguyen Ngoc Thanh Phuong** has done studies, guided and supervised by Pham Thi My Tram. **Pham Thi My Tram** and **Nguyen Ngoc Thanh Phuong** carried out the calculations. The paper was written by **Pham Thi My Tram**. All authors agreed to the final version of this manuscript.

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Conflict of Interest

Authors declare no conflict of interest.

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جلد ۲۲، شماره ۱، فروردین- اردیبهشت ۱۴۰۵، ص. ۴۹-۳۵

اثر شرایط استخراج بر میزان کل آنتوسیانین *Perilla frutescens* (L.) Britton و کاربرد آن در رنگ‌دهی بیسکویت کره‌ای

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چکیده

گرایش مصرف‌کنندگان به سوی غذاهای ایمن و سالم، پژوهش و کاربرد رنگ‌های طبیعی را در محصولات سنتی بیسکویت به‌منظور بهبود کیفیت و ارزش حسی افزایش داده است *Perilla frutescens* (L.) Britton .گیاهی از خانواده Lamiaceae است که به‌طور گسترده در کشورهای شرق آسیا یافت می‌شود و هم به‌عنوان ادویه در آشپزی و هم در طب سنتی مورد استفاده قرار می‌گیرد. این گیاه سرشار از آنتوسیانین‌ها، دسته‌ای از ترکیبات پلی‌فنولی است که به‌عنوان رنگ‌دهنده‌های طبیعی عمل کرده و دارای خواص آنتی‌اکسیدانی قوی هستند. هدف این مطالعه، ارزیابی فرآیند استخراج برگ پریلا برای افزایش میزان کل آنتوسیانین و کاربرد آن در رنگ‌دهی بیسکویت‌های کره‌ای بود. عوامل مورد بررسی شامل نسبت نمونه به آب (۱:۱۰، ۱:۱۵، ۱:۲۰ و ۱:۲۵ گرم:میلی‌لیتر)، دمای استخراج (۶۰، ۷۰، ۸۰ و ۹۰ درجه سانتی‌گراد) و زمان استخراج (۲۰، ۲۵، ۳۰ و ۳۵ دقیقه) بودند. عصاره به‌دست‌آمده از نظر میزان آنتوسیانین با استفاده از روش اختلاف pH مورد تجزیه‌وتحلیل قرار گرفت. نتایج نشان داد که نسبت نمونه به آب ۱:۲۰ گرم:میلی‌لیتر، دمای استخراج ۹۰ درجه سانتی‌گراد و زمان استخراج ۳۰ دقیقه، مناسب‌ترین شرایط برای دستیابی به بیشترین میزان کل آنتوسیانین (۱,۷۰ میلی‌گرم بر گرم وزن خشک) هستند. افزودن عصاره برگ پریلا (PLE) به‌طور معنی‌داری بر ویژگی‌های فیزیکی بیسکویت‌ها (قطر، بازده، روشنایی و سختی) تأثیر گذاشت؛ به‌طوری‌که با افزایش میزانPLE ، بیسکویت‌ها تیره‌تر و تردتر شدند. ارزیابی حسی نشان داد که بیسکویت‌های غنی‌شده با PLE از نظر بافت، رنگ و طعم امتیاز بالاتری نسبت به نمونه شاهد دریافت کردند. نتایج همچنین نشان داد که بیسکویت‌های دارای بیشترین امتیاز در تمامی شاخص‌ها، حاوی ۷,۵٪ PLE بودند. به‌طور کلی، این مطالعه نشان می‌دهد که با افزودن عصاره برگ پریلا می‌توان بیسکویت‌های کره‌ای با رنگی متمایز و ارزش تغذیه‌ای بهبودیافته تولید کرد.

واژه‌های کلیدی: آنتوسیانین، استخراج، بیسکویت کره‌ای، Lamiaceae، *Perilla frutescens*

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