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Research article

Development of Ultrasonic Extraction Techniques for Bioactive Compounds from Banana Peel Waste: A Comparative Study of Biological Activities

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Abstract: Banana peel (*Musa* spp.), an abundant agricultural by-product, represents a valuable source of bioactive compounds with potential applications in functional food and cosmeceutical industries. This study investigated the influence of ultrasound-assisted extraction (UAE) on total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and tyrosinase inhibitory capacity of peel extracts from four economically important cultivars: Cavendish, Bluggoe, Pisang Awak, and Red Dacca. Extraction was conducted using 70% (v/v) aqueous ethanol under ultrasonic conditions at three temperatures (30, 50, and 70 °C) and three time intervals (30, 60, and 120 min). The results demonstrated that Pisang Awak peel extract exhibited the highest TPC (4.63 mg GAE/g extract) and TFC (4.28 mg QE/g extract). Antioxidant capacity evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays revealed superior activity for Pisang Awak extract (IC_{50} = 1.27 μg/mL; FRAP = 3.71 mg TE/g extract). This cultivar also demonstrated significant tyrosinase inhibitory activity (IC_{50}= 1.34 mg/mL). Optimization studies identified 50 °C and 60 min as optimal extraction parameters, yielding maximum extraction efficiency across all cultivars. These findings establish UAE as an effective technology for recovering bioactive compounds from banana peel waste, particularly the Pisang Awak cultivar, which shows considerable promise for development into nutraceutical and cosmeceutical formulations. This valorization approach promotes sustainable waste management and circular bioeconomy principles in the agricultural sector.

Keywords: Ultrasonic waves; phenolic; antioxidant capacity; banana peel

1. Introduction

The fruit and vegetable processing industry generates a substantial amount of waste materials, more than 25 to 40 percent by weight, which include peels, seeds, press residues, and fruit pulp. Most of this waste is disposed of in the environment, contributing to air pollution. Moreover, the management of such waste remains a major concern and incurs high costs [1]. Bananas are one of Thailand's key economic fruits. The commercially cultivated varieties include Pisang Awak banana, Cavendish banana, Lady Finger banana, Bluggoe banana, and Red Dacca banana. Ripe bananas are rich in nutrients, providing approximately 105 kilocalories of energy per 100 grams, and are excellent sources of vitamin B6, vitamin C, essential minerals such as potassium, iron, magnesium, and manganese, and high levels of dietary fiber.

In recent decades, the utilization of agricultural byproducts as sources of bioactive compounds has gained significant attention. These compounds, particularly phenolic compounds, anthocyanins, flavonoids, vitamins, minerals, and antioxidants, exhibit a wide range of biological activities including antimicrobial, antioxidant, antidiabetic, antiaging, anticancer, and weight management effects [2]. Waste materials derived from banana processing are important sources of such bioactive compounds [3]. In particular, banana peel, accounting for approximately 30 to 40 percent of the fresh fruit weight, is rich in phenolic compounds, flavonoids, and antioxidants [4]. Attributed to its multifunctional bioactive nature, banana peel has been applied in various domains such as a prebiotic agent, a source of pectin, an ingredient in bakery products, and dietary supplements [5-7].

The extraction process of biologically active constituents derived from botanical sources directly influences the yield and quality of the target compounds [8]. Traditional solvent-based extraction methods techniques such as maceration and Soxhlet extraction, rely on the application of heat to raw materials in solvents at high temperatures. Although these methods often yield high extraction outputs, the extended extraction time may degrade active compounds and consume considerable energy [9]. In addition, traditional extraction methods involve large volumes of solvents, which can negatively impact the environment. Recently, the green technology concept has gained increasing attention, emphasizing the extraction of bioactive compounds without the use of toxic chemicals, thereby promoting environmentally friendly processes that are also safe for consumers [9-10]. Modern extraction technologies include employing techniques such as microwave-assisted, sonication-based, high-pressure, and pulsed electric field methods, and enzymes. Ultrasonic extraction is an efficient alternative technology that offers advantages such as reduced extraction time, lower solvent usage, and high yields [11]. The mechanism relies on the acoustic cavitation phenomenon, whereby ultrasonic waves generate microbubbles in a liquid medium. The collapse of these bubbles creates localized high-temperature and pressure zones, enhancing mass transfer and extraction efficiency [12]. However, the efficiency of ultrasonic extraction depends on several operational parameters, particularly thermal conditions and processing duration, which significantly influence efficiency and integrity of the recovered phytochemicals [13].

This study explored the influence of ultrasound-assisted extraction on phenolic and flavonoid composition, antioxidative potential, and the capacity to suppress tyrosinase activity in banana peel extracts. The findings of this research will provide valuable insights for identifying optimal extraction conditions and developing an efficient process for recovering bioactive compounds from banana peels. This can lead to broader applications across various industries and enhance the value of agricultural waste. Furthermore, this research supports the objectives of multiple UN Sustainable Development Goals, particularly Goal 9: Industries, Innovation and Infrastructure, by developing efficient and environmentally friendly extraction processes; Goal 12: Responsible Consumption and Production, through the utilization of agricultural waste and waste reduction; and Goal 15 Life on Land, by promoting the sustainable use of natural resources. Therefore, this study holds significance in scientific, technological, and sustainable development contexts at the global level.

2. Materials and Methods

This study investigated the effects of ultrasonic extraction on the total phenolic content, total flavonoid content, antioxidant activity, and tyrosinase inhibitory activity of banana peel extracts.

2.1 Sample Preparation and Extraction

The banana peels studied included Cavendish banana peel, Bluggoe banana peel, Pisang Awak banana peel, and Red Dacca banana peel, collected from bakeries, grilled banana vendors, and Thai dessert shops in Khlong Luang District, Pathum Thani Province.

Sample preparation began with washing each type of banana peel with clean water, followed by air drying at room temperature. The peels were sectioned into smaller fragments and dehydrated using a hot air oven at 45 °C until constant mass was achieved. The dried samples were then milled into uniform powder by a mechanical grinder. Each powdered sample was accurately weighed using a four-decimal analytical balance.

For extraction, 10 g of the powdered peel was combined with 100 mL of 70% (v/v) ethanol. The ultrasonic-assisted extraction process was carried out at three temperature levels (30, 50, and 70 °C) and three time intervals (30, 60, and 120 min). After sonication, the mixtures were subjected to filtration, and the resulting filtrates were concentrated under reduced pressure using a rotary evaporator operated at 45 °C. The extracts were stored in the dark at a temperature of minus 18 degrees Celsius until further analysis.

(a) (b) (c) (d)

Figure 1. Banana Peel: (a) Cavendish; (b) Bluggoe; (c) Pisang Awak; and (d) Red Dacca

2.2 Determination of Total Phenolic Content (TPC)

Gallic acid reference standards were prepared in a concentration series of 0, 20, 40, 60, 80, and 100 mg/L. For each concentration, each standard (0.5 mL) was placed in a test tube and combined with 2.5 mL of distilled water. Then, 0.5 mL of diluted Folin-Ciocalteu reagent (diluted 1:10) and 0.5 mL of Na_2CO_3 solution (7.5% w/v) were introduced to the mixture. After thorough mixing by vortex, the samples were kept in darkness at ambient temperature for 30 min, after which absorbance was measured at 765 nm using UV-Vis spectrophotometry. Each extract sample was prepared at a final concentration of 1 mg/L. Subsequently, 0.5 mL of each sample solution was processed following an identical protocol: combined with 2.5 mL distilled water, treated with 0.5 mL diluted Folin-Ciocalteu reagent (1:10), and 0.5 mL of Na_2CO_3 solution (7.5% w/v). Following vortex mixing, samples were incubated in the dark at room temperature for 30 min, and absorbance was measured at a wavelength of 765 nm using UV-Vis spectrophotometry. A blank control containing only distilled water underwent identical treatment procedures. Quantification of total phenolic compounds in crude extracts was achieved through calibration against the gallic acid reference curve, with results reported as mg GAE/g extract. Triplicate analyses were performed for all determinations [14].

2.3 Determination of Total Flavonoid Content (TFC)

Quercetin reference standards were established across a concentration range of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL using ethanol as solvent. Each reference standard (1 mL) was transferred to a test tube and combined with 1 mL of $AlCl_3$ (10% v/v). The resulting mixture underwent thorough agitation and was maintained under dark conditions at ambient temperature for 5 min. Then, 0.5 mL of CH_3COOH solution (10% v/v) was introduced, and the final volume was brought to 5 mL using distilled water. After vortex mixing, absorbance readings were obtained at 420 nm wavelength using UV-Vis spectrophotometry. Sample extracts were processed at a 1.0 mg/mL concentration. Each extract (1 mL) was placed in a test tube and treated with 1 mL of $AlCl_3$ (10% v/v) solution. The mixture was agitated and kept in the dark at room temperature for 5 min. An aliquot of 0.5 mL of CH_3COOH solution (10% v/v) was added, and the volume was adjusted to 5 mL with distilled water. The mixture was thoroughly homogenized using a vortex mixer and subsequently subjected to absorbance measurement at 420 nm using a UV–visible spectrophotometer. Total flavonoid concentrations in crude extract samples were determined using the quercetin calibration curve and reported as mg QE/g extract. All analytical determinations were performed in triplicate [15-16].

2.4 Determination of Antioxidant Capacity by DPPH Radical Scavenging Assay

A 100 μM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was prepared in absolute ethanol. Trolox reference standards were formulated across concentration levels of 25, 50, 250, 500, and 1,000 μg/mL. Each standard solution (1,000 μL) was combined with an equal volume (1,000 μL) of DPPH solution. The resulting mixtures underwent vortex homogenization and were maintained under dark conditions for 30 min before spectrophotometric analysis at 516 nm wavelength. Sample crude extracts were processed at equivalent concentrations of 25, 50, 250, 500, and 1,000 μg/mL. For each test concentration, 1,000 μL of extract was combined with 1,000 μL of DPPH solution. Following vortex mixing, the combinations were kept in darkness

at room temperature for 30 min before absorbance determination at 516 nm using UV-Vis spectrophotometry. Pure ethanol served as the control blank, replacing the sample solution while following identical procedural steps. Triplicate analyses were conducted for all samples [17-19].

Radical scavenging activity percentage was determined using the equation:

$$\% \text{ Radical scavenging} = [(Ac - As)/Ac] \times 100$$

Where:

Ac represents the absorbance of the DPPH solution alone.

As represents the absorbance of the sample – DPPH mixture.

The resulting data were utilized to establish the 50% inhibitory concentration (IC_{50}) through graphical analysis, plotting extract concentration versus % radical scavenging activity. Trolox served as the reference standard for comparative analysis.

2.5 Antioxidant Capacity Test by Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP working solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) dissolved in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ at a volumetric ratio of 10:1:1. The mixture was incubated at 37 °C for 4 min before use. Trolox was used as the reference standard at concentrations of 200, 400, 600, 800, and 1,000 μM. For the assay, 20 μL of each Trolox standard solution was mixed with 180 μL of FRAP working solution, followed by vortex homogenization and incubation in the dark at room temperature for 30 min. Absorbance was measured at 593 nm using a UV–Vis spectrophotometer, and a standard calibration curve was established to correlate Trolox concentration with absorbance. Crude banana peel extracts were dissolved at 2 mg/mL, and 20 μL of each sample solution was mixed with 180 μL of FRAP working solution under the same conditions. The antioxidant capacity was expressed as FRAP values, calculated from the Trolox standard curve and reported as mg TE/g extract, indicating the reducing potential of the samples (Fe^{3+} to Fe^{2+}). All analytical determinations were performed in triplicate [20].

2.6 Tyrosinase Inhibitory Capacity Test by Dopachrome Method

Kojic acid reference standards were established across concentration levels of 0.01, 0.05, 0.1, 0.5, and 1 mg/mL. Each standard level (40 μL) was transferred to a 96-well plate and combined with 80 μL of phosphate buffer (50 mM, pH 6.8) containing L-tyrosine at 90 mg/mL. Following thorough homogenization, 40 μL of L-DOPA (2.5 mM L-3,4-dihydroxyphenylalanine) was introduced. After complete mixing, the reaction mixture was maintained at 25 °C for 20 min. Spectrophotometric analysis was subsequently performed at 475 nm using a UV-Vis microplate reader. Sample extracts were processed at equivalent concentrations of 0.01, 0.05, 0.1, 0.5, and 1 mg/mL. For each test concentration, 40 μL of the sample was transferred into a 96-well plate and treated with 80 μL of phosphate buffer (50 mM, pH 6.8) containing L-tyrosine (90 mg/mL). After thorough mixing, the reaction mixture was incubated at 25 °C for 20 min. Absorbance determination was conducted at 475 nm using a UV-Vis microplate reader. Control solution preparation involved phosphate buffer substitution for the sample extract, while the blank solution employed buffer replacement for the enzyme component. Inhibition percentage was determined using the equation.

$$\% \text{ Inhibition} = [(Ac - As)/Ac] \times 100$$

Where:

Ac represents the control solution absorbance.

As a representative sample solution absorbance.

Tyrosinase inhibitory capacity was evaluated against Kojic acid as a reference standard. IC_{50} values were determined through graphical analysis, plotting extract concentration versus inhibition percentage[21].

2.7 Statistical Analysis

All experimental data, obtained from triplicate determinations, were subjected to statistical evaluation using analysis of variance (ANOVA). Mean comparisons were performed with Duncan's New Multiple Range Test (DMRT) at a significance level of $p = 0.05$, and results are presented with distinct superscript letters in the tables to indicate statistically significant differences among treatments. All analyses were conducted using a

statistical software package. In addition, correlation analysis was carried out to assess the relationships among total phenolic content, total flavonoid content, antioxidant capacity, and tyrosinase inhibitory activity of banana peel extracts obtained through ultrasonic extraction under varying temperature and time conditions. Pearson's correlation coefficient (r) was employed for this analysis.

3. Results and Discussion

3.1 Ultrasound-Assisted Extraction of Banana Peel: Yield and Cultivar Effects

The banana peel varieties examined in this investigation comprised Cavendish banana peel, Bluggoe banana peel, Pisang Awak banana peel, and Red Dacca banana peel. These specimens underwent ultrasonic extraction using 70% ethanol (v/v) as the solvent under ultrasonic conditions with frequencies above 50 kHz at temperatures of 30, 50, and 70 °C for durations of 30, 60, and 120 min. The extraction process demonstrated varying efficiency depending on the type of banana peel examined. Extract preparation involved vacuum drying and weight measurements under dark conditions. The crude extracts appeared as dark brown to black viscous liquids. Upon weighing the crude extracts and calculating the yield percentage based on the dry weight of the sample (10 g), the crude extract yields ranged from 12.21% to 18.57% (Table 1).

Table 1. Percentage yields (mean± SD) of crude banana peel extracts obtained by ultrasonic-assisted extraction.

Temperature (°C)	Extraction time (min)	Cavendish (%)	Bluggoe (%)	Pisang Awak (%)	Red Dacca (%)
30	30	14.23 ± 0.02^{A}	14.25 ± 0.06^{A}	14.25 ± 0.06^{A}	14.06 ± 0.03^{AF}
	60	13.23 ± 0.01^{B}	13.70 ± 0.04^{H}	13.87 ± 0.09^{L}	13.66 ± 0.01^{H}
	120	12.39 ± 0.05^{C}	13.23 ± 0.01^{B}	13.34 ± 0.03^{M}	13.03 ± 0.02^{P}
50	30	12.21 ± 0.01^{D}	12.23 ± 0.01^{D}	12.23 ± 0.01^{D}	12.36 ± 0.00^{D}
	60	12.35 ± 0.01^{D}	12.60 ± 0.00^{I}	12.28 ± 0.00^{D}	12.68 ± 0.00^{I}
	120	12.63 ± 0.01^{D}	12.71 ± 0.00^{I}	12.86 ± 0.01^{N}	12.82 ± 0.01^{N}
70	30	14.40 ± 0.00^{E}	13.97 ± 0.01^{O}	13.97 ± 0.01^{O}	14.03 ± 0.02^{OF}
	60	14.23 ± 0.00^{A}	14.34 ± 0.00^{J}	14.02 ± 0.01^{OF}	14.36 ± 0.03^{J}
	120	14.87 ± 0.00^{F}	14.67 ± 0.00^{K}	14.69 ± 0.00^{K}	14.56 ± 0.01^{R}

Note: Different superscript letters (A–R) within a column indicate significant differences at $p \leq 0.05$ (DMRT)

According to Table 1, at 70 °C for 120 min, Cavendish peel exhibited the highest crude extract yield (18.57%), which was significantly higher ($p \leq 0.05$, DMRT) than those of Red Dacca (17.54%), Bluggoe (16.89%), and Pisang Awak (15.26%). Prolonged extraction duration (120 min) in combination with elevated temperature enhanced solvent penetration and facilitated the release of intracellular compounds. In contrast, at 30 °C, the yields were markedly lower across all cultivars, indicating that mild thermal conditions were insufficient to disrupt plant tissues effectively. Ultrasonic extraction of banana peels yielded crude extracts ranging from 13.97% to 18.57% at optimal conditions. Regression analysis revealed that temperature significantly influenced extraction yields, with 70°C producing maximum yields for all varieties. Elevated temperatures enhance bioactive compound solubility and reduce solvent viscosity, improving mass transfer and solvent penetration into plant tissues [22-23]. Extraction duration also significantly affected yields, with 120 min at 70 °C producing optimal results. Extended extraction periods provide sufficient time for solvent penetration into cellular structures and bioactive compound release [24-25]. Varietal comparison showed Cavendish banana yielded the highest crude extracts (18.57% at 70 °C, 120 min), followed by Red Dacca (17.54%), Bluggoe (16.89%), and Pisang Awak (15.26%). These differences reflect variations in cell wall structure, phenolic content, and bioactive composition among cultivars, influenced by genetic diversity, maturation stages, and environmental factors [26-27]. Ultrasonic extraction enhanced efficiency through cavitation mechanisms, generating intense shock waves via microbubble collapse that rupture cell walls and facilitate intracellular compound release[28-29]. The cavitation phenomenon creates localized high temperatures and pressures, softening cellular tissues and increasing mass transfer rates. The yields obtained (12.21-18.57%) were comparable to recent banana peel extraction studies under similar conditions[24-25].

3.2 Results of Total Phenolic Content (TPC)

Total phenolic extraction yields from the peel of four banana varieties, including Cavendish banana peel, Bluggoe banana peel, Pisang Awak banana peel, and Red Dacca banana peel, were subjected to ultrasonic extraction with frequencies above 50 kHz at temperatures of 30, 50, and 70 °C, and extraction times of 30, 60, and 120 min. The TPC was determined using the Folin-Ciocalteu method, and the results were expressed as gallic acid equivalents per gram of extract (mg GAE/g extract). The total phenolic content of the extracts varied between 1.03 and 4.5 mg GAE/g extract. The Pisang Awak banana peel had the highest phenolic content at 4.63 ± 0.03 mg GAE/g extract at 60 min and 50 °C, which was significantly higher ($p \leq 0.05$, DMRT) than those of Cavendish banana peel, Bluggoe banana peel, and Red Dacca banana peel had phenolic contents of 4.02 ± 0.01, 3.65 ± 0.03, and 2.87 ± 0.01 mg GAE/g extract, respectively, under the same conditions (60 min, 50 °C) (Table 2).

Table 2. Total phenolic content (mg GAE/g extract) of crude extracts from various banana peel varieties**.**

Temperature (°C)	Extraction time (min)	Cavendish	Bluggoe	Pisang Awak	Red Dacca
30	30	2.31 ± 0.09^{B}	2.16 ± 0.02^{B}	2.56 ± 0.01^{A}	2.04 ± 0.01^{C}
	60	2.43 ± 0.01^{B}	2.32 ± 0.02^{C}	3.55 ± 0.01^{A}	2.27 ± 0.02^{C}
	120	2.27 ± 0.01^{B}	2.25 ± 0.04^{B}	2.77 ± 0.01^{A}	2.25 ± 0.01^{B}
50	30	3.66 ± 0.02^{B}	3.32 ± 0.03^{C}	4.05 ± 0.02^{A}	2.45 ± 0.02^{D}
	60	4.02 ± 0.01^{B}	3.65 ± 0.03^{C}	4.63 ± 0.03^{A}	2.87 ± 0.01^{D}
	120	3.89 ± 0.02^{B}	3.43 ± 0.01^{C}	4.24 ± 0.01^{A}	2.59 ± 0.02^{D}
70	30	2.24 ± 0.04^{B}	2.28 ± 0.01^{B}	2.31 ± 0.01^{A}	2.11 ± 0.01^{C}
	60	2.45 ± 0.01^{B}	2.50 ± 0.01^{B}	2.56 ± 0.01^{A}	2.36 ± 0.01^{C}
	120	2.32 ± 0.02^{B}	2.31 ± 0.01^{B}	2.41 ± 0.00^{A}	2.30 ± 0.02^{B}

Note: Different superscript letters (A–D) within a column indicate significant differences at $p \leq 0.05$ (DMRT)

Temperature optimization analysis revealed that 50 °C yielded the highest TPC for all four banana peel varieties compared to 30 °C and 70 °C. This trend is consistent with previous reports indicating that moderate heating (50–60 °C) enhances phenolic recovery. For instance, Vu et al. (2019)[30] demonstrated that microwave-assisted extraction of Cavendish banana peel achieved high phenolic yields at approximately 50–60 °C, while Wang et al. [26] profiled different banana cultivars and similarly noted improved phenolic extraction under moderate temperature conditions. The TPC values obtained in the present study fall within the ranges reported previously. Suleria et al. [27] reported wide variation among 27 Australian-grown banana cultivars, with TPC ranging from 0.15 to 55.5 mg GAE/g. Vu et al. [30] observed phenolic recovery up to 50.55 mg GAE/g from Cavendish peel using microwave-assisted extraction, whereas Wang et al. [26] showed cultivar-specific differences linked to maturity and ecotype. Such varietal differences reflect both genetic diversity and cultivation environments [27]. Regarding extraction time, 60 min provided optimal recovery for most varieties in this study. In contrast, Vu et al. [30] reported much shorter optimal extraction times (6 min) for microwave-assisted extraction, highlighting that advanced extraction techniques often require less time than conventional approaches. At 30 °C, insufficient thermal energy may limit cell wall disruption, whereas moderate temperatures (50–60 °C) promote tissue softening, solubility, and mass transfer, thereby improving extraction efficiency [30]. However, phenolic compounds are heat-sensitive, and degradation may occur at higher temperatures (>70 °C), as previously noted in banana peel extracts [27]. Under optimized conditions (50 °C, 60 min), Pisang Awak banana peel exhibited the highest phenolic content among the examined varieties, highlighting its potential as a promising source for food, pharmaceutical, and cosmetic applications.

3.3 Results of Total Flavonoid Content (TFC)

The total flavonoid content in crude preparations from four types of banana peels, including the peels of the Cavendish banana, Bluggoe banana, Pisang Awak banana, and Red Dacca banana, was determined using the Aluminium Trichloride ($AlCl_3$) colorimetric assay, with quercetin serving as the standard reference. Total flavonoid concentrations in these extracts, processed under varying temperatures and extraction times,

ranged from 1.96 to 4.28 mg QE/g extract. The extract from Pisang Awak banana peel exhibited the highest TFC of 4.28 ± 0.01 mg QE/g extract at 60 min and 50 °C, followed by the Cavendish banana peel, Bluggoe banana peel, and Red Dacca banana peel, which had values of 3.87 ± 0.02, 3.46 ± 0.02, and 2.81 ± 0.00 mg QE/g extract, respectively, at 60 min and 50 °C Statistical analysis ($p \leq 0.05$, DMRT) showed significant differences among cultivars, indicating Pisang Awak peel as the richest flavonoid source. (Table 3).

Table 3. Total flavonoid content (mg QE/g extract) of crude extracts from various banana peel varieties.

Temperature (°C)	Extraction time (min)	Cavendish	Bluggoe	Pisang Awak	Red Dacca
30	30	2.02 ± 0.01^{B}	2.02 ± 0.01^{B}	2.48 ± 0.02^{A}	1.96 ± 0.02^{C}
	60	2.31 ± 0.01^{B}	2.25 ± 0.00^{B}	3.23 ± 0.01^{A}	2.11 ± 0.01^{C}
	120	2.15 ± 0.03^{B}	2.10 ± 0.01^{B}	2.51 ± 0.01^{A}	2.03 ± 0.02^{C}
50	30	3.51 ± 0.02^{B}	3.28 ± 0.02^{C}	3.98 ± 0.01^{A}	2.35 ± 0.03^{D}
	60	3.87 ± 0.02^{B}	3.46 ± 0.02^{C}	4.28 ± 0.01^{A}	2.81 ± 0.00^{D}
	120	3.72 ± 0.02^{B}	3.36 ± 0.02^{C}	4.09 ± 0.02^{A}	2.40 ± 0.00^{D}
70	30	1.97 ± 0.03^{B}	2.08 ± 0.01^{B}	2.19 ± 0.01^{A}	1.94 ± 0.03^{C}
	60	2.32 ± 0.02^{B}	2.37 ± 0.01^{B}	2.42 ± 0.01^{A}	2.11 ± 0.01^{C}
	120	2.05 ± 0.01^{B}	2.23 ± 0.01^{B}	2.28 ± 0.02^{A}	2.03 ± 0.01^{C}

Note: Different superscript letters (A–D) within a column indicate significant differences at $p \leq 0.05$ (DMRT)

Flavonoid content analysis revealed patterns consistent with phenolic content, with 50 °C and 60 min optimal for all banana peel varieties. Quantitative comparison contextualizes these findings: Mahmood et al. [31] reported TFC of 13.99 mg QE/g DM using enzyme-assisted extraction at 55 °C, while Ishak et al. [32] obtained 29.0 mg RE/g from Cavendish peel at 60 °C. Pisang Awak whole fruit demonstrated TFC of 440 mg QE/100 g, significantly exceeding other cultivars [33], while Wanyo et al. [34] reported 196 mg QE/g from banana peel extracts. The TFC values obtained align with these ranges, confirming extraction effectiveness. Temperature significantly influenced flavonoid extraction, with 50 °C yielding optimal results. Reduced content at 70 °C reflects thermal sensitivity, as flavonoids exhibit greater lability than phenolic acids with degradation at elevated temperatures [35]. The 60-minute duration proved optimal, balancing efficiency with compound stability. Varietal comparison under optimal conditions revealed distinct differences reflecting genetic diversity, maturation stages, and environmental factors [27]. Positive correlations between flavonoid and phenolic content were evident, with elevated phenolic levels corresponding to increased flavonoid accumulation. This establishes Pisang Awak banana peel as a valuable natural source of flavonoids for food, pharmaceutical, and cosmetic applications.

3.4 Results of Antioxidant Capacity using DPPH Radical Scavenging Methodology

Antioxidant capacity evaluation of crude preparations from four types of banana peels, including Cavendish banana peel, Bluggoe banana peel, Pisang Awak banana peel, and Red Dacca banana peel, employed the DPPH radical scavenging assay with Trolox serving as the reference solution. The IC_{50} values for these crude extracts, obtained under varying temperatures and extraction times, ranged from 1.27 to 2.06 μg/mL. The Pisang Awak banana peel extract demonstrated superior antioxidant performance, with an IC_{50} of 1.27 ± 0.08 μg/mL at 60 min and 50 °C, statistically lower ($p \leq 0.05$, DMRT) than Cavendish banana peel extract, Bluggoe banana peel extract, and Red Dacca banana peel extract, which showed IC_{50} values of 1.31 ± 0.04, 1.35 ± 0.03, and 1.55 ± 0.10 μg/mL, respectively, at 60 min and 50 °C. The IC_{50} value of Trolox at the same concentration was 1.28 ± 0.34 μg/mL (Table 4).

Table 4. DPPH Radical scavenging capacity (IC_{50}, mg/mL) of crude extracts from various banana peel varieties.

Temperature	IC_{50} (μg/mL)

(°C)	Extraction time (min)	Cavendish	Bluggoe	Pisang Awak	Red Dacca
30	30	1.59 ± 0.07^{B}	1.44 ± 0.04^{A}	1.66 ± 0.02^{C}	1.70 ± 0.06^{D}
	60	1.46 ± 0.11^{A}	1.42 ± 0.04^{A}	1.57 ± 0.02^{B}	1.65 ± 0.05^{C}
	120	1.65 ± 0.05^{B}	1.64 ± 0.03^{B}	1.67 ± 0.01^{B}	1.87 ± 0.03^{C}
50	30	1.35 ± 0.01^{A}	1.57 ± 0.05^{C}	1.44 ± 0.04^{B}	1.68 ± 0.08^{D}
	60	1.31 ± 0.04^{A}	1.35 ± 0.03^{A}	1.27 ± 0.08^{A}	1.55 ± 0.10^{B}
	120	1.66 ± 0.03^{B}	1.78 ± 0.11^{C}	1.66 ± 0.04^{B}	1.83 ± 0.04^{D}
70	30	1.97 ± 0.03^{C}	1.93 ± 0.05^{C}	1.65 ± 0.05^{A}	1.69 ± 0.05^{B}
	60	1.74 ± 0.02^{B}	1.63 ± 0.03^{A}	1.35 ± 0.04^{A}	1.63 ± 0.04^{A}
	120	2.06 ± 0.05^{C}	1.85 ± 0.05^{B}	1.80 ± 0.04^{B}	1.92 ± 0.07^{B}
Trolox IC_{50} (μg/mL)	-	1.28 ± 0.34			

Note: Different superscript letters (A–D) within a column indicate significant differences at $p \leq 0.05$ (DMRT)

Pisang Awak banana peel extract obtained at 50 °C for 60 min achieved superior antioxidant performance with an IC_{50} value comparable to Trolox standard, indicating potential as a natural antioxidant. Quantitative comparison with recent studies contextualizes these findings. Islam et al. [31] reported DPPH scavenging activity of 81.59% and ABTS activity of 88.25% from banana peel extracts using enzyme-assisted extraction at 55 °C. [36] documented DPPH values of 52.38 ± 2.04 mg Trolox/g sample from Nam Wa banana peel using methanol extraction. For Barangan banana peel (Musa acuminata), IC_{50} values of 32.52 μg/mL and 83.60 μg/mL were reported using ethanol extraction [37]. Additionally, Musa sapientum variety Muraru demonstrated IC_{50} values of 73.70 μg/mL and 147.99 μg/mL for methanolic extracts [38]. The IC_{50} values obtained in this study align with these reported ranges, confirming comparable antioxidant efficacy. Temperature significantly influenced antioxidant activity, with 50 °C optimal for all banana peel varieties. Activity decreased at 70 °C, consistent with phenolic and flavonoid degradation patterns at elevated temperatures. This aligns with recent findings that excessive heat compromises bioactive compound stability [24-25]. The 60-minute extraction duration maximized antioxidant activity, with both shorter and longer durations yielding reduced efficacy, reflecting the balance between extraction efficiency and oxidative degradation. Varietal comparison under optimal conditions (50 °C, 60 min) revealed positive correlations between antioxidant capacity and phenolic-flavonoid concentrations. Peels with elevated phenolic and flavonoid content demonstrated correspondingly higher antioxidant activity, consistent with established relationships between these bioactive compounds [26, 33]. These compounds exert antioxidant effects primarily through radical-scavenging mechanisms involving hydrogen atom transfer (HAT) and single electron transfer (SET), which neutralize reactive oxygen species (ROS) and prevent oxidative damage [38]. The findings establish Pisang Awak banana peel as a promising natural antioxidant source for food, pharmaceutical, and cosmetic applications.

3.5 Results of Antioxidant Capacity Assessment using the Ferric Reducing Antioxidant Power (FRAP) Assay

Antioxidant performance was evaluated utilizing the Ferric Reducing Antioxidant Power (FRAP) assay, which quantified the electron-donating ability of each sample. This method is based on the reduction of the Fe^{3+}-TPTZ (ferric tripyridyltriazine) complex to the Fe^{2+}-TPTZ (ferrous tripyridyltriazine) complex by antioxidant compounds. The results were reported as FRAP values (mg TE/g extract), with higher FRAP values indicating greater reducing potential of the crude preparations. The crude extracts from the peels of four banana varieties, including Cavendish banana peel, Bluggoe banana peel, Pisang Awak banana peel, and Red Dacca banana peel, exhibited antioxidant activity at various extraction times and temperatures, with FRAP values ranging from 2.09 to 3.71 mg TE/g extract. The crude extract from Pisang Awak banana peel showed the highest antioxidant activity, with a FRAP value of 3.71 ± 0.03 mg TE/g extract at 60 min and 50 °C, significantly higher ($p \leq 0.05$, DMRT) than Cavendish banana peel (3.49 ± 0.08 mg TE/g extract), Bluggoe banana (3.50 ± 0.07 mg TE/g extract), and Red Dacca banana peel (3.21 ± 0.03 mg TE/g extract), all under the

same conditions (60 min, 50 °C). Trolox standard exhibited 3.65 ± 0.16 mg TE/g extract 3.65 ± 0.16 mg TE/g extract (Table 5).

Table 5. Ferric reducing antioxidant power (FRAP, mg TE/g extract) of crude extracts from various banana peel varieties

Temperature (°C)	Extraction time (min)	FRAP (mg TE/g extract)			
		Cavendish	Bluggoe	Pisang Awak	Red Dacca
30	30	2.34 ± 0.04^{B}	2.38 ± 0.13^{B}	2.29 ± 0.07^{B}	2.09 ± 0.08^{C}
	60	2.58 ± 0.04^{B}	2.52 ± 0.02^{B}	2.53 ± 0.14^{B}	2.28 ± 0.16^{C}
	120	2.52 ± 0.10^{B}	2.42 ± 0.08^{B}	2.52 ± 0.11^{B}	2.14 ± 0.06^{C}
50	30	2.87 ± 0.61^{A}	3.14 ± 0.02^{A}	3.38 ± 0.06^{A}	3.06 ± 0.05^{A}
	60	3.49 ± 0.08^{A}	3.50 ± 0.07^{A}	3.71 ± 0.03^{A}	3.21 ± 0.03^{A}
	120	3.39 ± 0.13^{A}	3.42 ± 0.10^{A}	3.36 ± 0.08^{A}	3.11 ± 0.02^{A}
70	30	2.43 ± 0.03^{B}	2.33 ± 0.02^{B}	2.43 ± 0.06^{B}	2.13 ± 0.13^{C}
	60	2.73 ± 0.10^{B}	2.54 ± 0.05^{B}	2.80 ± 0.15^{B}	2.28 ± 0.03^{C}
	120	2.58 ± 0.05^{B}	2.42 ± 0.03^{B}	2.55 ± 0.15^{B}	2.11 ± 0.05^{C}
Trolox FRAP (mg/mL)	-	3.65 ± 0.16			

Note: Different superscript letters (A–C) within a column indicate significant differences at $p \leq 0.05$ (DMRT)

All banana peel extracts demonstrated ferric reducing capacity (Fe^{3+} to Fe^{2+} conversion), with Pisang Awak exhibiting particularly high antioxidant potential through effective electron donation [39]. These findings aligned with Sulaiman et al. [4] and Darsini et al. [33], who reported comparable FRAP activity in Pisang Awak to synthetic antioxidant BHT. Extraction at 50 °C yielded optimal FRAP values across all varieties, consistent with recent optimization studies. Hernández-Carranza et al. [40] and Granella et al. [24] reported maximum bioactive compound extraction at 50–60 °C, with polyphenol recovery of 355.11 ± 4.99 μg GAE/g achieved at 60 °C. Chaudhry et al. [41] confirmed that moderate temperatures produced the highest phenolic content (31.45 mg GAE/g) and FRAP activity (29.51%). At 70 °C, FRAP values decreased substantially due to thermal degradation of antioxidant compounds. Cegledi et al. [25] observed reduced hydroxycinnamic acid and flavonoid content beyond 60°C, while Khalangre et al. [42] demonstrated accelerated degradation at 70 °C through kinetics analysis. Vu et al. [30] explained that excessive temperatures decrease recovery yield despite initially enhanced compound release. These results supported Fidrianny et al. [43] and Islam et al. [31], who established positive correlations between phenolic content and FRAP activity at optimal temperatures of 35–60 °C. The FRAP values of Pisang Awak extract exceeded the Trolox standard, indicating exceptional potential for development as a natural antioxidant agent when extracted at optimized temperatures of 50–60 °C to maximize bioactive recovery while minimizing thermal degradation.

3.6 Results of Tyrosinase Inhibitory Capacity Assessment Employing the Dopachrome Assay

The tyrosinase inhibitory capacity of crude preparations from peels of four banana varieties, including Cavendish banana peel, Bluggoe banana peel, Pisang Awak banana peel, and Red Dacca banana peel, was determined utilizing the Dopachrome assay, employing Kojic acid as the reference compound. The crude preparations from all four banana peel types, extracted under varying temperatures and durations, demonstrated tyrosinase inhibitory potential, with IC_{50} values ranging from 1.34 to 2.06 mg/mL. The crude extract from Pisang Awak banana peel exhibited superior tyrosinase inhibitory performance, with an IC_{50} concentration of 1.34 ± 0.03 mg/mL at 60 min and 50 °C, statistically lower ($p \leq 0.05$, DMRT) than Cavendish banana peel, Bluggoe banana peel, and Red Dacca banana peel, with IC_{50} values of 1.46 ± 0.03, 1.55 ± 0.04, and 1.62 ± 0.03 mg/mL, respectively, under the same extraction conditions (60 min, 50 °C). The IC_{50} concentration of the reference Kojic acid standard was 1.34 ± 0.21 mg/mL (Table 6).

Table 6. Tyrosinase inhibitory capacity (IC_{50}, mg/mL) of crude extracts from various banana peel varieties.

Temperature (°C)	Extraction time (min)	IC_{50} (mg/mL)			
		Cavendish	Bluggoe	Pisang Awak	Red Dacca
30	30	1.69 ± 0.08^{B}	1.75 ± 0.06^{B}	1.73 ± 0.04^{B}	1.82 ± 0.02^{A}
	60	1.56 ± 0.02^{B}	1.55 ± 0.02^{B}	1.52 ± 0.02^{C}	1.60 ± 0.02^{A}
	120	1.66 ± 0.06^{B}	1.68 ± 0.09^{B}	1.60 ± 0.01^{C}	1.73 ± 0.01^{A}
50	30	1.54 ± 0.03^{B}	1.84 ± 0.05^{A}	1.55 ± 0.03^{B}	1.85 ± 0.05^{A}
	60	1.46 ± 0.03^{B}	1.55 ± 0.04^{A}	1.34 ± 0.03^{C}	1.62 ± 0.03^{A}
	120	1.63 ± 0.04^{B}	1.71 ± 0.02^{A}	1.42 ± 0.02^{C}	1.73 ± 0.02^{A}
70	30	1.83 ± 0.02^{A}	1.73 ± 0.05^{B}	1.64 ± 0.04^{C}	1.82 ± 0.02^{A}
	60	1.58 ± 0.02^{B}	1.63 ± 0.03^{B}	1.53 ± 0.02^{C}	1.79 ± 0.02^{A}
	120	1.71 ± 0.03^{C}	1.63 ± 0.03^{C}	1.66 ± 0.03^{BC}	1.94 ± 0.04^{A}
0Kojic acid IC_{50} (mg/mL)	-	1.34 ± 0.21			

Note: Different superscript letters (A–C) within a column indicate significant differences at $p \leq 0.05$ (DMRT)

Pisang Awak banana peel crude extracts demonstrated tyrosinase inhibitory activity (66.39–77.05%) exceeding kojic acid (44.68–59.93%), attributed to flavonoids, particularly ferulic acid (906.62 mg/100 g), which suppresses tyrosinase through copper chelation and competitive substrate binding [44-45]. Extraction temperature significantly influenced enzyme inhibition, with 50 °C yielding optimal activity across all cultivars, correlating with maximum phenolic (25.37 mg GAE/g DM) and flavonoid (13.99 mg QE/g DM) recovery. These findings aligned with Islam et al. reporting 55 °C optimal for enzyme-assisted extraction and Granella et al. [24] identifying 52.3 °C for ultrasound-assisted polyphenol recovery (355.11 ± 4.99 μg GAE/g). The temperature-dependent enhancement reflects improved cell wall disruption without thermal degradation [43]. Temperatures exceeding 60 °C accelerated phenolic compound degradation [25,30]. Extraction duration of 60 minutes maximized tyrosinase inhibitory capacity, suggesting complete extraction of key bioactive constituents. This optimal timeframe aligned with kinetic studies demonstrating maximum polyphenol recovery between 30–90 min [24, 46]. Under optimized conditions (50 °C, 60 min), tyrosinase inhibitory capacity correlated strongly with phenolic and flavonoid contents. These bioactive compounds inhibit tyrosinase through copper chelation at the binuclear active site and competitive L-tyrosine binding [44, 47]. Molecular docking revealed rosmarinic acid interactions with tyrosinase residues (HIS263, VAL283, SER282, MET280) at a binding energy of 5.05 kcal/mol [45]. The correlation between antioxidant capacity and tyrosinase inhibition confirms dual functionality of banana peel polyphenols in free radical scavenging and melanogenesis suppression [6, 39]. Phacharapiyangkul et al. [45] demonstrated a dose-dependent reduction in tyrosinase activity and melanin content through MITF downregulation via the p38 signaling pathway. Similarly, Linsaenkart et al. [44] confirmed suppression of MITF, TYR, TRP-1, and DCT gene expression, achieving effects comparable to kojic acid and arbutin. These findings indicate that banana peel extracts extracted at optimized temperatures (50–60 °C) represent promising natural alternatives to synthetic tyrosinase inhibitors for cosmetic and pharmaceutical applications.

4. Conclusions

This investigation evaluated the influence of ultrasound-assisted extraction (UAE) on total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and tyrosinase inhibitory activity of peel extracts from four economically important banana cultivars (*Musa* spp.) cultivated in Thailand: Cavendish, Bluggoe, Pisang Awak, and Red Dacca. UAE, utilizing 70% (v/v) aqueous ethanol as extraction solvent, recognized for its superior efficiency in recovering bioactive phytochemicals, was applied throughout the experimental procedures. The results demonstrated that Pisang Awak banana peel extracts exhibited significantly superior bioactive profiles compared to other cultivars ($p < 0.05$). This cultivar displayed the highest TPC and TFC values, accompanied by optimal antioxidant activity (DPPH, ABTS, and FRAP assays)

and maximal tyrosinase inhibitory capacity. These findings underscore the substantial potential of Pisang Awak peel extracts for valorization into high-value functional ingredients, including nutraceutical formulations, cosmeceutical applications, and pharmaceutical preparations targeting dermatological conditions such as hyperpigmentation disorders and photoaging. Moreover, this research aligns with the United Nations Sustainable Development Goals (SDGs), specifically contributing to SDG 12 (Responsible Consumption and Production) through agricultural waste valorization, SDG 3 (Good Health and Well-being) via development of natural bioactive ingredients, and SDG 9 (Industry, Innovation and Infrastructure) by promoting circular bioeconomy principles. Optimization studies identified 50 °C and 60 minutes as the optimal extraction parameters, yielding maximum extraction efficiency across all investigated banana peel cultivars, thereby establishing a reproducible and scalable methodology for industrial applications.

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