

Effects of the Ripening Time of Porcelain Banana (*Musa spp. Abb cv. Pisang awak*) on the total Polyphenol Contents and Bioactivities

Hue Thi Ha^{1#}, Son The Mai^{1#}, Nhi Tuyet Mai¹, Dao Thi Anh Phan^{1*} 

[#]These authors contributed equally to this work

¹Ho Chi Minh City University of Technology and Education, Vietnam

*Corresponding author. Email: daopta@hcmute.edu.vn

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ABSTRACT

To investigate the influence of the banana ripening stage on the change in the content of bioactive components and their bioactivities, ripening stages of porcelain banana peels were screened for the total phenolic content (TPC) and antioxidant activities using three methods, including DPPH scavenging assay, ferric reducing/antioxidant power (FRAP) assay, and inhibition of polyphenol oxidase activity (PPO assay). The Pearson correlation analysis of antioxidant parameters of banana peel extracts for each ripening stages with TPC data showed that the TPC values in the sample extracts were strongly negatively correlated with the IC₅₀ values of the DPPH assay ($r = -0.905$). They positively correlated with the Fe³⁺ reduction capacity of Fe²⁺ ($r = 0.723$) and the ability to inhibit PPO enzyme at a concentration extract of 100 µg/mL ($r = 0.786$). During the ripening of bananas, the TPC at stage 1 (M1, light green, 228 mg GAE/100 g DW) was highest, and decreased to a minimum at stage 4 (M4, yellow color, accounts for 50% to 85% of the total banana peel surface area), and increase in stage 6 (M6, yellow color and appearance of black points, 199.9 mg GAE/100 g DW). The banana peel extract in stage 6 showed intense activities, having the highest extraction yield (15.18%) and was analyzed chemical constituents by HPLC-MS, which showed that it contains valuable group compounds such as phenolic acids, flavonols, flavan-3-ols, catecholamines, lignans, and antibacterial compounds. This shows the potential of using banana peels to prepare polyphenol extracts containing high antioxidant activities, enhancing the use value of bananas.

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1. Introduction

Banana peel is considered one of the sources of many phenolic compounds with high biological activity, anti-microbial activity, and PPO enzyme inhibition. Still, different cultivars, growing conditions, or pretreatment methods also affect the content of biologically active phenolic compounds. Porcelain banana is one of Vietnam's most commonly grown bananas [1], [2]. Although banana peel is considered a waste product, it has high biological value, is rich in fiber (accounting for 50% of the total dry matter content), protein, some essential amino acids, saturated fatty acids, and potassium [3]. Banana peel contains many phenolic compounds, especially dopamine, L-DOPA, and catecholamine with significant content, which have a potent antioxidant capacity, so there are many potentials for antioxidant applications in foods [4]. Banana peel and banana pulp extracts can inhibit the activity of the PPO that catalyzes the chain reaction that produces brown pigment [5]. Banana peel extract was also shown to have a high capability to inhibit 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radicals [6]. Devatkal *et al.* (2011) also demonstrated that banana peel extract could inhibit lipid peroxidation similar to commercial butylated hydroxytoluene. In addition, there are antibacterial compounds in banana peels, such as β-sitosterol, 12-hydroxystearic acid, and malic acid. These substances can inhibit microorganisms found in food, such as *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella enteritidis* [7]. The phenolic compounds in banana peels are diverse and depend on the banana variety. In addition, the total polyphenol content also

change according to the ripening stages of bananas [8]. Most green banana peels have a higher phenolic compound content than ripe banana peels. This ratio is up to 1.6 times in the banana variety *M. paradisiaca* [9].

However, the current research on banana peel extract is still limited, mainly focusing on quantifying phenolic compounds present in the extract and comparing the content of bioactive substances in the extract between different banana varieties or different cultivation conditions. Still, there has been no study on how banana peel composition and antioxidant activity change during the transition from green to ripe bananas. Therefore, this study focused on investigating the influence of ripening time on the change of antioxidant activities and inhibiting polyphenol oxidase (PPO) in banana peels and finding out when the banana peels are of high quality.

2. Materials and Methods

2.1. Plant Materials and Chemicals

The Porcelain banana (*Musa spp. ABB cv. Pisang awak*) used in the study was obtained from Thu Duc Farmers Market, Ho Chi Minh City, Vietnam, in March 2020.

Ethanol (Chemsol, Vietnam); Aluminium chloride, sodium hydroxide, ferric chloride, hydrochloric acid, chloroform, sulfuric acid, sodium chloride, potassium iodide, iodine, and sodium carbonate (Xilong Chemical, China). Gallic acid, catechin, and Folin-Ciocalteu (FC) reagents were obtained from Merck (Germany). Methanol and acetonitrile used in HPLC were obtained from Fisher (USA).







2.2. Extraction

Six banana peel samples were obtained from six stages during the ripening stages of bananas (**Table 1**). All samples were dried, ground to size around 1 mm, and extracted by soaking with ethanol 96% solvent with raw material: solvent ratio of 1:3 (*w/v*) at room temperature (35 ± 3 °C) for 24 hours and repeated three times. Liquid extracts from each study sample were collected and evaporated (100 rpm, 50 °C) to get the crude extract. The extracts were stored in a dark glass jars at room temperature before using further studies. The extraction yields of the extracts were estimated using the expression:

$$H (\%) = \frac{m}{m'} \times 100 \quad (1)$$

H: Extraction yield (%); m: The weight of the extract (g); m': The weight of the material powder (g)

Table 1. The color description and experimental images of the six banana samples [10]

Stage	Color Description	Image
M1	Light green	
M2	Light green with a bit of yellow. Yellow color accounts for 8% to 25% of the total banana peel surface area	
M3	Yellow with a bit of green. Yellow color accounts for 25% to 50% of the total banana peel surface area	
M4	Yellow, but still green at the top and the end. Yellow color accounts for 50% to 85% of the total banana peel surface area.	
M5	Yellow color accounts for over 85% of the total banana peel surface area	
M6	Yellow color and appearance of black points	

2.3. Determination of total polyphenol contents (TPC)

The extract (0.2 g) was mixed with 9.80 mL of ethanol: water extract (70° v/v), incubated at 70°C for 10 min, and mixed by vortex for 5 min. The mixture was centrifuged at 3500 rpm for 10 min to collect the supernatant. Mixing 0.2 mL of the supernatant with 9.80 mL of distilled water, 1300 µL of the extract, 1 mL of FC reagent (10%), and incubation for 5 min, adding 700 µL of Na₂CO₃ 10% (w/v). The mixture was stirred and incubated at room temperature in the dark for 30 min, and the absorbance was measured at 760 nm using a UV spectrometer (UH5300, Hitachi, Japan). The TPC value was calculated and expressed in mg gallic acid per 100 gram dry weight (GAE/100g DW) [11].

2.4. DPPH free radical scavenging assay

The extract was diluted to various concentrations (10, 25, 50, 100 µg/mL). 1.5 mL of the diluted extract sample was mix with 1.5 mL of 0.15 mM DPPH (DPPH is diluted in 96% ethanol). Shake the mixture and incubate in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm a UV spectrophotometer (UH5300, Hitachi, Japan). Control samples at each concentration were prepared similarly but replaced the extract with 96% ethanol.

The percentage of DPPH free radical inhibition (I%) was calculated as:

$$I(\%) = \frac{As - Ac}{Ac} \times 100\% \quad (2)$$

I: The percentage of DPPH free radical inhibition (%)

Ac: The absorbance of the control sample

As: The absorbance of the banana peel extract

The half maximal inhibitory concentration (IC₅₀) was determined using the mean values from three measurements at 100, 50, 25, and 10 µg/mL concentrations in 96% ethanol. Gallic acid was used as a positive control with IC₅₀ of 5.6 µg/mL [12].

2.5 Ferric reducing/antioxidant power assay

The extract was diluted with distilled water to different concentrations (0.1, 0.5, 1.0 mg/mL). Mix 1.0 mL of the diluted extract with 2.5 mL of 2.0 M phosphate pH = 6.6 and 2.5 mL of potassium ferricyanide 1%. The mixture was incubated at 50°C for 20 min. Then add 2.5 mL of TCA 10% and centrifuge at 2000 rpm for 10 minutes. 2 mL of the supernatant was mixed with 2 mL of distilled water and 0.4 mL of ferric chloride 0.1%. The absorbance was measured at 700 nm using a UV spectrophotometer (UH5300, Hitachi, Japan). The control samples at each concentration were made similarly, but the diluted extraction was replaced with distilled water of the same volume. Vitamin C was used as the reducing agent in positive control samples [13].

2.6. Evaluation of the ability to inhibit PPO

Dissolve 0.01 g of the extract in 9.99 mL of distilled water and mix well with a vortex mixer for 30 s. The extract was diluted in phosphate buffer (pH 6.8) mixed with 100 µg/mL PPO 300 U/ml and incubated for 30 min at cold temperature (1 °C - 3 °C). Then, 1 ml L-DOPA 1.5 mM was added, and the reaction was carried out for 7 min. The absorbance was measured at 475 nm using a UV spectrometer (UH5300, Hitachi, Japan). The percentage of PPO inhibition (I%) was calculated similarly to the DPPH assay. Kojic acid was used as a positive control with an IC₅₀ of 49.9 µM [14].

2.7. HPLC-EIS-MS analysis

In this study, the chemical compositions of banana peel extract were analyzed by HPLC-ESI-MS. Liquid chromatography was carried out as follows: the stationary phase using ACE3- C18 column (4.6 × 150mm; 3.5 µm), stabilized at 40 °C. The gradient mobile phase of solution A was mixed with solution B in the following ratios: 0 min 95:5; 2 min 95:5; 25 min 0:100; 30 min 0:100 (v:v). Solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol). Next, inject 20 µL of sample volume into the HPLC system with a 0.5 mL/min flow rate. For ESI-MS, the gradient mobile phase is sprayed out of the microtubule at a slow nebulization rate of 10 - 20 µL/min. Precision ion m/z was adjusted in ESI - L turning mix by direct syringe pump 200 (µL/h)

2.8. Statistical analysis.

All experiments were replicated three times, enhancing the results expressed as mean \pm standard deviation. Experimental data were analyzed by analysis of variance (ANOVA) with Tukey's test ($p < 0.05$) using an SPSS package (SPSS 22 for Windows Evaluation Version, IBM Corporation, USA).

3. Results and Discussion

3.1. Effect of banana ripening stages on extraction efficiency

The results of the extraction yields are represented in Table 2. The mass loss relative to the material's moisture content decreases in the order of M2 (5.42%) and M3, M6 (5.1%). The peel extract M6 had the highest extraction efficiency of 15.18% and the lowest yield of peel extract M1 of 4.65%. The results suggested that extraction yield increased gradually with the ripening stages. This can be explained by the fact that during ripening, the starch content in the banana peel is broken down into sucrose, glucose, and fructose by enzymes such as amylase, glycosidase, sucrose synthase, and invertase [3]. These monosaccharides and disaccharides have good solubility in ethanol, leading to a high yield of banana peel extract towards the end of ripening [15], [16].

Table 2. Extraction yield of Banana peel extract

No	Sample	Raw material moisture (%)	Extraction Yield (%)
1	M1	5.43	6.37
2	M2	5.45	9.14
3	M3	5.10	10.83
4	M4	5.42	12.59
5	M5	5.15	13.93
6	M6	5.10	15.18

3.2. Effect of banana ripening stages on the total phenolic content

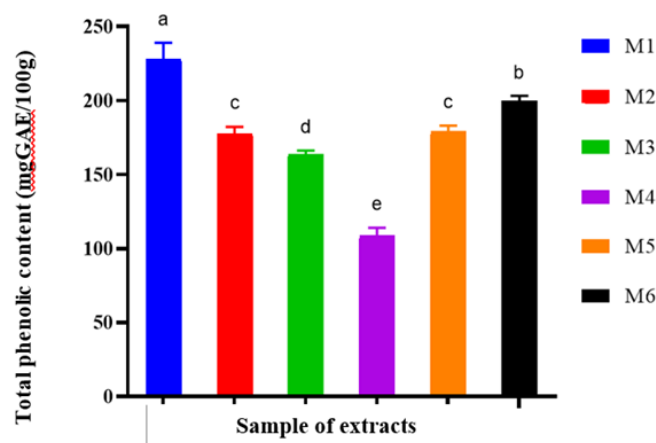


Figure 1. The TPC in banana peel extract by stages

The change of total polyphenol content (TPC) synthesis in banana peel extracts is presented in **Figure 1**. The results showed that the TPC in banana peel extract was highest in sample M1 228 ± 11 (mg GAE/100 g DW), decreased continuously to the minimum at stage 4 (M4), and increased at stage 6 (M6, 199.9 ± 3.4 mg GAE/100 g DW). This result may be due to flavonoid synthesis occurring through three pathways: shikimate pathway, phenylpropanoid pathway, and flavonoid pathway. These three pathways produce several phenolic compounds such as lignans, tyrosine, caffeic acid, gallic acid, phenylalanine, flavonoids, etc [17], [18].

3.3. Effect of banana ripening stages on the DPPH and FRAP assay

For the DPPH assay, the IC_{50} values of banana peel extracts changed gradually from sample M1 to sample M4 and then decreased in the following samples (**Figure 2a**). According to Blois (1958), samples with IC_{50} lower than $50 \mu\text{g/mL}$ in DPPH measurement indicate strong antioxidant activity; IC_{50} from $50 \mu\text{g/mL}$ to $100 \mu\text{g/mL}$ are samples with weak antioxidant activity [19], so banana peel extracts M1 ($17.29 \mu\text{g/mL}$), M2 ($43.56 \mu\text{g/mL}$), M6 ($49.66 \mu\text{g/mL}$) are classified as substances with intense antioxidant activity. Samples M5 ($IC_{50} = 50.51 \mu\text{g/mL}$), M3 ($69.46 \mu\text{g/mL}$), and M4 ($79.27 \mu\text{g/mL}$) are classified as substances with weak antioxidant activity.

For the FRAP assay, the absorbance at 700 nm increased as the sample concentration increased from 0.1 to 1.0 mg/mL ($p < 0.05$) (**Figure 2b**). At a 1 mg/mL concentration, the M1 extract had the most excellent absorbance of 0.567 ± 0.015 , showing the best reducing power, followed by samples M2 and M6 with absorbances of 0.365 ± 0.029 and 0.2870 ± 0.0050 , respectively. Samples M3, M4, and M5 show weak reducing capacities with lower absorbances: M3 (0.2150 ± 0.0038), M4 (0.2180 ± 0.0012), and M5 (0.2290 ± 0.0060). However, the Fe^{3+} reduction capacity to Fe^{2+} of the extracts was lower than that of the positive control (Vitamin C) with the absorbance of 1.0890 ± 0.0053 at 0.1 mg/mL concentration and previous studies [20].

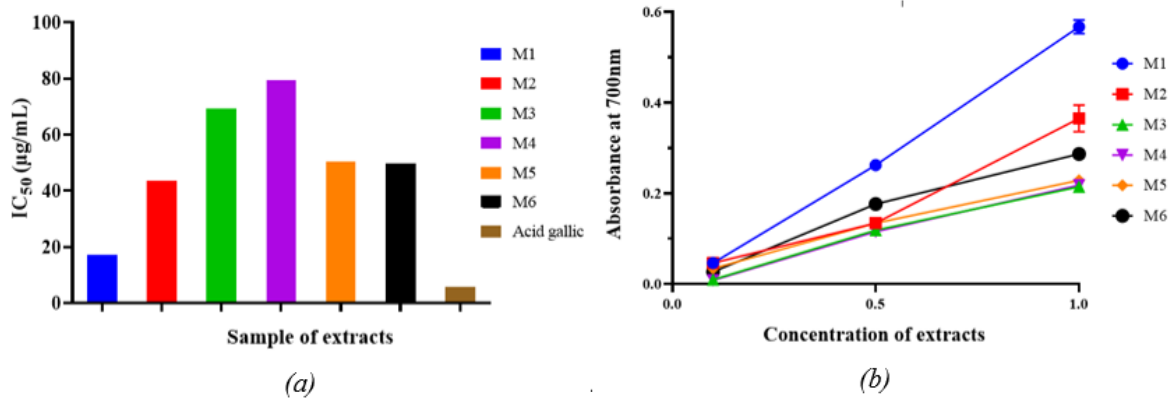


Figure 2. IC_{50} ($\mu\text{g/mL}$) in DPPH assay (a) and the absorbance at 700 nm in FRAP assay (b) of banana peel extracts in 6 stages of ripening

The results show that all banana peel extracts have antioxidant capacity in two categories. The samples with strong antioxidant capacities were those of ripe bananas M1, M2, M6, and the samples with weak antioxidant activities were those of ripe bananas M3, M4, and M5.

3.4. Effect of banana ripening stages on the inhibition of PPO enzyme of the extracts

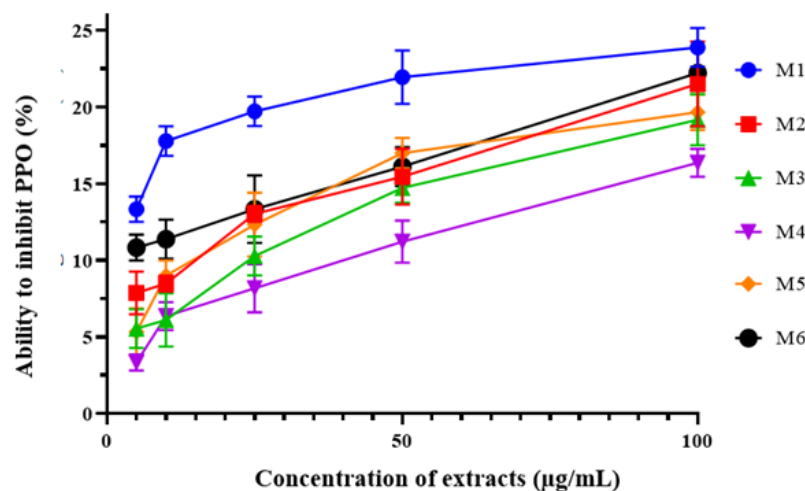


Figure 3. The percentage of PPO inhibition of the extracts at different concentrations

The percentage of PPO inhibition of the extracts is shown in Figure 3. The research results show that all samples of banana peel extract can inhibit PPO. At the 100 µg/mL concentration, sample M1 had the best ability to inhibit the PPO enzyme of $23.9 \pm 1.3\%$. However, also at this concentration, the ability to inhibit the PPO enzyme of sample M1 had no statistically significant difference compared with other samples such as sample M6 ($22.22\% \pm 0.48\%$), sample M2 ($21.5\% \pm 2.8\%$) and sample M5 ($19.7\% \pm 1.2\%$). The remaining samples with the ability to inhibit PPO enzyme decreased gradually such as sample M3 has a value of $19.2 \pm 1.7\%$ and the lowest value ($16.36 \pm 0.91\%$) belong to sample M4.

These results can be explained by the fact that banana peel extract containing polyphenols, flavonols, and flavan-3-ol monomers such as quercetin, catechins, and catechin derivatives, which can inhibit PPO by forming a chelating complex. Furthermore, the hydroxyl groups of phenolic compounds and organic acids which present in the structure of the hydroxycarboxylic acids, such as caffeic acid, ferulic acid, and ascorbic acid, also affect the reduction of DOPA-chrome to DOPA by donating electrons to DOPA-chrome [21].

3.5. Pearson correlation analysis between TPC and antioxidant activities

Table 3. Pearson correlation of TPC with antioxidant parameters of banana peel extract M6

Methods	TPC	DPPH (IC ₅₀)	FRAP ₁	PPO ₁₀₀
TPC	1			
DPPH (IC ₅₀)	-0,905**	1		
FRAP ₁	0,723**	0,901**	1	
PPO ₁₀₀	0,786**	0,766**	0,552*	1

* The correlation has a significance level of 0.01.

** The correlation has a significance level at 0.05.

TPC Synthetic content of phenolic compounds

DPPH (IC₅₀) A high concentration of extract inhibits 50% of DPPH free radicals

FRAP₁ The ability to reduce Fe³⁺ to Fe²⁺ at a concentration of 1 mg/mL

PPO₁₀₀ The ability to inhibit PPO enzyme at a concentration of 100 µg/mL

The results of Pearson correlation analysis of antioxidant parameters of banana peel extracts through the ripening stages are shown in Table 3. The study results showed that the TPC content in the high sample extract was strongly negatively correlated with the IC₅₀ index of the ability to inhibit DPPH free radicals ($r = -0.905$). They strongly positively correlated with the Fe³⁺ reduction capacity of Fe²⁺ ($r = 0.723$) and the ability to inhibit PPO enzyme ($r = 0.786$). Based on the correlation coefficient, it can be seen that the TPC content in the high-extracted sample has a strong to powerful linear correlation with the indicators of antioxidant capacity and PPO enzyme inhibition. Therefore, it can be concluded that the TPC content in the extracts is an important part, greatly influences the antioxidant capacity and melanosis in the banana peel extracts.

3.6. Chemical composition of banana peel extract.

From the indicators of TPC content, antioxidant capacity, and ability to inhibit the PPO enzyme of the extract during ripening, we found that M1, M2, and M6 had high TPC content and strong bioactivities. Furthermore, food processing plants use the ripe banana pulp at stage 6, and the banana peel is considered a by-product. Therefore, taking advantage of banana peel by-products is an advantage regarding cost-available raw materials, thereby the strengths of ripe banana raw materials should be fully exploited. For the above reasons, our team selected the M6 banana peel sample to analyze the bioactive components.

The results of the chemical composition analysis of porcelain banana peel extracts by HPLC-MS have identified 17 compounds, which can be divided into 6 compound groups such as phenolic acids, flavonols, flavan-3-ols, catecholamines, lignans, and antibacterial compounds. These compounds have many valuable bioactivities as antioxidant, anti-tumor, anti-mitotic, anti-viral and antibacterial (**Table 4**).

Table 4. Chemical composition of M6 sample

Compound groups	Compound	Predicted formula	m/z	Biological activity
Phenolic acid	Ferulic acid	C ₁₀ H ₁₀ O ₄	208.0305	Ferulic acid has an antioxidant capacity, slowing down the brain's aging process and limiting cognitive decline in humans.
	p-coumaric acid	C ₉ H ₈ O ₃	163.8449	p-coumaric acid is a major intermediate in the polyphenol biosynthesis pathway and has antioxidant potential
	Caffeic acid	C ₉ H ₈ O ₄	179.0551	Caffeic acid has antioxidant, anti-inflammatory, and anti-cancer properties. In addition, caffeic acid also can resist oxidation caused by H ₂ O ₂ on brain tissue.
	Caffeic-hexoside acid	C ₁₅ H ₁₈ O ₉	34.1077	
	Gallic acid	C ₇ H ₆ O ₅	169.8854	Gallic acid has antioxidant and anti-cancer properties
Flavonol	Quercetin	C ₁₅ H ₁₀ O ₇	303.1465	Quercetin has antioxidant, anti-inflammatory, antiviral, antibacterial, and anti-cancer properties—increased antioxidant capacity when combined with metal ions.
Flavan-3-ol	Gallocatechin	C ₁₅ H ₁₄ O ₇	452.0314	Gallocatechin also prevents lipid and protein oxidative damage in cortical and cerebellar tissues.
	Dopamine	C ₈ H ₁₁ NO ₂	154.0385	Dopamine is involved in nitrogen fixation, the phosphorylation of chloroplasts. In humans, a lack of dopamine can cause mental decline.
Catecholamine	L-DOPA	C ₉ H ₁₁ NO ₄	196.8102	L-DOPA has antioxidant and disease-prevention capabilities thanks to its ability to convert into dopamine
	Tyrosine	C ₉ H ₁₁ NO ₃	182.1073	Tyrosine helps protect cells, preventing lipid oxidation on nerve cell membranes.
	Tyramine	C ₈ H ₁₁ NO	138.0574	Tyramine is an antioxidant formed from the amino acid tyrosine
Lignan	Secoisolariciresinol	C ₂₀ H ₂₆ O ₆	361.1208	Lignan has anti-tumor, anti-mitotic, anti-viral, and specific inhibition of some enzymes.
	Podophyllotoxin	C ₂₂ H ₂₂ O ₈	413.0320	
	Honokiol	C ₁₈ H ₁₈ O ₂	265.9665	
Antibacterial compounds	Malic acid	C ₄ H ₆ O ₅	133.0499	Malic acid can inhibit yeast, mold, and bacteria based on its effect on pH.
	β-sitosterol	C ₂₉ H ₅₀ O	415.0518	β-sitosterol is a natural micronutrient in higher plants and can inhibit microorganisms such as <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> and <i>Klebsiella pneumonia</i> .
	12-hydroxystearic acid acid	C ₁₈ H ₃₆ O ₃	319.1279	12-hydroxystearic acid was evaluated as a potential compound for inhibiting the activity of microorganisms such as <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Salmonella enteritidis</i> , and <i>E. coli</i> .

4. Conclusions

The research results show that the extract of porcelain banana peel has many biological activities and potential applications for food industry and medicine. The study also showed that the ripening stages of porcelain bananas have a strong influence on the high extraction yield, TPC content, antioxidant capacity, and ability to inhibit PPO enzyme. During the ripening period of the banana, the high ethanol extraction yield gradually increased, but the TPC and bioactivities gradually decreased from stage 1 to stage 4 and increased again until stage 6. Besides, food processing plants use the ripe banana pulp at stage 6, and the banana peel is considered a by-product. It was so that using banana peel at stage 6 to produce antioxidant compounds to apply for food, aquaculture, preservation, and medicine has significant potentials.

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Conflict of Interest

The authors declare no conflict of interest.

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Ha Thi Hue received the Degree in Home Economic Engineer from Ho Chi Minh City University of Technology and Education in 2006; the Master of Engineering in Food Technology from Ho Chi Minh City University of Technology and Education in 2019. She is a Ho Chi Minh City University of Technology and Education, Vietnam lecturer. Her research interest includes phytochemistry, the bioactivities of plants, and application in food and aquaculture. Email: hueht@hcmute.edu.vn.




Mai The Son received a Bachelor's in Food Technology from Ho Chi Minh City University of Technology and Education in 2020. He is currently working at P&G Vietnam.



Mai Tuyet Nhi received a Degree in Food Technology from Ho Chi Minh City University of Technology and Education in 2020. She is currently working in the Food Business at C.P. Vietnam Corporation.



Phan Thi Anh Dao received the Degree in Chemistry Bachelor in the University of Science, Viet Nam National University, Ha Noi, in 2006; the Master of Engineering in Chemistry from Ho Chi Minh City University of Technology in 2009, and the Doctor of Philosophy in Chemistry in Vietnam National University, HCM in 2016. She is a Ho Chi Minh City University of Technology and Education, Vietnam lecturer. Her research interest includes phytochemistry, the bioactivities of plants, and application in food and aquaculture. Email: daopta@hcmute.edu.vn. ORCID:  <https://orcid.org/0000-0002-8482-6306>