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Bacterial acrylamidase

Phenolic Content and Antioxidant Capacities at Different Stages of Berangan Banana Ripening

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ABSTRACT

Bananas are widely consumed for their nutritional value, but the optimal ripening stage for maximizing health benefits remain unclear. This study investigated the total phenolic content (TPC) and antioxidant capacities of Berangan bananas across seven ripening stages. TPC was measured using the Folin-Ciocalteu assay with gallic acid as the standard, while antioxidant activities were evaluated via 2,2-diphenyl1-picryl-hydrazyl-hydrate (DPPH) and cupric reducing antioxidant capacity (CUPRAC) assays, with ascorbic acid as the standard. Results indicated that stages 1 (8.543±0.268 mg GAE/g), 2 (8.646±0.034 mg GAE/g), and 7 (8.99±0.059 mg GAE/g) had the highest phenolic content, while stage 5 had the lowest (6.756±0.069 mg GAE/g). The strongest DPPH scavenging activity was observed at stages 5 and 6, with IC_{50} values of 25.92 µg/mL and 25.28 µg/mL, respectively. The highest CUPRAC value was found at stage 2, measuring 34.027±0.027 mg AAE/g at 100 µg/mL. These findings suggest that bananas consumed at different ripening stages offer varying antioxidant benefits, which could influence dietary choices for optimal health. This study highlights the therapeutic potential of bananas as a functional food, providing natural antioxidants that beneficial consumer health and nutrition. Further research may expand on these findings to explore therapeutic applications of banana antioxidants.

INTRODUCTION

The roles of fruits and vegetables in enhancing human health and preventing chronic diseases have gain global attention. Epidemiological studies have frequently demonstrated the apparent link between eating fruits and reduce the risk of chronic diseases [1]. Research into phenolic compounds such as gallic acid and chlorogenic acid indicates that these compounds contribute significantly to the antioxidant capacities of fruits, thus enhancing their chemoprotective potential [2]. Phenolic antioxidants significantly reduce free radicals from the body, halt their synthesis, and protect cells from further cellular damage or death [3]. Despite several reports of regularly found fruits such as blueberries and apples on phenolic content and antioxidant capacity, there is little information accessible regarding indigenous fruit varieties that are currently underappreciated. Previous studies on Australian bananas, such as Bashmil et al, highlight the antioxidant potential of banana phenolics, reinforcing the importance of studying these compounds in tropical varieties like Berangan [4]. Moreover, studies like Vu et al. have shown that banana ripening stages significantly affect antioxidant availability, with phytochemical changes observed in the peel across stages of ripeness [5]. Such research supports the significance of investigating ripening stages in optimizing antioxidant intake.

These underappreciated fruits may be rich in phytochemicals or even unusual compounds that are beneficial to health. Their antioxidant capacity might be on par with or perhaps better than that of the more thoroughly researched fruits. USDA Human Nutrition Center has discovered that the highest antioxidant capacity among the 40 fresh fruits and vegetables, was found in blueberries [6]. Blueberries have gained popularity as a commercial fruit crop due to their higher antioxidant capacity and other indications of health-promoting characteristics [7].
Berangan banana is a Malaysian banana variety that belongs to the Musaceae family, botanically known as *Musa Paradisiaca*. *Berangan* banana is also known as Ang Bak Chio in Hokkien,

spoken in Singapore. In Malaysia, *Berangan* banana is the most widely farmed as they are essential for the production for local use and international export. About 50% of banana plantations in Malaysia are planted with *Berangan* and Cavendish varieties, primarily for export. Domestic banana prices vary according to variety, with *Berangan* banana fetching the highest farm price (RM2.25/kg) in 2018.

Bananas are used as staple food in some countries worldwide after maize and rice and contain higher bioactive compounds that play essential roles in health. The presence of phenolic and antioxidant compounds has made this tropical fruit an excellent source of nutritive ingredients for health benefits [8]. Polyphenols, also known as phenolic compounds, are a secondary metabolite found in plants essential for the human diet [9]. As antioxidants, phenolic compounds such as carotenoids and flavonoids belong to an important class of secondary metabolites. In addition, both banana pulp and peels are also high in polyphenols such as catechin, epicatechin, lignin, and tannins [10]. Bananas are rich in polyphenols and antioxidant compounds. Antioxidant compounds are substances, including vitamin E, vitamin C, β-carotene, carotenoids, phenolic, and flavonoids that may protect cells from reactive oxygen species which can cause an oxidative damage. Antioxidant also contribute to lowering the risk of chronic diseases such as inflammation, heart diseases, and cancers. These compounds could provide protection and improvement in health, including immunity from critical diseases and a high nutritional supply to keep the body healthy [11]. Moreover, antioxidant compounds have the potential to neutralize free radicals that are responsible for oxidative stress in skin cells.

The ripening stages of the banana can be indicated by the changes in the physical structure of the banana, such as the colour from green to yellow and differences in biochemical content within the banana, especially the antioxidants and phenolic compounds. During the ripening stages, the change of peel and pulp colour of bananas can be examined roughly by using naked eyes. Bananas are usually classified according to 7 different stages of ripening as follows; stage 1 (dark green), stage 2 (green + yellow traces), stage 3 (more green than yellow), stage 4 (more yellow than green), stage 5 (yellow + green), stage 6 (entirely yellow), and stage 7 (yellow + brown spot areas) [5]. However, different stages of banana ripening contain different nutritional compositions that may provide health benefits to the human health. Therefore, the study aimed to investigate the total phenolic content and antioxidant capacities at different stages of banana ripening. By examining the antioxidant potential across various stages, this study aims to guide consumers on selecting the optimal ripening stage for maximizing antioxidant intake, providing valuable insights into dietary choices for enhanced health benefits.

MATERIALS AND METHODS

Banana sample collection and preparation

Fresh bananas (*Berangan*) were purchased from the local market in Kuantan, Pahang, Malaysia. The bananas were grouped into seven ripening stages by observing three different parts of the banana fruit, including the stalk, middle, and tip (**Fig.** 1). The banana samples were cleaned and washed thoroughly to eliminate foreign particles, and the pulps were cut and freeze-dried before ground into powder. The ground samples were stored at 4 °C in an airtight container prior to use.

Extraction of the banana sample

The banana samples were extracted using methanol in the Soxhlet extractor. Methanol (250 mL) was added to a Soxhlet extractor and condenser on a heating mantle. The banana sample (50 g) for each ripening stage was placed inside the thimble of the Soxhlet extractor, respectively. The solvent was heated by the heating mantle and evaporates as it moves to the condenser. Subsequently, the condensate dripped into the thimble (reservoir). The solvent level reached the siphon and poured back into the flask. This cycle was repeated 5 times. After completing the process, the methanol was evaporated using a rotary evaporator. The crude extract of the banana was stored at 4 °C prior to use.

Determination of total phenolic content (TPC)

The TPC was determined using Folin- Ciocalteu colorimetric assay following methods adapted from Phuyal et al. [12]. The Folin-Ciocalteu reagent (10%) was prepared by adding 15 mL of Folin-Ciocalteu reagent and 150 mL of distilled water. The freshly prepared Folin-Ciocalteu reagent was stored in a dark place prior to use. Sodium carbonate (7%) was prepared by dissolving 35g of Na_2CO_3 into 500 mL of distilled water. A gallic acid standard was used as a standard. Banana extracts were prepared and analyzed at concentrations of 0, 25, 50, 75, and 100 µg/mL. In brief, 10% Folin-Ciocalteu reagent (5 mL) and 7% Na_2CO_3 (4 mL) were added to 1 mL of banana extract concentration to obtain a 10 mL final volume. The mixture obtained was homogenized and incubated in the water bath at 40°C for 30 minutes. The colour changes from pale blue to dark blue were observed at different concentrations. Subsequently, the absorbance reading was measured in triplicates at 760 nm against blank using a UV-Vis spectrophotometer. The total phenolic content of the extracts was calculated as mg Gallic Acid Equivalent (GAE) per gram of sample in dry weight (mg/g) as follows [12];

$$C = c\frac{v}{m}$$

Where,
C = total phenolic content (mg) GAE/g dry extract,
c = concentration of Gallic Acid obtained from calibration curve in mg /mL,
V = volume of extracts in mL,
m = mass of extract in gram.

Determination of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay

DPPH free radical scavenging assay was conducted to determine antioxidant activity of the banana extract [13]. The stock solution of DPPH was prepared by dissolving 6.4 mg of DPPH in 160 mL of methanol. The different concentrations of banana extracts were prepared by dissolving the required amount in methanol (0, 5, 10, 15, 20, and 25 µg/mL), making the final volume 100 µL. Ascorbic acid was used as a control. Methanol was used as a blank, and the negative control was prepared by only mixing 1 ml methanol and 1 ml DPPH solution. All the solution was incubated in a dark for 30 minutes. Then, the absorbance reading was measured at 517 nm. All the experiment was conducted in triplicates. The percentage of inhibition for radical scavenging activity was calculated. The percentage of inhibition was calculated by using the formula as follows;

$$I\% = \frac{A_C - A_O}{A_C} \, x \, 100$$

Where,
I% = percentage of inhibition,
A_C = the absorbance for the control (1mL methanol + 1 mL DPPH solution),
A_O = absorbance of the sample solution.

IC_{50} values were used to express the radical scavenging activities of banana extracts.

Determination of cupric ion reducing antioxidant capacity (CUPRAC)
CUPRAC assay was conducted following the method described by Apak et.al.using 10 mM $CuCl_2$ (1.0 mL), 7.5 mM neocuproine (1.0 mL), 7.7% (w/v) $AcONH_4$ (1.0 mL), and banana extract (1.1 mL) [14]. The mixture was incubated for 90 minutes. The absorbance was measured at 450 nm using UV-Vis spectrophotometer.

Statistical Analysis.
SPSS software (SPSS statistical software version 23, IBM Crop., NY) was employed to carry out statistical analysis. The one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test was used to determine the mean differences. The data were expressed as mean ± SEM values of triplicates. Mean with different superscripts were significantly different ($p<0.05$).

RESULTS AND DISCUSSION

The physical changes of bananas during the ripening process
The banana was expected to ripen from 24 hours to 5 days at room temperature. The mechanism of ripening involves the degradation of chlorophyll into carotenoids as the result the peel turns yellowish [15]. The colour difference of bananas can be observed using the naked eye referring on three different parts: stalk, middle, and tip (**Fig.** 1). As the *Berangan* banana ripens, the dark green colour significantly changes into yellow. Chlorophyll is a pigment that gives plants their green colour and aids in photosynthesis, which allows plants to create their own food [16]. This indicates that during *Berangan* banana ripening, the chlorophyll compound degrades and loses its ability to express the green colour in *Berangan* bananas [15-17]. Most chlorophylls had vanished by ripening stage 4, however, flavonoids and carotenoids had gradually increased [5]. Therefore, a yellow colour banana was observed. Moreover, the Berangan banana pulp on stage 1 is firm compared to stage 7. On stage 7 of *Berangan* banana ripening, the pulp has a mushy and softer texture. This may be due to the increase in sugar content as the banana ripens [18].

Total phenolic content (TPC)
Table 1 presents the TPC of banana extract at different concentrations from the different stages of *Berangan* banana ripening. The higher concentration, the higher the TPC value. The TPC values at stage 1, stage 2 and stage 7 were significantly higher compared to stages 3, 4, 5 and 6, at concentration of 100 µg/mL. This suggests that bananas at these stages may have a higher potential for phenolic extraction compared to other stages. The TPC obtained ranging between 2.907 ± 0.119 to 1.567 ± 0.157 mg GAE/g and 5.554 ± 0.035 and 3.01 ± 0.103 mg GAE/g at 50 µg/mL and 75 µg/mL, respectively. Both concentrations showed a similar trend of TPC content indicating that the TPC content is not significantly affected by the specific concentration within this range.

Moreover, the phenolic content measured at 100 µg/mL ranged from 8.99 ± 0.059 to 6.756 ± 0.069 mg GAE/g at stage 7 of banana ripening. This suggests that fully ripe bananas (stage 7) may have a higher potential for phenolic extraction compared to other stages at this specific concentration. Overall, these results indicate that the ripening stage and concentration of the extract play an important role in determining the TPC content and phenolic content of banana extracts. Further studies may be necessary to investigate the potential health benefits of these phenolic compounds and optimize the extraction process.

Table 1. Total phenolic content (TPC) of *Berangan* banana at different ripening stages.

Ripening stage	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
1	0.536 ± 0.059^{ab}	2.873 ± 0.034^{de}	5.141 ± 0.091^{cd}	8.543 ± 0.268^{c}
2	0.227 ± 0.000^{a}	1.121 ± 1.157^{a}	3.01 ± 0.103^{a}	8.646 ± 0.034^{c}
3	0.261 ± 0.034^{a}	1.567 ± 0.157^{b}	3.835 ± 0.059^{b}	7.271 ± 0.034^{a}
4	0.845 ± 0.119^{bc}	2.543 ± 0.048^{cd}	5.347 ± 0.034^{de}	7.89 ± 0.034^{b}
5	0.983 ± 0.035^{c}	2.392 ± 0.059^{c}	4.9 ± 0.091^{c}	6.756 ± 0.069^{a}
6	0.914 ± 0.034^{c}	2.907 ± 0.119^{de}	5.45 ± 0.035^{de}	6.859 ± 0.137^{a}
7	0.673 ± 0.067^{bc}	2.355 ± 0.155^{e}	5.554 ± 0.035^{e}	8.99 ± 0.059^{c}

Data presented are mean ± SEM for triplicates, assessed by one-way ANOVA followed by Tukey's post hoc test. Mean with different superscripts are significantly different ($p<0.05$).

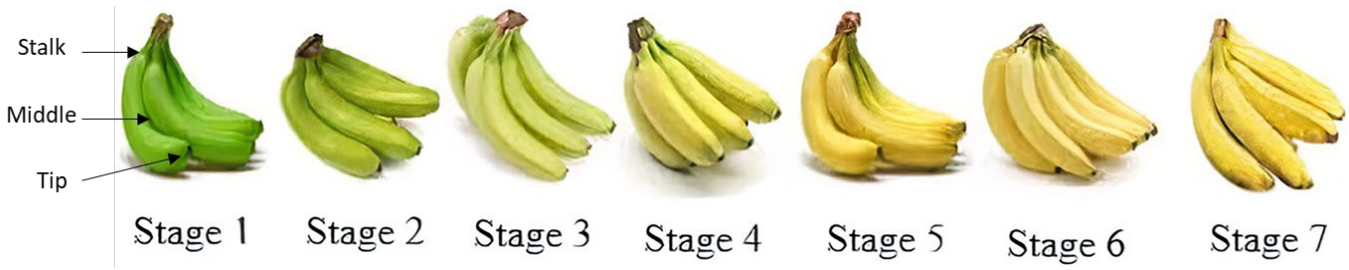

Fig. 1. Stages of banana ripeness [19].

Tannins, in particular, which are responsible for the astringent flavour of unripe fruits, generally decreased with ripening due to polymerization, which rendered them intractable and tasteless. Tannin concentrations in green bananas typically range from 122.6 mg to 241.4 mg [20]. In the current study, ripening stages 3, 4, and 6 showed a decline in phenolic concentration with maturation. Similar circumstances have been described in several studies [5, 20]. As bananas ripen, their tannin level decreases and melds with the pulp, mirroring the low total phenolic content seen in repining stages 3, 4, and 6. Phenolic concentration did, however, appear to rise as ripening progressed, especially at stage 7 of ripening. The increase in the phenolic content might be attributed to the accumulation of anthocyanins and other flavonoids [21].

Antioxidant activity using DPPH method

The DPPH assay has been conducted to assess the scavenging ability and reducing power of bioactive metabolites, mainly polyphenols. The reducing power was calculated using their concentration providing 50% inhibition (IC_{50}) values. Ascorbic acid, also known as vitamin C, is the most powerful natural water-soluble antioxidant was used as a control in this study, as they are able to measure antioxidant capacity and is widely available in a variety of food sources.

DPPH provides hydrogen ions or act as a free radical's scavenger in the biological system. The mixture of DPPH with banana extracts accepts hydrogen atoms and diminishes violet colour. The DPPH values of *Berangan* banana at different ripening stages increased in a concentration-dependent manner, indicating that higher concentrations of the extract have higher antioxidant activity. The IC_{50} value, which represents the concentration of the extract required to scavenge 50% of the DPPH free radicals, varied between different ripening stages of the banana. The IC_{50} value obtained for the *Berangan* banana extract at stage 1, 2, 3, 4, 5, 6, and 7 was 36.31, 35.51, 33.88, 30.9, 25.92, 25.28, and 30.9 µg/mL, respectively. At the highest concentration of 25 ug/mL, the percentage of inhibition at stage 5 and 6 was significantly higher than in the other ripening stages. The results showed that the highest antioxidant activity was observed in stages 5 and 6, as indicated by the lower IC_{50} values. These finding align with research by Borges et. al., who noted that antioxidant levels in bananas vary significantly with ripening and cooking processes, which my influence nutritional retention after consumption [22]. Additionally, the higher flavonoid levels observed in later stages may explain the increased antioxidant activity, as reported by Amri et al. in studies on ripe bananas [9]. These observations suggest that the Berangan banana's antioxidant potential is closely tied to ripening stages, offering distinct health benefits at different stages.

The IC_{50} value obtained from stage 1 of the *Berangan* banana was the highest, with a value of 36.31 µg/mL. This finding suggested that in stage 1 of banana ripening, the *Berangan* banana's pulp is low in antioxidant capacity. Meanwhile, at stage 6 of the *Berangan* banana, low IC_{50} values were recorded at 25.28 µg/mL which is the closest to the IC_{50} of ascorbic acid (22.5 µg/mL). This result indicates a higher antioxidant activity at stage 6. The higher IC_{50} value closest to the IC_{50} value obtained for the ascorbic acid (positive control) indicates the higher antioxidant activity presents in the extracts [23]. A causative correlation between total phenolic compounds and antioxidant activity may be indicated through the proportional of their phenolic content [4].

Antioxidant capacity using CUPRAC method

CUPRAC values were expressed as ascorbic acid equivalent in **Table 3**. The CUPRAC value obtained ranged from 34.027 ± 0.027 to 28.357 ± 0.013 mg AAE/g at 100 µg/mL concentration. The CUPRAC value at ripening stages 3, 4, and 7 were significantly lower than ripening stage 2. The CUPRAC increased gradually as the concentration increased. At a high concentration of 25 µg/mL, the CUPRAC value at stages 3 and 4 ripening stages were significantly lower than in stage 2 of banana ripening. While at the concentration 10 µg/mL, the CUPRAC value obtained ranged from 14.996 ± 0.024 to 12.635 ± 0.037 mg AAE/g (**Table 3**). The trend of CUPRAC scavenging activities investigated shows that the CUPRAC value increases as the concentration increases.

Table 2. DPPH scavenging activity of *Berangan* banana at different stages of ripening.

Ripening stage	0 µg/mL	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL	25 µg/mL	IC_{50} µg/mL
1	0.509 ± 0.235^{a}	8.084 ± 0.227^{b}	10.975 ± 0.194^{bc}	20.159 ± 2.246^{ab}	28.731 ± 1.064^{ac}	34.494 ± 0.474^{a}	36.31
2	0.305 ± 0.337^{a}	7.697 ± 0.105^{ab}	12.319 ± 0.102^{bc}	23.335 ± 0.035^{bc}	28.629 ± 0.989^{ac}	34.514 ± 0.423^{a}	35.51
3	0.448 ± 0.041^{a}	1.364 ± 0.194^{a}	12.869 ± 0.836^{c}	23.555 ± 1.450^{bc}	27.103 ± 0.041^{a}	36.754 ± 0.204^{b}	33.88
4	0.489 ± 0.000^{a}	6.903 ± 0.554^{ab}	9.204 ± 0.163^{b}	29.287 ± 0.174^{cd}	32.743 ± 0.000^{c}	40.102 ± 0.000^{c}	30.90
5	0.448 ± 0.041^{a}	6.638 ± 3.524^{ab}	16.554 ± 0.807^{d}	29.607 ± 0.114^{d}	40.949 ± 1.064^{d}	46.487 ± 0.382^{d}	25.92
6	0.916 ± 0.035^{a}	4.398 ± 0.382^{ab}	0.460 ± 1.496^{a}	17.817 ± 0.134^{a}	31.765 ± 0.162^{bc}	62.452 ± 0.143^{e}	25.28
7	0.733 ± 0.000^{a}	6.455 ± 0.607^{ab}	9.204 ± 0.163^{b}	26.288 ± 0.174^{cd}	32.743 ± 0.000^{c}	40.012 ± 0.000^{c}	30.90

Data presented are mean ± SEM for triplicates, assessed by one-way ANOVA followed by Tukey's post hoc test. Mean with different superscripts are significantly different ($p<0.05$).

Table 3. CUPRAC activity of *Berangan* banana at different stages of ripening.

	Concentration (µg/mL)					
Ripening stage	0	10	25	50	75	100
1	12.200 ± 0.144^{bc}	14.871 ± 0.020^{c}	16.413 ± 0.206^{a}	19.883 ± 0.027^{ab}	23.897 ± 0.013^{a}	29.147 ± 0.035^{bc}
2	10.900 ± 0.140^{a}	12.635 ± 0.037^{a}	16.040 ± 0.000^{a}	24.507 ± 0.353^{c}	29.94 ± 0.716^{b}	34.027 ± 0.027^{e}
3	11.593 ± 0.048^{ab}	13.537 ± 0.325^{abc}	15.996 ± 0.026^{a}	21.247 ± 0.262^{cd}	23.967 ± 0.017^{a}	28.357 ± 0.013^{a}
4	13.000 ± 0.000^{c}	14.996 ± 0.024^{c}	15.857 ± 0.013^{a}	19.607 ± 0.027^{a}	23.693 ± 0.153^{a}	28.357 ± 0.013^{a}
5	11.303 ± 0.013^{a}	13.218 ± 0.680^{ab}	15.747 ± 0.043^{a}	19.883 ± 0.027^{ab}	23.553 ± 0.013^{a}	29.883 ± 0.267^{d}
6	12.370 ± 0.106^{bc}	14.579 ± 0.363^{bc}	16.537 ± 0.822^{a}	20.457 ± 0.083^{bc}	24.050 ± 0.076^{a}	29.370 ± 0.023^{c}
7	11.972 ± 0.162^{bc}	14.315 ± 0.124^{bc}	18.343 ± 0.132^{b}	21.717 ± 0.994^{d}	24.010 ± 0.236^{a}	28.953 ± 0.190^{b}

Data presented are mean ± SEM for triplicates, assessed by one-way ANOVA followed by Tukey's post hoc test Mean with different superscripts are significantly different ($p<0.05$).

At stage 2 of *Berangan* banana ripening, the CUPRAC scavenging activity showed the highest value. It was suggested that the antioxidant compounds of catechin and quercetin are high as they both significantly contributed to the antioxidant activity of *Berangan* banana [22]. However, we did not measure the catechin and quercetin to confirm the findings.

In the CUPRAC reaction, the complex Cu (I) – neocuproine has a yellow colour solution and exhibits characteristic absorption at 450 nm. The intensity of the yellow colour is determined by the amount of Cu (II) that is reduced to Cu (I) [22]. Because of its rapid colour development, ascorbic acid was selected as a standard in the CUPRAC assay, as well as its availability in several food sources, particularly citrus fruit extracts [24]. A previous study suggested that if the sample has a lower reduction potential than Cu (II)/Cu (I), which is 0.159 V, it will behave as an antioxidant in the CUPRAC assay [22].

These results suggest that the ripening stage of Berangan bananas affects their antioxidant potential, highlighting opportunities for consumers to select specific stages for desired health benefits. Given the substantial antioxidant capacity observed, there is also potential for Berangan bananas to be incorporated into functional foods or supplements targeted at enhancing dietary antioxidant intake. Such applications may provide accessible options for improving health through natural dietary sources.

CONCLUSION

This study investigated the TPC and antioxidant capacities of *Berangan* bananas at seven different stages of ripening. The results revealed that the phenolic content of banana extracts varied significantly at different stages of ripening. The highest phenolic content was observed at stages 1 (8.543± 0.268 mg GAE/g), 2 (8.646 ± 0.034 mg GAE/g), and 7 (8.99 ± 0.059 mg GAE/g), while the lowest was observed at stage 5 (6.756 ± 0.069 mg GAE/g). The banana extracts also demonstrated a high capacity for scavenging free radicals, with the highest activity observed at stages 5 and 6 with IC_{50} values of 25.92 µg/mL and 25.28 µg/mL, respectively. Furthermore, the CUPRAC value of banana extract at ripening stage 2 (34.027 ± 0.027 mg AAE/g) at 100 µg/mL concentration was significantly greater than those of other ripening stages. These findings suggest that the optimal stage of banana ripening for consumption may vary depending on the desired nutritional benefits. While earlier stages of ripening may be ideal for a higher phenolic content, later stages of ripening may offer greater antioxidant activity. Given these results, Berangan bananas at specific ripening stages could be utilized in the development of functional foods or dietary supplements aimed at enhancing antioxidant intake. Such applications could make this nutrient-dense fruit more accessible to consumers looking to improve their health through natural dietary antioxidants. Therefore, consumers should consider the ripening stage when selecting bananas for optimal nutritional benefits. The study highlights the potential health benefits of consuming *Berangan* bananas at different ripening stages. As bananas are a widely available and affordable fruit, the findings could have significant implications for public health recommendations. Incorporating bananas into the diet at the appropriate ripening stage could provide an easy and accessible means of obtaining essential nutrients and antioxidants. Overall, the study contributes to our understanding of the nutritional content of bananas and their potential health benefits. Incorporating bananas into diets at specific ripening stages could offer an accessible and affordable way to support public health by providing essential nutrients and antioxidants, particularly in regions where Berangan bananas are widely available.

LIST OF ABBREVIATIONS

AAE: ascorbic acid equivalent
CUPRAC: cupric reducing antioxidant capacity
DPPH: 2,2-diphenyl1-picryl-hydrazyl-hydrate
GAE: Gallic acid equivalent;
TPC: total phenolic content

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REFERENCES

1. Smith L, López Sánchez GF, Veronese N, Soysal P, Oh H, Barnett Y, Keyes H, Butler L, Allen P, Kostev K, et al. Fruit and vegetable intake and non-communicable diseases among adults aged ≥50 years in low- and middle-income countries. *J Nutr Health Aging*. 2022;26(8):1003-1009.
2. Kaur S, Singh V, Chopra HK, Panesar PS. Extraction and characterization of phenolic compounds from mandarin peels using conventional and green techniques: a comparative study. *Discov. Food*. 2024;4:60.
3. Tumilaar SG, Hardianto A, Dohi H, Kurnia D. A comprehensive review of free radicals, oxidative stress, and antioxidants: overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *J Chem*. 2024;2024:5594386.
4. Bashmil YM, Ali A, Bk A, Dunshea FR, Suleria HAR. Screening and characterization of phenolic compounds from Australian grown bananas and their antioxidant capacity. *Antioxidants (Basel)*. 2021;10(1):8.
5. Vu HT, Scarlett CJ, Vuong QV. Changes of phytochemicals and antioxidant capacity of banana peel during the ripening process; with and without ethylene treatment. *Sci Hortic*. 2019;253:255-262.
6. Kalt W, Cassidy A, Howard LR, Krikorian R, Stull AJ, Tremblay F, Zamora-Ros R. Recent research on the health benefits of blueberries and their anthocyanins. *Adv Nutr*. 2020;11(2):224-236.
7. Vahapoglu B, Erskine E, Gultekin Subasi B, Capanoglu E. Recent studies on berry bioactives and their health-promoting roles. *Molecules*. 2022;27(1):108.
8. Amini Khoozani A, Birch J, Bekhit AEA. Production, application, and health effects of banana pulp and peel flour in the food industry. *J Food Sci Technol*. 2019;56(1):548-559.
9. Amri FSA, Hossain MA. Comparison of total phenols, flavonoids, and antioxidant potential of local and imported ripe bananas. *Egypt J Basic Appl Sci*. 2018;5(4):245-251.
10. Vu HT, Scarlett CJ, Vuong QV. Phenolic compounds within banana peel and their potential uses: A review. *J Funct Foods*. 2018;40:238-248.
11. Kumari P, Gaur SS, Tiwari RK. Banana and its by-products: A comprehensive review on its nutritional composition and pharmacological benefits. *eFood*. 2023;4(1):e110.
12. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *Sci World J*. 2020;2020:8780704.
13. Islam MR, Kamal MM, Kabir MR, Hasan MM, Haque AR, Hasan SMK. Phenolic compounds and antioxidant activity of banana peel extracts: Testing and optimization of enzyme-assisted conditions. *Measurement Food*. 2023;10:100085.
14. Apak R, Güçlü K, Özyürek M, Çelik SE. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim Acta*. 2008;160(3-4):413-419.
15. Maduwanthi SDT, Marapana RAUJ. Induced ripening agents and their effect on fruit quality of banana. *Int J Food Sci*.

2019;2019:2520179.
16. Liu P. Uncovering the mechanism of banana "green ripening". *Plant Cell*. 2023;35(6):1294-1295.
17. Maduwanthi SDT, Marapana RAUJ. Comparison of pigments and some physicochemical properties of banana as affected by ethephon and acetylene-induced ripening. *Biocatal Agric Biotechnol*. 2021;33:101997.
18. Cordenunsi-Lysenko BR, Nascimento JRO, Castro-Alves VC, Purgatto E, Fabi JP, Peroni-Okyta FHG. The starch is (not) just another brick in the wall: The primary metabolism of sugars during banana ripening. *Front Plant Sci*. 2019;10:391.
19. Khodabakhshian R, Baghbani R. Classification of bananas during ripening using peel roughness analysis—An application of atomic force microscopy to food process. *J Food Process Eng*. 2021;44(5):e13857.
20. Youryon P, Supapvanich S. Physicochemical quality and antioxidant changes in 'Leb Mue Nang' banana fruit during ripening. *Agric Nat Resour*. 2017;51(1):47-52.
21. Kritsi E, Tsiaka T, Sotiroudis G, Mouka E, Aouant K, Ladika G, Zoumpoulakis P, Cavouras D, Sinanoglou VJ. Potential health benefits of banana phenolic content during ripening by implementing analytical and in silico techniques. *Life (Basel)*. 2023;13(1):35.
22. Borges CV, Maraschin M, Coelho DS, Leonel M, Gomez HAG, Belin MAF, Diamante MS, Amorim EP, Gianeti T, Castro GR, Lima GPP. Nutritional value and antioxidant compounds during the ripening and after domestic cooking of bananas and plantains. *Food Res Int*. 2020;132:109061.
23. Rivero-Cruz JF, Granados-Pineda J, Pedraza-Chaverri J, Pérez-Rojas JM, Kumar-Passari A, Diaz-Ruiz G, Rivero-Cruz BE. Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of Mexican brown propolis. *Antioxidants (Basel)*. 2020;9(8):651.
24. Apak R, Güçlü K, Ozyürek M, Bektas Oğlu B, Bener M. Cupric ion reducing antioxidant capacity assay for food antioxidants: vitamins, polyphenolics, and flavonoids in food extracts. *Methods Mol Biol*. 2008;477:163-193.