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Original Research

Extraction of Natural Colorants from Banana (*Musa* spp.) Peel and Determination of Total Carotenoids, β-Carotene and Antioxidant Activity of the Extracts

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ABSTRACT

Purpose: *The banana (Musa spp.) peel, which is rich in many bioactive compounds, is not utilized effectively. This study was conducted to extract natural colorants from banana peel and to determine the total carotenoid and β-carotene concentration, and antioxidant activity of the extracts.*

Research Method: *Ambul, Puwalu , and Cavendish banana varieties were selected and yellow colorant was extracted by heat refluxing (50°C for 30 minutes) with 50 mL of 1:1 hexane-acetone mixture (S1). The concentrated extracts produced from 10g of peel material were used to calculate the total carotenoid pigment concentration using the spectrophotometric method at 460 nm. The colorant was extracted from Ambul banana peel (the variety with the highest carotenoid concentration with S1) using ethanol as the solvent (S2), following the same procedure. The β-carotene concentrations of each variety's extract were measured using the spectrophotometric method at 440 nm. 2,2-diphenyl-1-picryhydrazylhydrate (DPPH) assay was used to determine the antioxidant activity and expressed as mg/g equivalents of Trolox.*

Findings and Values: *The highest total carotenoid concentration (0.03 ±0.08 mg/g) was recorded in the Ambul variety with S1. The carotenoid concentration in the Ambul variety was further improved by using S2 solvent (0.04 ±0.01mg/g). The β-carotene level in the peel of all three tested banana varieties was between 1.03×10-4 to 2.45×10-4 mg/g, with the highest in the Ambul variety with S1 solvent. DPPH assay values were between 0.01±0.00 to 1.81±0.03 mg Trolox/g. Antioxidant activity was highest in the Puwalu variety with the S1. The study concludes that Ambul banana peel is a highly potential source for the extraction of natural yellow colorant. Findings can be used to initiate a novel industrial procedure to extract yellow colorant from banana peel for potential applications in food and non-food industries.*

Keywords: *Banana peel, β-carotene, Carotenoid, Extraction, Yellow colorant*

INTRODUCTION

Banana, which belongs to the genus Musa, is one of the most consumed fruits all over the world. The annual global production of bananas in 2020 was 120 metric tons.

Amongst more than 1000 banana varieties cultivated worldwide, Cavendish has become the most consumed and exported variety (IISD 2023). In Sri Lanka, the most cultivated and consumed fruit is the banana. Approximately 60,000 hectares of land in Sri Lanka are used

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for banana cultivation, which occupies the largest cultivated area among fruit cultivation in Sri Lanka. Sri Lanka produces around 780,000 metric tons of bananas annually (Athukorala *et al.* 2020). Bananas are fruits rich in various bioactive compounds, including carotenoids. Recent studies reveal that carotenoids decrease the risk of many non-communicable diseases, such as cancer, diabetes, and heart disease, due to their biological activities, which include antioxidant, anti-inflammatory, anti-diabetic, and anti-aging effects. In addition, carotenoids act as photo-protectors and as a precursor to vitamin A (Crupi *et al.* 2023). Due to the antioxidant capacity of carotenoids, they serve as preservatives that can increase the shelf life of certain foods. Moreover, carotenoids are highly favored as natural food colorants in the food industry, generating colors that range from yellow to red (Ayala-Zavala *et al.* 2011).

In most natural sources of carotenoids, such as fruits and vegetables, very low concentrations of carotenoids are found (Lin *et al.* 2016). According to the literature, α-carotene (10.9%), β-carotene (14.9%), lutein (52.3%), and zeaxanthin are the major carotenoids found in bananas (Borges *et al.* 2019). The biosynthesis of carotenoids is affected by cultivar, maturity level, genetic factors, and geographic conditions (Subagio & Morita 1997; Facundo *et al.* 2015). According to Ayala-Zavala *et al.* (2011), banana peel contains (3–4 μg/g) more carotenoids than the edible portion (0.75 μg/g). This fact suggests that banana peel is a potential source for the extraction of carotenoids.

Out of the entire weight of the banana fruit, the peel weighs over 40%. But, being inedible, it is disposed of without being used for any useful purpose. When banana fruit production is increased, the generation of peel waste in landfills is also increasing causing disposal problems (Ansari *et al.* 2023). For the long-term sustainability of the banana industry, setting up an efficient way to collect banana peels and utilize them as a value-added product is timely and crucial. Being a rich source of carotenoids, the extraction of yellow colorant from banana peel will be a productive way to utilize it. Recently, the consumer preference for natural food colorants has increased significantly due to health concerns (Crupi *et al.* 2023). Hence, carotenoid extracts from natural sources would be much more desirable to use in dietary supplements and as additives in processed foods.

Using a food-grade extraction method would make it highly potential to be used as a food colorant in future applications. In previous studies, various solvents such as hexane, acetone, methanol, and ethanol have been used solely or in combination to extract carotenoids from various banana varieties. Further, determination of total carotenoid concentrations in extracts was conducted with spectrophotometric or chromatographic techniques (Subagio & Morita 1997, Ayala-Zavala *et al.* 2011, Ekesa *et al.* 2015). However, there is a lack of studies about the extraction of color compounds from the peel of banana varieties available in Sri Lanka. Thus, this study was piloted to investigate the possibility of extracting natural yellow color pigment from banana peel waste, which is a cheap substrate. In the first stage, a hexane-acetone mixture (1:1) was used to identify the banana variety with the highest total carotenoid concentration. Secondly, the selected variety was tested for its potential to extract carotenoids with ethanol, which is the least toxic solvent used in food-grade applications. The specific objectives of the current study were to perform the preliminary characterization of each extract, such as the determination of total carotenoid and β-carotene concentrations and antioxidant capacity, which will be important to promote its use in potential industries.

MATERIALS AND METHODS

The study was conducted at the Herbal Technology Section of the Industrial Technology Institute, Colombo, Sri Lanka from July 2021 to June 2022.

Sample preparation

Yellow color naturally ripe (completely yellow, easily peelable, without brown spots) banana samples (*Ambul* , *Puwalu* , and Cavendish varieties) were collected from the Colombo district (6.5354° N; 79.5213° E) and all were washed with deionized water and soaked in 5% (w/w) citric acid solution (25°C, pH = 2.25) for 5 minutes to avoid potential browning reactions. Afterward, citric acid residues were washed away and samples were air-dried. Next, the bananas were hand-peeled, and the outermost layer was scraped off using a surgical blade for the extraction of yellow colorants.

Experimental design

All analyses were conducted using a Complete Randomized Design (CRD) with triplicate data points, representing mean and standard deviation. Three banana fingers from each banana variety were used for replication purposes.

Extraction of carotenoids

Separated color-containing parts of banana peels (10 g) were finely ground using a mortar and pestle to make a fine pulp. Equal portions of 99.99% acetone (Merck, Germany) and 99.99% hexane (Merck, Germany) (S1) were mixed to make a 50 mL solvent mixture and used to extract the pigments from the samples. Samples were heat refluxed at 50°C for 30 minutes, which involved distillation of the solvent and returning it to the extraction vessel. Refluxing was repeated two times by adding 10 mL of the new solvent mixtures each time.

The refluxed sample was then added with anhydrous sodium sulfate gradually to the organic solvent mixture, and the formed clumps were removed with filtration using a Whatman no. 42 filter paper to remove the residual moisture. The extracted samples were concentrated 15 times by evaporating in a rotary evaporator (Huanghua F-305, China) attached to a vacuum pump and weighed. Final samples were sealed and stored in an air-tight container at 4°C until further analysis.

The possibility of extracting yellow color pigments using ethanol (the least toxic solvent utilized in food-grade colorant extractions) was tested by using 50 mL of 99.99% ethanol (Merck, Germany) as the solvent (S2) following the above-mentioned procedure. However, this step was carried out only for the *Ambul* variety, which generated the highest total carotenoid concentration according to the first step of this experiment.

Determination of total carotenoids and β-carotene

Total carotenoid concentration in banana peel samples was determined using the spectrophotometric method described by Cho (2018). Spectrophotometric (Thermo Fisher Scientific, USA) values obtained at 460 nm and calculated values were presented as mg of carotenoids in 1 g banana peel sample (Equation 1).

$$\text{Total carotenoid pigment concentration} = \frac{\text{Absorption at 460 nm} \times V \times 10000}{\text{Absorption coefficient} \times \text{Sample weight}} \quad (1)$$

The absorption coefficient was taken as 2500 for total carotenoids, while $V = 10$ mL (total volume of the sample used).

β-carotene concentration in the extract of each variety was calculated using the spectrophotometric method (Thermo Fisher Scientific, USA) at 440 nm. Final data were explained using the β-carotene standard (Sigma-Aldrich Co, USA) curve and presented as mg of β-carotene in 1 g banana peel sample.

Determination of the color of extracted yellow colorants

Banana peels (before using them for the extraction of colorant) and the extracted yellow colorant samples were tested for their colorimetric values using a reflective chromameter (Konica Minolta, Japan). The middle length position of the banana finger before peeling was used for data recording. Extracted yellow colorant samples were transferred to a Petri dish for data recording. L*, a*, and b* values (Hunter system) were obtained in triplicate for each sample. By using the above a* and b* values in Equations 2 and 3, respectively, the hue angle (h^*) and chroma (C^*) were calculated (Mclellan *et al.*, 1995).

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \quad \text{(Equation 2)}$$

$$C^* = \sqrt{(a^{*2} + b^{*2})} \qquad \text{(Equation 3)}$$

Determination of antioxidant activity

The antioxidant capacity of banana peel extracts was measured using the DPPH radical scavenging assay as explained by Bopitiya & Madhujith (2012). For the DPPH radical scavenging assay, 50 µL of sample was mixed with 50 µL DPPH radical (0.1 M), and incubated at room temperature for 10 minutes. Then, the absorbance was recorded at 517 nm using a UV-visible spectrophotometer (Thermo Fisher Scientific, USA). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using Equation 4, where A_{sample} is the sample absorbance and A_{DPPH} is the absorbance of the DPPH solution devoid of extract.

$$RSA(\%) = \left(\frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}}\right) \times 100 \qquad \text{(Equation 4)}$$

The DPPH radical scavenging activity was expressed as an IC_{50} value, which denotes the concentration of the sample required to scavenge 50% of the DPPH radicals. A Trolox standard curve was prepared using a concentration series (1–0.001 mg/mL) and the final antioxidant value was expressed in mg/g equivalents of Trolox.

Evaluation of application possibility in foods

Developed banana peel extracts were diluted with 99.99% ethanol and mixed with a vortex mixer (Thermo Fisher Scientific, USA) to make a liquid colorant sample of 5 mL. This was added to UHT-treated whole milk samples (25°C, pH = 6.5) of 5 mL stored in labeled test tubes (triplicates of milk samples were used for each extract). The amount of banana peel extract required to be added to obtain a visible color change was recorded. A sensory evaluation test was performed employing 30 semi-trained panelists (male: female; 1:1) to understand the sensory acceptability of those milk samples concerning color, odor, and overall acceptability (a five-point hedonic scale ranging from 1 = extremely dislike to 5 = extremely like).

Data analysis

All analyses were conducted using a Complete Randomized Design (CRD) with triplicate data points, representing mean and standard deviation. A one-way ANOVA procedure with Tukey's pairwise comparison was performed to determine significant differences ($p<0.05$) between varieties. Statistical analysis was conducted using the SPSS Software package.

RESULTS AND DISCUSSION

Total carotenoid content, β-carotene concentration, and antioxidant activity

Total carotenoid concentration (mg/g), ***β***-carotene concentration (mg/g), and antioxidant activity (mg Trolox/g) in the peel of *Ambul* , *Puwalu* , and Cavendish banana varieties are depicted in Table 1. There are very limited studies on total carotenoid concentration in banana peels. In a previous study, 0.003–0.004 mg of carotenoids per gram of peel as lutein equivalent were reported (Subagio *et al.* 1996). Ekesa *et al.* (2015) reported 0.078 to 0.106 mg per gram of peel (fresh weight basis) of seven banana cultivars. In the present study, the highest concentration of carotenoid pigments of the selected varieties was extracted from *Ambul* banana peel (0.03±0.08 mg/g), followed by *Puwalu* and Cavendish banana, when a hexane-acetone mixture was used as the solvent (Table 1). The total carotenoid concentration was significantly higher in the *Ambul* variety than in the other two. When the colorant was extracted from *Ambul* banana peel using ethanol, the total carotenoid concentration was further increased (0.04±0.01 mg/g), revealing that *Ambul* banana peel can be successfully used for food-grade carotenoid extractions. Moreover, the above observation concludes that the carotenoid concentration in the extract varies with the solvent used for the extraction. Extractable carotenoid concentration can be increased when ethanol is used as the solvent instead of hexane and acetone.

β-carotene concentration in the hexane–acetone extracts of the three varieties was between 1.03×10^{-4} mg/g to 2.45×10^{-4} mg/g. *Ambul*

Table 1. Total carotenoid concentration (mg/g), β-carotene concentration (mg/g), and antioxidant activity (mg Trolox/g) in banana peel extracts.

Banana variety	Solvent	Total carotenoid concentration (mg/g)	β-carotene concentration (mg/g)	Antioxidant activity (mg Trolox/g)
Ambul	S1	0.03 ± 0.08^a	$2.45 \times 10^{-4} \pm 0.04^a$	0.01 ± 0.00^a
Puwalu	S1	0.02 ± 0.06^b	$1.54 \times 10^{-4} \pm 0.03^b$	1.81 ± 0.03^b
Cavendish	S1	0.01 ± 0.04^b	$1.03 \times 10^{-4} \pm 0.02^c$	0.41 ± 0.15^c
Ambul	S2	0.04 ± 0.01^c	$2.01 \times 10^{-4} \pm 0.01^d$	0.32 ± 0.00^d

variety showed the highest β-carotene level (2.45×10^{-4} mg/g) of the three tested varieties. When the colorant was extracted from *Ambul* banana peel using ethanol as the solvent, β-carotene concentration was $2.01 \times 10^{-4} \pm 0.01$ mg/g. Accordingly, it can be concluded that extractable β-carotene content significantly decreases when ethanol is used instead of a hexane–acetone mixture as the solvent.

The antioxidant activities of banana peel extracts in the three selected varieties are shown in Table 1. Antioxidant activity is directly proportional to the Trolox equivalent value. Accordingly, the highest antioxidant activity was recorded in peel extract from *Puwalu* variety, when extracted with hexane–acetone, which was followed by Cavendish and *Ambul* . In a study by Babbar *et al.* (2011), 5.67 mg/g of Trolox equivalent value was recorded in methanolic extracts of banana peel, which was very high compared to the values recorded in the present study. Carotenoids that contain more than seven double bonds show higher scavenging radical activity and most of the carotenoids, such as lutein, α- and β-carotene, violaxanthin, and auroxanthin in banana peel, are soluble in *n*-hexane (Sulaiman *et al.* 2011, Darsini *et al.* 2012).

This would be a possible reason for recording a significantly higher antioxidant activity in *Puwalu* peel extract when a hexane–acetone solvent mixture was used. Moreover, Sulaiman *et al.* (2011) exposed that β-carotene level has a significant influence on the antioxidant capacity by scavenging free radicals. However, according to Babbar *et al.* (2011), the antioxidant activity of banana peel is correlated to phenolic compounds rather than carotenoids.

Determination of Color

According to the literature, total carotenoid concentration in banana peel varies with the maturity stage (Subagio & Morita, 1997). The initial color measurements of the selected banana samples are depicted in Table 2.

Color evaluation of yellow colorants extracted from selected banana peels

Table 3 summarizes the colorimetric values of the yellow color extracts of banana peel samples from the three different varieties.

According to the received color measurements, it can be concluded that the solvent used for the color extraction from banana peel impacts the colorimetric values of the extract. Overall, the lightness was almost similar in all three banana varieties when extracted with a hexane-acetone mixture. When comparing the *Ambul* banana peel extracts with the two tested solvents, it is revealed that the ethanolic extract is lighter than the hexane-acetone extract. Based on the chroma and hue values, it can be concluded that the *Ambul* banana peel sample extracted with ethanol had the maximum yellowness, although it was duller than the hexane-acetone extract.

Evaluation of application possibility in foods

Observable color change started to appear at the point of adding 2 mL of diluted extract to 5 mL of fresh milk in all the samples. Colorimetric values of the fresh milk samples mixed with banana peel extracts at the very first point of receiving an obvious color change is displayed

Table 2. Colorimetric values for the ripe banana peel samples of *Ambul* , *Puwalu* , and Cavendish varieties. Data represent the mean±standard error of luminosity (L*), hue angle (h*), and chroma (C*) values.

Variety	L*	h* ()	C*
Ambul	68.34±0.24	23.02±0.54	17.81±0.76
Puwalu	54.62±0.45	14.19±0.78	9.05±0.41
Cavendish	47.67±0.12	14.76±0.32	7.42±0.95

Table 3. Colorimetric values of the banana peel extracts of *Ambul* , *Puwalu* , and Cavendish varieties. S1: hexane–acetone (1:1) mixture as the solvent to extract color compounds from banana peel, S2: ethanol as the solvent to extract color compounds from banana peel. Data represent the mean±standard error of luminosity (L*), hue angle (h*), and chroma (C*) values.

Variety	Solvent	L*	h* ()	C*
Ambul	S1	42.55±0.25	15.04±0.16	11.81±1.17
Puwalu	S1	42.63±0.32	13.23±0.16	10.05±0.42
Cavendish	S1	42.97±0.14	11.76±0.56	6.42±1.36
Ambul	S2	47.33±0.11	47.51±0.19	3.87±0.32

Table 4. Colorimetric values of fresh milk samples (5 mL) added with banana peel extracts (2 mL) of *Ambul* , *Puwalu* , and Cavendish varieties. S1: hexane–acetone (1:1) mixture as the solvent to extract color compounds from banana peel, S2: ethanol as the solvent to extract color compounds from banana peel. Data represent the mean±standard error of luminosity (L*), hue angle (h*), and chroma (C*) values.

Variety	Solvent	Color		
		L*	h* ()	C*
Ambul	S1	32.56±0.24	6.02°±0.14	7.41±1.17
Puwalu	S1	29.45±0.32	6.51°±0.16	6.23±0.42
Cavendish	S1	24.56±0.15	4.34°±0.59	6.56±1.36
Ambul	S2	33.33±0.01	8.11°±0.11	7.84±0.21

Table 5. Sensory acceptability of the milk samples added with banana peel extracts. S1: hexane–acetone mixture as the solvent to extract color compounds from banana peel, S2: ethanol as the solvent to extract color compounds from banana peel. Values represent the median. * above the P value means p<0.05, significant according to the Kruskal–Wallis test.

Sensory Property	*Ambul* S1	*Puwalu* S1	Cavendish S1	*Ambul* S2	P value
Color	4.0	2.0	2.5	3.0	0.0000*
Odor	2.0	2.5	2.5	3.5	0.0003*
Overall Acceptability	3.0	2.5	2.5	3.5	0.0001*

in Table 4. And the sensory evaluation test results with respect to the color, odor, and overall acceptability are summarized in Table 5.

Sensory evaluation of banana peel extracts in milk samples

The sensory evaluation test confirmed that the most acceptable color was observed in the hexane–acetone extract of *Ambul* banana peel, which was followed by the ethanol extract of the same variety. However, regarding the odor, all hexane–acetone extracts received poor acceptability. According to the sum of ranks received, the highest overall acceptability was reported in the ethanolic extract of *Ambul* banana peel.

CONCLUSION

The study concludes that banana peels can be used as a source to extract carotenoids, including β-carotene, using either a hexane–acetone mixture or ethanol as the extraction solvent. The highest total carotenoid and β-carotene concentrations were extracted from the peel of the *Ambul* banana variety (0.04 ± 0.01 mg/g and $2.45 \times 10^{-4} \pm 0.04$ mg/mL, respectively), revealing the potential for further experiments to exploit its industrial-scale applications. Nevertheless, this study concluded that ethanolic extracts of *Ambul* banana peel generate better colorimetric values as well as sensory acceptability in food applications, highlighting its potential to serve as a food-grade colorant.

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