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Carotenoid Analysis of Ripe Banana Flesh and Peel from Three Cultivars of Banana

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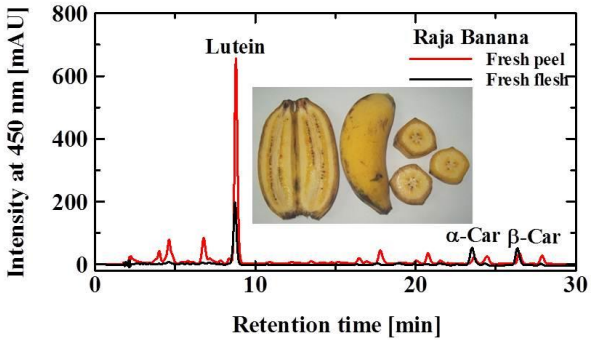
Abstract

Banana peel is a promising source to be utilized in fortification of food products due to the high content of carotenoids (Cars) which are active as provitamin A. The dominant Car, total and relative concentrations of Cars were determined from fresh flesh and peel in three cultivars of banana which are obtained from Raja, Ambon Kuning, and Kepok Kuning. To evaluate the difference in Car compositions principal component analysis (PCA) was also performed. Based on chromatographic, spectroscopic, and mass spectrometric analyses, dominant Cars were separated and identified to be lutein, *α*-carotene, and *β*-carotene. Lutein was the major Car of fresh peel, while the other two Cars were found in fresh flesh in addition to lutein. Raja banana had the highest total concentration of Cars among three banana samples used. PCA results generated from the absorption spectra showed three clusters of the different banana samples. PCA results are correlated to their Car compositions and this method might be applicable for the determination of dominant Cars.



Short Description
The highest total carotenoid was found in the Raja banana among three cultivars tested. Three carotenoids, lutein, *α*-Car, and *β*-Car, were separated and identified from the extracts of fresh flesh and peel by HPLC and LCMS analyses. The lutein was the major carotenoid present in the both fresh flesh and peel.

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Keywords: carotenoid, carotenoid composition, banana, RP-HPLC, PCA

INTRODUCTION

Bananas are one of the primary commodities of Indonesia. In 2014, Banana production was the highest, around 7 million ton, compared to the other fruits [1]. Furthermore, due to their low cost and their potential as functional and nutraceutical foods, bananas are the most widely consumed fruit. Both high production and number of consumption levels are ensured that the banana peel waste is abundantly present. Therefore, banana peel is a promising source to be utilized in the fortification of food products. Both fresh flesh and peel of banana contain carotenoids (Cars) which are highly responsible for yellow color.

The main Cars found in banana fruit are alpha-carotene (*α*-Car), beta-carotene (*β*-Car), and lutein [2-4], whereas lutein is the dominant Car in banana peel [5]. Many reports justified that banana Cars play important roles in human health. *β*-Car and *α*-Car are the most important Cars having provitamin A activity. These provitamin A Cars are converted into retinol in the body and considered as a way to solve the vitamin A deficiency problem [6,7]. Slattery et al. (2000) reported that lutein reduces the risk of some chronic diseases such as cancer, heart disease and age-related [8].

Color change from green to yellow during the ripening of banana peel is associated with the presence of chlorophylls (Chls) and Cars. The Chls decrease rapidly and finally do not present in the ripe banana, while at the same stage Cars increase [3]. The studies of Arora et al. (2008) and Lokesh et al. (2014) suggested the variation of Cars concentrations in

different cultivars of banana [9,10]. The Cars biosynthesis in a banana is also influenced by other factors such as genetic and geographic [11]. A recent study by Facundo et al. (2015) showed that Car composition from two cultivars of banana was differentially affected by the low storing condition [4].

Up to date, there is limited report on the Car concentrations of fresh peel bananas. In this study, we determine the total concentration of Cars by spectrometric method, Car composition and relative concentrations of the dominant Cars by reversed-phase high-performance liquid chromatography (RP-HPLC) and liquid chromatography-mass spectrometry (LC-MS) of fresh flesh and peel from three cultivars of banana. Raja, Ambon Kuning, and Kepok Kuning bananas were selected for this study due to their native Indonesian and highly commercial. Also, principal component analysis (PCA) was used to evaluate the differences in the composition of dominant Cars in the samples.

EXPERIMENTAL

General

Raja, Ambon Kuning, Kepok Kuning bananas were purchased from a traditional market of Sukun, Malang, Indonesia. Three cultivars of banana have the same ripening level without showing green color in the banana peel (Figure 1).

Acetone, acetonitrile, *n*-hexane, ethanol (EtOH), methanol (MeOH), methyl tert-butyl ether (MTBE), water (H_2O), calcium carbonate ($CaCO_3$), formic acid and sodium ascorbate were purchased from Merck (Darmstadt, Germany). H_2O and acetonitrile were liquid chromatography grade. These chemicals were used without further purification. Standard Cars such as lutein, α-Car, and β-Car were obtained from NATChrom (Malang, Indonesia).

Figure 1. Fresh flesh and peel from three cultivars of banana, Ambon Kuning (A); Kepok Kuning (B); and Raja (C) bananas

Car Extraction

Fresh flesh and peel were only ground by a blender and used without further treatment. Fresh peel was extracted with 100% acetone, while a mixture of EtOH and *n*-hexane (4:3, v/v) was used as an extraction solvent for fresh flesh homogenate. Car extraction was carried out by stirring with a magnetic bar at 120 rpm for 30 min in the presence of $CaCO_3$ and sodium ascorbate. The Car extract was separated from residue by centrifugation at 10,000 rpm for 10 min and extraction was repeated until the residue became colorless. Car extract was then dried under a stream of nitrogen gas and stored at −80˚C.

Determination of Total Concentration of Cars

Absorption spectra of Car extracts in *n*-hexane were measured by a UV-Vis spectrophotometer (UV 1700, Shimadzu, Kyoto, Japan) in the range of 350–600 nm. Total concentrations of Cars were calculated according to an equation of Gross (1991) [12].

Separation, Identification and Quantification of the Dominant Cars

Prior to injection, the dried Car extract was dissolved in acetone and then filtrated by a membrane filter (Nylon, Whatman, 20 μm). Car extract (20 μL) was injected to HPLC. Cars were separated by RP-HPLC (Shimadzu) equipped with quaternary pumps (LC-20AD), an online degasser (DGU-20A5), a Rheodyne injection valve (Rheodyne LLC, Rohnert Park, USA) with a 20 μL of sample loop, a column oven (CTO-20A) at 30˚C and a diode array detector (LC20A). RP-HPLC analysis was performed using a C30 column (YMC; 4.6 I.D. x 150 mm) with a tertiary gradient elution from MeOH, MTBE and Water (81:15:4, by vol.) to (6:90:4, by vol.) for 70 min at a flow rate of 1 mL/min. Chromatographic and spectroscopic properties of the separated Cars were used for the identification of the Cars. Further identification of dominant Cars was carried out an electron spray ionization (ESI) method by LCMS-8030 (Shimadzu) equipped with dual pumps (LC-20AD XR), an online degasser (DGU-20A3R), an autosampler (SIL-20AC XR) at 5˚C, a column oven (CTO-20AC) at 30˚C, and a diode array detector (SPD-M20A). The dominant Cars were analyzed directly by LC-MS with a mobile phase of 10% A and 90% B (A = 0.1% formic acid in H_2O; B = 0.1% formic acid in acetonitrile) for 2 min at a flow rate of 0.3 mL/min. Other parameters of LC-MS analysis were 250˚C of DL temperature, 400˚C of heat block temperature, 3 mL/min of nebulizing gas flow, 15 mL/min of drying gas flow, and Q1 scan (+) mode.

PCA Analysis

Absorption spectra of Car extracts from the samples and also standard Cars in *n*-hexane were analysed by PCA from Past 3.06 software [13]. Before PCA analysis, the absorption spectrum was initially corrected with the sample mass, water content and dilution factor.

RESULTS AND DISCUSSION

Absorption spectra of Car extracts from fresh flesh and peel of Raja banana and also the fresh peel from three cultivars of banana showed a typical fine structure of Car (Figure 2). Fresh flesh and fresh peel of Raja banana had a similar spectral shapes with the different maximum absorption wavelength (λ_{max}) at 444 nm and 440 nm. The absorption spectrum of fresh peel was blue-shifted compared with the former absorption spectrum. These results indicated that Car compositions of the fresh flesh might be slightly different from that of the fresh peel (Figure 2A).

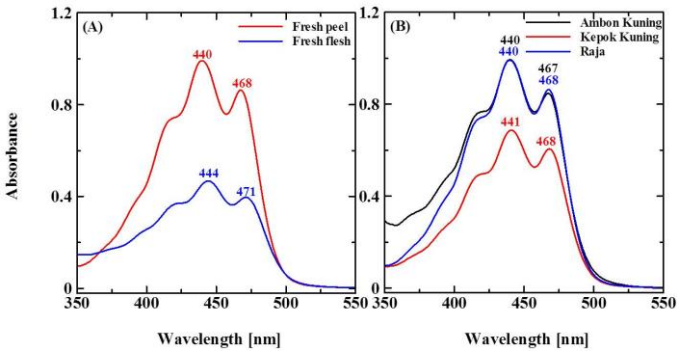

Figure 2. Absorption spectra of Car extracts from fresh flesh and fresh peel of Raja banana (A) and fresh peel from three cultivars of banana (B) in *n*-hexane.

The differences in absorbance value from the fresh peel of three bananas suggested that their Car concentrations were varied. The fresh peel of Ambon Kuning and Raja had the same and highest Car concentration in dry weight basis followed by Kepok Kuning banana from the absorbance values. By comparing the absorption spectra of the fresh peel (Figure 2B), however, three cultivars of banana peel had the same Car composition from their spectral shapes and λ_{max}. The same trends of composition and concentration of Cars were also observed for fresh flesh of samples.

Car extracts from fresh peel and fresh flesh of Raja banana were separated by a C30 column within 35 min (Figure 3). Three dominant Cars found in fresh flesh of Raja banana were detected not only in other parts of Raja banana but also among three cultivars of banana (see Table 3).

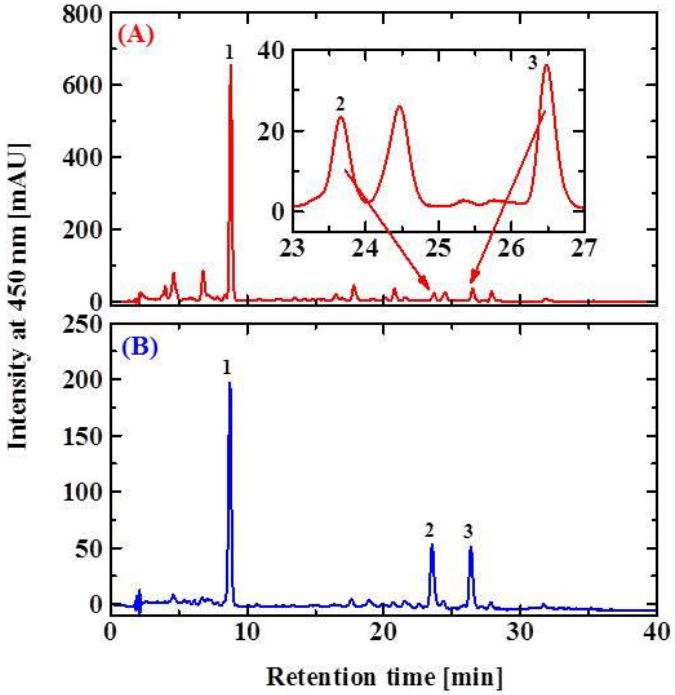

Figure 3. HPLC chromatograms of Cars extracts from fresh peel (A) and fresh flesh (B) of Raja banana detected at 450 nm. Peak numbers in the elution profile correspond to those in Table 1. Inset figure 3A: HPLC chromatogram of Car extract from fresh peel in the range of 23–27 min.

The identification of the dominant Cars was based on the chromatographic and spectroscopic properties such as retention time (t_R), co-chromatography results with the standard Cars, λ_{max} in HPLC eluent and other solvents compared to the references [14,15]. Also, Car identifications were confirmed by LC-MS analyses (Table 1). The first peak eluted at 8.7–8.8 min was identified as lutein by RP-HPLC method. This high polarity of lutein is probably due to the presence of two hydroxyl groups at the cyclic end groups compared to other dominant Cars. Co-chromatography results also confirmed our identification in terms of the same t_R and sum of peak intensity (data not shown). λ_{max} in EtOH and *n*-hexane of the first peak isolated from HPLC were in agreement with those values of the references [14,15]. As shown in Table 1, the mass spectrum of lutein showed the molecular ion $[M]^+$ at *m/z* 568.6 with the fragment ion detected at *m/z* 551.6 that indicates the loss of one hydroxyl group $[M-OH]^+$.

The second and third peaks of dominant Cars have a close polarity with t_R at 23.5–23.7 min and 26.4–26.5 min, respectively. According to the results of co-chromatography and λ_{max}, these peaks were identified as *α*-Car and *β*-Car, respectively. The different position of conjugated double bonds between *α*-Car and *β*-Car, especially one double bond at *β*-ring, causes shortening of λ_{max} for *α*-Car by around 5 nm compared to the latter one [16]. These dominant Cars have the same mass number, therefore, the mass spectra of *α*-Car and *β*-Car appeared $[M]^+$ at *m/z* 536.7. The adduct ion of *β*-Car was detected at *m/z* 567.6 $[M+OCH_3]^+$ which indicates the addition of OCH3 from MeOH. HPLC analysis showed that fresh peel contained only lutein as the dominant Cars, while in fresh flesh three dominant Cars were present as similar to the previous report of lutein, *α*-Car, and *β*-Car as major Cars in banana flesh [2,4].

The averages of the total concentration of Cars from Raja banana were the highest in two parts, while Kepok Kuning banana had the lowest total concentration (Table 2). The present result on total Cars of fresh flesh in Raja banana was in the range of other reports. The different cultivars of banana had a total concentration of Cars ranging from 0.6-21.0 µg/g fresh weight (fw) [10]. Arora et al. (2008) found the highest Car concentration (4 µg/g dry weight (dw)) in the pulp of Red banana [9]. Total concentrations of Cars of fresh peels for Raja and Ambon Kuning bananas were in the range of 20.3-20.6 µg/g dw (or 3.5-3.7 µg/g fw). These values were in a line with the report of Subagio et al. (1996) where the total Cars of banana peel were in the range of 3-4 µg/g fw as lutein equivalent [5].

In fresh peel of bananas, lutein was the dominant Car and had almost 70% of the total area of all Cars, although other dominant Cars were only less than 3.3% (Table 3). The studies of Subagio et al. (1996) and Sheikhzadeh et al. (2015) revealed that lutein was abundantly found in the banana peel. In fresh flesh, high values of % area of lutein (48.8%), *α*-Car (35%), *β*-Car (46.6%) were found in Raja, Ambon Kuning, and Kepok Kuning bananas, respectively [5,17]. In the case of fresh flesh, lutein content was close to that of *α*-Car in Ambon Kuning and Kepok Kuning bananas.

Table 1. Chromatographic, spectrophotometric and mass spectrometric properties of the dominant Cars from banana. Peak number corresponds to those in HPLC chromatograms of Figure 3.

Peak No	t_R [min]	λ_{max} [nm]			Molecular mass [*m/z*]	Fragment ion [*m/z*]	Car
		HPLC eluent	EtOH	Hexane			
1	8.7-8.8	–, 445, 473	–, 446, 474	–, 445, 474	568.6 $[M]^+$	551.6 $[M-OH]^+$	Lutein
2	23.5-23.7	423, 446, 474	–, 445, 474	422, 445, 473	536.7 $[M]^+$	-	*α*-Car
3	26.4-26.5	–, 451, 478	–, 451, 479	–, 450, 477	536.7 $[M]^+$	567.6 $[M+OCH_3]^+$	*β*-Car

Table 2. Total concentrations of Cars (µg/g dw ± SE) of fresh flesh and fresh peel from three cultivars of banana, Raja, Ambon Kuning, and Kepok Kuning bananas

Cultivar	Total concentration of Cars (µg/g dw ± SE)	
	Fresh flesh	Fresh peel
Ambon Kuning	7.29 ± 1.37	20.33 ± 0.55
Kepok Kuning	3.49 ± 1.05	13.61 ± 0.32
Raja	9.54 ± 0.62	20.63 ± 0.83

Table 3. Relative concentrations of the dominant Cars from three cultivars of banana calculated from their peak areas detected at λ_{max} and percentage of peak area (% area) detected at 450 nm

	Cultivar	Peak area at λ_{max}			% peak area at 450 nm		
		lutein	*α*-Car	*β*-Car	lutein	*α*-Car	*β*-Car
Fresh flesh	Ambon Kuning	1,618	1,761	545	31.4	35.0	12.1
	Kepok Kuning	288	434	123	17.8	15.5	46.6
	Raja	2,924	970	865	48.8	18.5	16.7
Fresh peel	Ambon Kuning	11,702	35	71	70.6	0.2	0.4
	Kepok Kuning	5,774	30	49	72.9	1.1	2.2
	Raja	11,655	332	482	72.2	2.4	3.3

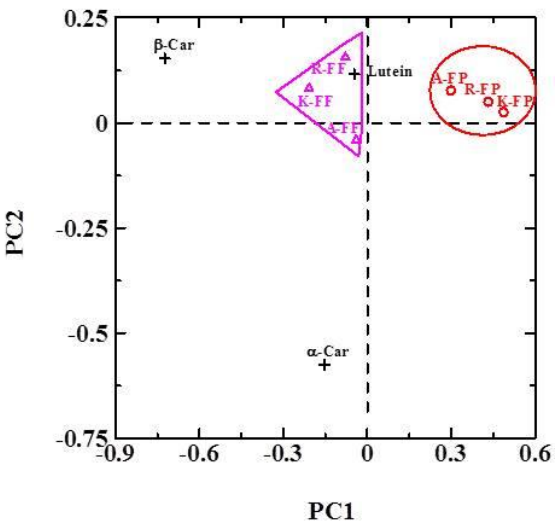

Figure. 4. The score plots of PC1 versus PC2 from the absorption spectra of banana Car extracts, and the standard Cars. Note: R (Raja banana); K (Kepok Kuning banana); A (Ambon Kuning banana); FF (fresh flesh); and FP (fresh peel). Standard carotenoids are expressed as a symbol (+).

Absorption spectra of Car extracts were used to evaluate the differences in the composition of dominant Cars from the banana samples by using PCA analysis. Recently, Sarungallo et al. (2015) revealed that nine clones of *Pandanus conoideus* could be classified into 3 groups using PCA analysis based on the proximity of its Car content [18]. Also, PCA was applied to analyze correlations between the concentrations of seven Cars from three sweet oranges grown in six different areas [19]. In the present PCA analysis, two principal components (PC1 and PC2) were adequate to resolve the absorption spectra from fresh flesh and fresh peel in three cultivars of banana. The score plots between PC1 and PC2 of the absorption spectra of Car extracts from banana samples and the standard Cars are shown in Figure 4.

Banana samples are possibly classified into 2 groups by the results of PCA, namely fresh peels (group 1; circle) and fresh fleshes (group 2; triangle). This reflects the Car compositions in the samples which correlate the ratio of the relative concentrations of dominant Cars. The close location of each group in this study suggests the presence of similar Car compositions and relative concentrations of dominant Cars in three cultivars of banana. By respecting to the quadrant and also the position of the standard Cars, fresh peels contain lutein as the dominant Car (see above explanation), were located in the quadrant closely with the lutein. Fresh flesh was localized in between three standard Cars and closely to lutein. This PCA result is also in agreement with the chromatographic data.

Besides the purpose of sample grouping based on the Car composition, PCA analysis might be useful for the determination of dominant Cars and its relation to other carotenoid species according to the PC1 and PC2 values. Heriyanto et al. (2011) reported that the degree of fucoxanthin stability during the treatments could be determined by PC1 value [20]. From the PCA analysis, the relation between lutein and *β*-Car and between lutein and *α*-Car may be estimated by PC 1 value. On group 1, PC 1 values were much higher than group 2, indicating that this group is dominated by lutein than another group. Concerning to group 2, PC 1 value was almost similar for R- and A-FF, indicating a close ratio of lutein and *β*-Car. While K-FF had the lowest PC 1 value that is closest to *β*-Car. This suggests the highest *β*-Car content. PC 2 values were, however, different in three samples. This means that ratios between lutein and *α*-Car are different. A-FF had the lowest PC 2 value that is closest to *α*-Car, indicating that this sample has a high content of *α*-Car.

CONCLUSION

The results of HPLC and LC-MS analyses showed that lutein, *α*-Car, and *β*-Car were found as the dominant Cars present in banana samples. Raja banana had the highest total Car concentrations among the three cultivars studied by the spectrometric method. In fresh peels of banana cultivars, lutein was the dominant Car and had almost 70% of the total Cars, although the other Cars, *α*-Car, and *β*-Car, were only less than 3.3%. PCA analysis could be used for the classification of banana samples and the determination of the dominant Cars according to their Car composition.

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Abstrak
Kulit pisang merupakan sumber yang menjanjikan untuk digunakan dalam fortifikasi produk makanan karena tingginya kandungan karoten yang aktif sebagai provitamin A. Karotenoid dominan serta total karotenoid dan konsentrasi relatif karotenoid ditentukan dari daging segar dan kulit segar pada tiga kultivar pisang, yaitu Raja, Ambon Kuning, dan Kepok Kuning. Analisis komponen utama (*principal component analysis* atau PCA) juga dilakukan untuk mengevaluasi perbedaan dalam komposisi karotenoid. Berdasarkan analisis kromatografi, spektroskopi, dan spektrometri massa, karotenoid dominan dipisahkan dan diidentifikasi sebagai lutein, *α*-karoten, dan *β*-karoten. Lutein merupakan karotenoid utama pada kulit pisang segar, sedangkan 2 karotenoid lainnya ditemukan dalam daging segar selain lutein. Pisang Raja memiliki total karotenoid tertinggi di antara tiga sampel pisang yang digunakan. Hasil PCA dari spektra serapan menunjukkan tiga kelompok sampel pisang yang berbeda. Hasil PCA berkorelasi dengan komposisi karotenoid dari pisang dan metode ini mungkin dapat digunakan untuk penentuan karotenoid dominan.

Kata kunci: karotenoid total, KCKT fase terbalik, komposisi karotenoid, PCA, pisang