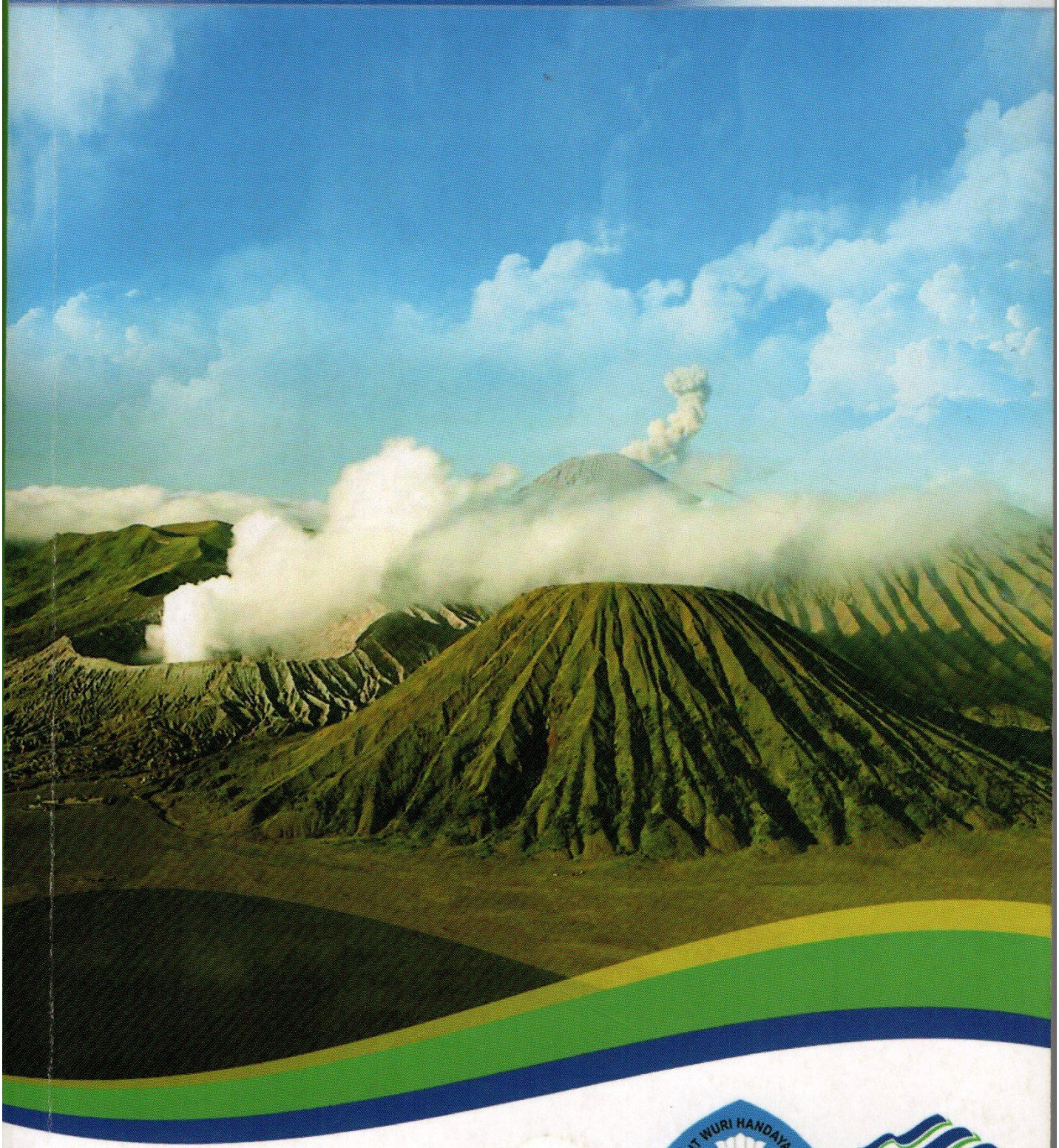
SYNERGY, NETWORKING AND
THE ROLE OF FUNDAMENTAL
RESEARCH DEVELOPMENT IN ASEAN

in conjunction with :

THE INTERNATIONAL CONFERENCE
ON NATURAL SCIENCES (ICONS) 2011

Alexander von Humboldt
Stiftung/Foundation



The Bureau
of Planning and
International Cooperation,
Ministry of National Education

TUT WURI HANDAYANI

UNIVERSITAS
MA CHUNG

Proceeding of Humboldt Kolleg:
SYNERGY, NETWORKING AND THE ROLE OF FUNDAMENTAL RESEARCH DEVELOPMENT IN ASEAN
in conjunction with:
THE INTERNATIONAL CONFERENCE ON NATURAL SCIENCES (ICONS) 2011

Cover design: Aditya Nirwana, S.Sn
Layout editor: Irfan Tri Raharjo, SE

Publisher : Ma Chung Press
Villa Puncak Tidar N-01 Malang

ISBN: 978-602-9155-05-1

Proceeding of Humboldt Kolleg:
SYNERGY, NETWORKING AND THE ROLE OF FUNDAMENTAL RESEARCH DEVELOPMENT IN ASEAN

in conjunction with:
THE INTERNATIONAL CONFERENCE ON NATURAL SCIENCES (ICONS)

Publisher : Ma Chung Press
Villa Puncak Tidar N-01
Malang

TABLE OF CONTENT

ISOLATION AND IDENTIFICATION OF CAROTENOID PIGMENTS OF YELLOW AMBON BANANA PEEL (*Musa paradisiaca sapientum* L.)

Suparmi[1*] and Harka Prasetya[1,2]
[1] Medical Faculty; Sultan Agung Islamic University
[2] Semarang Eye Center Sultan Agung Islamic Hospital
Jl. Raya Kaligawe KM. 4 PO BOX 1054 Semarang Central Java Indonesia ,
Phone (+6224) 65833584, Fax. (+6224) 6594366
**email:amik_achsy@yahoo.com*

ABSTRACT

Exploration of banana peel potency in the health sector has not been much done, although some researchers reported that banana peel contains essential nutrients for health that is not inferior to the flesh. The potency of banana peel carotenoids as provitamin A for eye health should be developed in Indonesia in line with the increasing cases of Vitamin A Deficiency (VAD), such a emerging lately. This study aimed to isolate and identify the carotenoids in the Ambon banana peel (Musa paradisiaca sapientum L.). Ambon banana peel samples of 200 grams grinded, then dissolved by acetone 100% with the sample and solvent ratio 1:10 w/v. Provitamin A carotenoids were identicated by chemical test of carotenoids, thin layer chromatography (TLC), Double Beam UV-Visible Spectroscopy, and epoxides and hydroxy group test. Peel yellow banana (Musa parasidiaca sapientum L.) containing carotenoid pigments, among others zeaxantin, xantofil, and β-carotene.

Keywords*: banana peel, Musa paradisiaca sapientum L., carotenoid*

INTRODUCTION

Banana is one of the Indonesian fruit commodities. Annual production of bananas gained 40-45% of the national fruit production. Java Island including Central Java dominates national banana production by producing more than 50% of the total production. The average of annual bananas production in Central Java was 440.283 tons [1]. Yellow banana (*Musa paradisiaca sapientum* L.) is one of banana varieties that have high economic value and much-loved by the community either as the fresh fruit, processed product, raw material of industries or for animal feed. On the other hand, the increasing of banana production caused the increasing of banana peels. Although the bananas peel including organic waste, if not used optimally, this waste could potentially pollute the environment.

In Indonesia, the utilization of banana peel is limited as a mixture of animal feed (20-30%), as well as manure and compost (60-70%) [2]. Exploration of banana peel potency in the health sector has not been much done, although some researchers reported that banana peel contains essential nutrients for health that is not inferior to the flesh. The focus of previous research more on the characterization of banana peel nutrient content and activity of banana peel extract for gaining antioxidant, antimicrobial, and a natural antibiotic, while the carotenoid content on a banana peel has not been much studied [3].

Carotenoid pigment is one source of pro vitamin A that very promising, because it proved to be converted into vitamin A by the body. Yellow fruits, including the banana are the source of carotenoid pigments [4,5]. Researchers from the Chung Shan Medical University Taichung, Taiwan reported that banana peel extract useful in protecting the retina from light damage due to degeneration of the retina [6]. However, whether the protective effect to retina caused by carotenoids content of these extracts as manifested in yellow color of banana peel is not known with certainty. Therefore, it needs further study regarding the content of provitamin-A carotenoids on bananas peel.

The potency of banana peel carotenoids as provitamin A for eye health should be developed in Indonesia in line with the increasing cases of Vitamin A Deficiency (VAD), such a emerging lately. The survey result of Nutritional and Health Status Monitoring during 1998-2002 showed that until 2002, nearly 50% of Indonesian children under five (about 10 million children) are threatened by sub-clinic VAD with serum retinol <20 μg/dl. Health Office of Central Java in 2003 recorded about 665.000 children (age 6-60 months) in Central Java suffered a mild degree of VAD. The VAD patients number of children under five, approximately 32% of the total number of children under five in Central Java [7]. Efforts to rescue children

from the threat of blindness caused by VAD has become the main agenda in the National Action Plan for Food and Nutrition (RANPG) 2006-2010 through supplementation of high dose vitamin A by Blue capsule (SI 100 000 doses) to infants (6-11 months) and Red capsules (SI 200 000 doses) for children (12-59 months) [7,8,9]. However, to date the government still imports vitamin A capsule supplement [9]. This reality is thus opening great opportunities to explore and utilize the carotenoid pigment in the yellow bananas peel (*Musa paradisiaca sapientum* L.) as an alternative source of natural vitamin A (*Sumber Vitamin A Alami*, SUVITAL).

The result of this research is expected to contribute the technology of banana peel processing, so as to reduce environmental pollution caused by the buildup of banana peel waste. Commercialization of a banana peel as a SUVITAL alternative expected to contribute in improving the economy of farmers and traders and will stimulate the development of banana plantations and the cultivation of bananas in Indonesia through increased value added processing such as focusing on a banana peel into the source of carotenoids. In addition, the use of supplements of vitamin A from banana peel carotenoids is expected to reduce the problem of malnutrition in Indonesia, especially the problem of VAD. Supplements of vitamin A that produced are expected to contribute in preparing the community with enough vitamin A, especially in order to rescue children from the threat of blindness. This study aimed to isolate and identify the carotenoids in the Ambon banana peel (*Musa paradisiaca sapientum* L.).

RESEARCH METHOD

The sample used are the Ambon yellow banana peel (*M. paradisiaca sapientum* L.) taken randomly from banana farmers or traders in the area around campus Islamic University of Sultan Agung, Semarang. Criteria used for the banana peel samples include: comes from the optimal-old banana with fresh and good condition, full leather sides, not flat shape, color and condition of the peel is still intact and smooth, no black or brown spots.

Pigment Isolation of Yellow Ambon Banana Peel

Ambon banana peel samples of 200 grams grinded, then dissolved by acetone 100% with the sample and solvent ratio 1:10 w/v. At the extraction, added $CaCO_3$ as a neutralizing agent and ascorbic acid as an antioxidant to prevent oxidation. The extraction was done in a dark room at a temperature of -15 $^{\circ}$C to prevent oxidation or enzymatic degradation. Furthermore, the extract was filtered by Whatman filter paper #42, the residue obtained was extracted again with the same solvent until all the pigment taken up (banana peel becomes colorless). The extract is partitioned with hexane, and then filtered with the multilevel Whatman filter paper 42, 0.45 m and 0.2 m membrane filters in a cold state. Subsequently, the extract is added into anhydrous Na_2SO_4 to remove water content in the extract. Pigment extract resulted by filtration then was concentrated using a rotary evaporator. Concentrated extract obtained is stored into the sample bottles and dried using N_2 gas [10, 11].

Purification of carotenoid pigments by column chromatography using stationary phase silica gel Si-60 and a hexane mobile phase. Crude extract of the pigment dissolved in solvent hexane, then packed into chromatography columns that had been prepared. Each fraction collected in sample bottles and dried using N_2 gas [10].

Identification of Provitamin A Carotenoids

Chemical Test of Carotenoids

Fraction of the isolated carotenoids by column chromatography of 5 ml drops 2-3 drops $SbCl_3$ 10% $^{w}/_{v}$ in chloroform and H_2SO_4. Solution color change from yellow to blue indicates positive carotenoids [12].

Thin Layer Chromatography (TLC)

Identification of carotenoid pigments using TLC method, with the stationary phase silica gel 60 F_{254} (Merck). Each sample spotted on TLC plates and then elucidated with hexane solution. Separation pattern of pigment were observed by color spots that formed, then count the value of retardation factor (Rf) on each spot. Rf is the ratio between the distance of the solute and the distance of a mobile phase [13, 14]. For comparison also used marker β-carotene (E-Merck, No. 1.02236).

Double Beam UV-Visible Spectroscopy

Fractions of the isolated pigment was analyzed by spectroscopy using a Varian Cary double beam 50 spectrophotometer at a wavelength of 300-600 nm. Spectral pattern formed in each of the pigment compared to the spectral pattern of marker β-carotene (E-Merck, No. 1.02236). The pattern of absorption spectra is described by program Origin 6.1.

Epoxides and Hydroxy Group Test

Fraction isolated by column chromatography of 5 ml drops 2-3 drops of concentrated HCl, and then performed the measurement of spectral patterns. The positive test of epoxide group existence is if there was a hipsocromic shift. While chemical tests to confirm the presence of hydroxy group carried by dripping pyridine and acetic acid anhydride to the solution of carotene pigment fraction that isolated. Batocromic shift in the pattern of absorption spectra indicate the presence of hydroxy groups [4].

RESULTS AND DISCUSSION

Isolation and Identification of Carotenoid Pigments on Yellow Ambon Banana Peel Samples (*M. paradisiaca sapientum* L.)

Extraction of 200 g yellow banana peel in acetone solvent producing 1.24 mg of concentrated extract, so the total yield of the pigment extracts from yellow banana peel is 0.01%. This means that every 100 g of banana peel yellow pigment extract containing 0.01 g of banana peel.

The crude extract of pigment of the yellow banana peel that has been partitioned using hexane produces crude extract preparations. Thin layer chromatography (TLC) with hexane mobile phase and silica gel stationary phase of crude extract of pigment produces 3 spot, as shown in **Figure 1**. Crude extract of the pigment is a mixture of several carotenoid pigments that exist in the yellow banana peel, so the number of spots that formed more than 1, because it is possible there are many different types of carotenoid pigments. Based on the calculation of Rf values (**Table 1**). The first spot bright yellow and has a Rf value of 0.2 were identified as zeaxantin. Second spot is grayish yellow with Rf value 0.46 and identified as xantofil. The third spot is yellow and has a value of Rf 0.53 were identified as β-carotene.

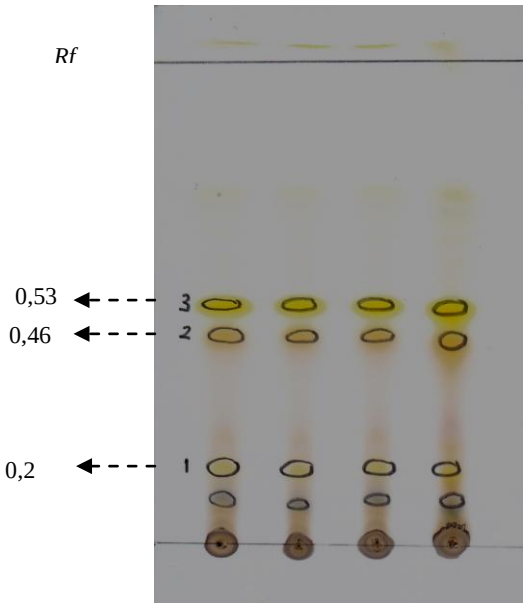


Figure 1. The separation pattern of pigments and Rf value of each spots on the TLC fractions of column chromatography (1) 1st fraction, (2) 2nd fraction, (3) 3rd fraction.

Table I. Rf value on TLC from crude extract of yellow banana skin samples and identification of pigments based on reference

Spot	*Rf* range		Spot colour	Pigment
	Result	Reference		
1	0.2	0.2 – 0.37 [15, 16]	Light yellow	Zeaxantin
2	0.46	0.4 – 0.5 [16]	Yellow-gray	Xantofil
3	0.53	0.5 – 0.92 [17, 10]	yellow	β-caroten

Identification of pigments was also performed by measuring the spectra patterns of fractions resulting from columns chromatography. Fractions resulted by column chromatography separation each having a maximum absorbance at a wavelength of 440, 450 and 442 nm, shown in **Table 2**, **Figure 2**. The identification results of this spectroscopic approach the maximum absorbance of zeaxantin, xantofil, and β-carotene those dissolved in acetone by references.

Table II.The maximum wavelength of absorption spectra from column chromatography fractions in hexane

Sample	λ_{maks} (nm)		Pigment
	Results	Reference	
Crude Extract	440	-	-
1st Fraction	440 457 476	440, 459, 479 [15, 16]	zeaxantin
2nd Fraction	452 477 506	453, 475, 507 [16]	xantofil
3rd Fraction	442 455 474	440, 453, 471 [17, 10]	β-caroten

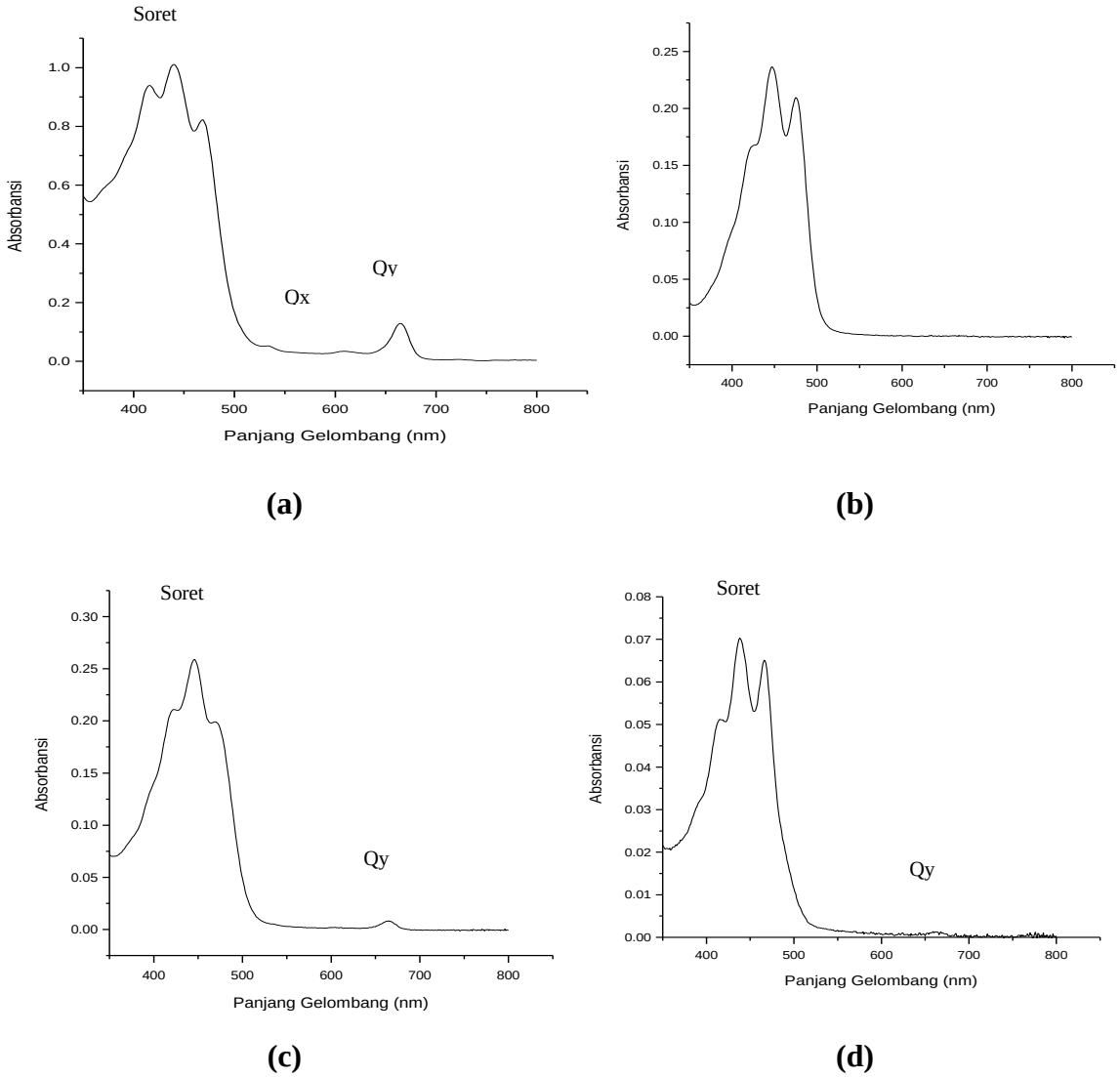


Figure 2. Pattern spectra crude extract and fractions of column chromatography in the solvent acetone, which (a) crude extract, (b) 1st fraction, (c) 2nd fraction, (d) 3rd fraction.

Presumption that fraction 3 is β-carotene is strengthened by the test chemical reaction of fraction 3 in the acetone solvent with the initial color is yellow, after added by $SbCl_3$ 10% $^{w/}_{v}$ in chloroform and H_2SO_4, the solution color changed to blue, as shown in **Figure 3**. Thus the fraction 3 was identified as β-carotene [11].

(a) (b)

Figure 3. The results of the identification of β-carotene, (a) before SbCl3 plus 10% $^{w}/_{v}$ in chloroform and H_2SO_4 and (b) after $SbCl_3$ plus 10% $^{w}/_{v}$ in chloroform and H_2SO_4.

CONCLUSION

Peel yellow banana (*Musa parasidiaca sapientum* L.) containing carotenoid pigments, among others zeaxantin, xantofil, and β-carotene.

ACKNOWLEDMENT

This research was supported by research grants from Region VI Kopertis Ministry of National Education in accordance Penelitian Dosen Muda dan Studi Kajian Wanita Number: 002/006.2/PP/SP/2010, dated March 1, 2010.

REFERENCES

[1] Anonim, *Pemanfaatan Limbah Kulit Pisang sebagai Substituen Tepung*, http://himdikafkipuntan.blogspot.com, 2008.

[2] Husni, I., *Prespektif Industri Pisang Terpadu (Guna Peningkatan Nilai Tambah Komoditas,* Direktorat budidaya Tanaman Buah Direktorat Jenderal Hortikultura Departemen Pertanian, 2009, http://ditbuah.hortikultura.deptan.go.id//index.php?option=com_content&task=view&id=118&Itemid=1.

[3] Mokbel, M.S and F. Hashinaga. 2005. Antibacterial and Antioxidant Activities of Banana (*Musa*, AAA cv. Cavendish) Fruits Peel. *American Journal of Biochemistry and Biotechnology* 1 (3): 125-131.

[4] Gross, J., 1991. *Pigment in Vegetables, Chlorophyll and Carotenoids*, Van Nostrand Reinhold, New York.

[5]Anwar, F., *Minyak Sawit Bikin Jantung Sehat Dan Awet Muda,* Kompas, 2002, http://www.kompas.com/kesehatan/news/senior/gizi/0207/25/gizi.htm.

[6] Kompas, *Khasiat Kulit Pisang untuk Depresi dan Kesehatan Retina*, 2008, www.kompas.com.

[7] Departemen Kesehatan RI, *Deteksi dan Tata Laksana Kasus Xeroftalmia* : *Pedoman Bagi Tenaga Kesehatan,* 2003.

[8] Badan Perencanaan Pembangunan Nasional. 2007. *Dokumen Rencana Aksi Nasional Pangan dan Gizi 2006-2010*. ISBN 978-979-3764-27-6.

[9] Naibaho, P.M., Pemisahan Karotena (Provitamin A) dari Minyak Sawit dengan Metode Adsorbsi, 2007, http://seafast.ipb.ac.id/seafast.info/Seafast.info0/produk_detail.php?categoryID=4&produkID=107.

[10] Britton, G., Liaaen-Jensen, S., and Pfander, H., Carotenoids Volume IA: Isolation and Analysis, Birkhauser Verlag. Basel. Boston. Berlin, 47p., 1995.

[11] Rodriguez-Amaya, D.B. and M. Kimura, HarvestPlus Handbook for Carotenoid Analysis, Washington, DC and California: International Food Policy Reseach Institute (IFPRI) and International Center for Tropical Agliculture (CIAT), 2004.

[12] Tobing, R.L., *Kimia Bahan Alam*, Depdikbud Direktorat Jenderal Pendidikan Tinggi. Proyek Pengembangan Lembaga Pendidikan Tenaga Kependidikan, Jakarta, 1989.

[13] Khopkar, S.M., *Konsep Dasar Kimia Analitik*, Terjemahan, Jakarta: Universitas Indonesia, 1990.

[14] Sastrohamidjojo, H., *Kromatografi*, Liberty, 2000, Yogyakarta.

[15] Rodriguez and Chischest, Carotenoid and food preparation: The retention of Provitamin A Carotenoids in Prepared, Procedded, and Stored Foods, John Snow, Inc / OMNI Project. U.S., 2001, *A Guide to Carotenoid Analysis in Food.* Washington: International Life Science Institute. . 1997. 71p.

[16] Foppen, F. H. 1971. *Tables for the Identification of Carotenoid Pigments.* Chromatographic Reviews, 14: 133-298.

[17] Ritter, E. D. and Purcell, A. E. 1981. *Carotenoid Analytical Method. In Carotenoid as Colorants and Vitamin A.* Precursors: Technological and Nutritional Applications, Bauernfeind, J.C., Ed, Academic Press Inc: New York, pp 815 – 882

SYNERGY
leave a legacy

PT. PANGAN AGRO LESTARI

RnD
Smart Solution

PT DITEK JAYA
Analytical & Measuring Instruments

Bakti Pendidikan
DJARUM foundation

SENSIENT

BPKLN

ISBN 978-602-9155-05-1
9 786029 155051

UNIVERSITAS
MA CHUNG

Universitas Ma Chung,
Jl. Villa Puncak Tidar N-01, Malang
Jawa Timur Indonesia - 65151
Telp : (0341) 550171 Faks : (0341) 550175
Website : http://humboldt-icons.machung.ac.id
Email : humboldt-icons@machung.ac.id