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DETERMINATION OF BETA-CARROT LEVELS OF CAMPOLAY FRUIT (POUTERIA CAMPECHIANA) BY METHOD HPLC

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Abstract

Besides its sensory characteristics, campolay fruit has the potential to be functional food. The yellow color of campolay fruit indicates the presence of carotenoids. One highly beneficial group of carotenoids is beta-carotene, which is abundantly available in agricultural products and makes this compound one of the most advantageous components in the prevention and treatment of various types of eye diseases. HPLC, which stands for High-Performance Liquid Chromatography, is a chemical analysis technique used to separate, identify, and measure the components in a solution mixture. The beta-carotene testing process in campolay fruit is conducted using High-Performance Liquid Chromatography (HPLC) equipment with a visible light detector at a wavelength of 446 nm. From the HPLC analysis, the regression equation obtained is y = 366032x - 6388. From the standard beta-carotene solution, a linear relationship between absorbance and concentration was obtained with a correlation coefficient (r) of 0.9996. The peak area of the campolay fruit extract was obtained. The sample concentration is calculated by comparing the peak area of the sample with the peak area of the standard beta-carotene, resulting in the beta-carotene concentration of the campolay fruit extract using the high-performance liquid chromatography method. Based on the beta-carotene concentration of the campolay fruit obtained from this study, it can be concluded that the beta-carotene concentration of the campolay fruit is 0.15 mg/L.

Keywords: Beta-carotene, campolay, HPLC, tropical fruit

INTRODUCTION

Campolay fruit is classified as a rare fruit that has not been widely used and cultivated by people in Indonesia (Do et al., 2023). This causes not many people to consume this fruit due to limited information about its characteristics. The flesh of a ripe campolay fruit will be yellow to orange, the flesh is soft, has a strong aroma, and tastes sweet (Aminullah, Purba, Rohmayanti, & Pertiwi, 2020) (Mangunsong, Assiddiqy, Sari, Marpaung, & Sari, 2019).

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Figure 1. Campolay Fruits and Pulp
(Personal Documentation, 2024)

In addition to its sensory characteristics, according to several research results, this fruit can have the potential to be a functional food. The yellow color of the campolay fruit is an indicator of the presence of carotenoid content in the fruit. Carotenoid compounds are substances that function as precursors for the formation of vitamin A (Dzulhijjah, Sarli, & Shabayek, 2022) (Juniarti & Hasnelly, 2016). Of the many groups of carotenoid compounds, only three types of carotenoids correlate with the formation of vitamin A (retinol) in the human body. The three types are α-carotene, β-carotene and β-cryptoxanthin (Pertiwi, Rohmayanti, Aminullah, Apriani, & Silpia, 2022). Of the three types of carotenoid compounds, β-carotene is the compound that has the best function as a precursor to vitamin A or provitamin A. β-carotene is also a carotenoid component that is available in large quantities in agricultural products which makes it one of the most beneficial components in the prevention and treatment of several types of eye diseases (Syukri, 2021) (Adyas & Dzulhijjah, 2022).

HPLC stands for *High-performance liquid chromatography* or High Performance Liquid Chromatography (KCKT) is a chemical analysis technique used to separate, identify, and measure the content of components in a mixture of solutions. In HPLC, the mixture of solutions to be analyzed is injected into a column containing a stationary phase, which can be resin or small particles. The solution is then passed through a column with the help of a phase of motion or solvent (*mobile phase*)(*Adiyaman [2016] Identification and quantification of poly phenolic compounds in underutilized fruits (Star fruit and egg fruit) using HPLC.pdf*, n.d.) (Mudrikah, Hidayah, Amelia, & Helsen, 2024).

Each component in the solution mixture interacts with the stationary phase and the motion phase in different ways, resulting in the separation of the components. Eventually, these components can be detected and their concentrations measured using sensitive detectors, such as ultraviolet detectors or mass detectors.

The high-performance liquid chromatography technique is a liquid-liquid chromatography method that can be used for both imaging analysis and quantitative analysis. Quantitative analysis with high-performance liquid chromatography techniques is based on standard area measurements. With the knowledge of beta carotene levels with analysis using HPLC instruments that have accurate results, it is hoped that it can be a reference for the utilization and development of campolay fruit processing technology

RESEARCH METHOD

1. Tool

The tools used include a 0.45 micron filter, glassware, filter paper, micropipettes, and analytical scales. The analysis instruments consisted of HPLC Shimadzu LC20AT, LiChrospher®100RP-18 column endcapped (5 μm) 25cm long, inner diameter 4mm (E.Merck), KLT silica gel plate 60 F254, Chamber, Microscale (Sartorius Dragon), Blender (Philips), Rotavapor (Buchi V-800), Separator funnel, Glass tools, Whatman filter paper No. 40.

2. Material

The chemicals used are standard beta carotene (quality p.a. from Merck), acetonitrile *(HPLC grade,* Merck), isopropanol *(HPLC-grade,* Merck), nitrogen gas (technical), methanol (p.a, Merck), petroleum ether (p.a, Merck), distilled water, potassium hydroxide (p.a, Merck), sodium sulfate anhydrous (p.a, Merck), chloroform p.a, ethyl acetate p.a, methanol p.a, antimony trichloride.

3. Prepare sample

A quantity of the pulp of the campolay is mashed in a blender, then weighed in as much as 50 grams, put into a sealed Erlenmeyer covered with aluminum foil on the outside and protected from light. Ethyl acetate solvent was added, beaten for 30 minutes with *a magnetic* stirr, and filtered with a Buchner funnel. The non-polar part is taken and subsequently used for quantitative testing.

4. Method of Determining Optimum Conditions HPLC/KCKT

Determination of maximum absorption wavelength β-carotene: A total of 20 mg of β-carotene isolate is put into a 50-mL flask, dissolve and dilute with chloroform until mark. Then pipetted a total of 2.5 mL inserted into the flask 10-mL diluted with chloroform until the mark. Next, a spectrum was created using a UV-Vis spectrophotometer at a wavelength of 420-500 nm.

5. Identification of **β-Carotene in Campolay**

Extract/Isolate Manufacturing

Fresh campolay fruits that have been cut into pieces and pureed, weighed in the amount of 100 g (campolay fruit), 50 g blended using mineral water solvent, then filtered. The residue is rinsed with Filtrate obtained given calcium salt, centrifuged at 3000 rpm for 15 minutes. The residue as pellets is beta carotene, concentrated using rotavapor at 400 C. Stored in cold temperatures.

a) Solution manufacturing

Preparation of raw solution: A quantity of 10 mg of β-carotene is put into a 50-mL flask, dissolved and diluted with chloroform to a mark. Then pipetted a 2.5 mL amount is inserted into a 10-mL flask diluted with chloroform up to the mark line (Standard beta carotene).

b) How to identify

A number of test solutions were injected as much as 20 μl into the KCKT device, then the retention time was compared with the standard retention time of β-carotene.

6. Quantitative analysis by high-performance liquid chromatography

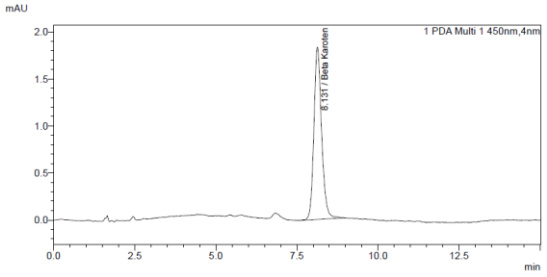
a) System conformity test The system conformity test is carried out to determine whether tools, methods and conditions form a single analysis system. A total of 20 μl of β-carotene raw solution was injected 5 times into the KCKT device, then the peak area

was measured with optimal KCKT conditions, then the relative standard deviation value was calculated.

b) Determination of β-carotene levels in campolay fruit extract by KCKT. The test solution is sonicated for 10 minutes. Each of them was injected as much as 20 µl into the KCKT device and the peak area was measured with the optimal KCKT condition.

RESULTS AND DISCUSSION

Measurement of absorbance of beta carotene raw solution with a concentration of 0.08 ppm; 0.1 ppm; 0.15 ppm; 0.2 ppm; 0.25 ppm; 0.5 ppm; 0.75 ppm; 1 ppm; 2.5 ppm; and 5 ppm at a maximum wavelength of 446 nm was injected as much as 20 µl into the KCKT device using the chloroform:methanol (95:5) motion phase and a flow rate of 1.0 ml/min with a visible light detector at a wavelength of 446 nm. So that the area and retention time are obtained.



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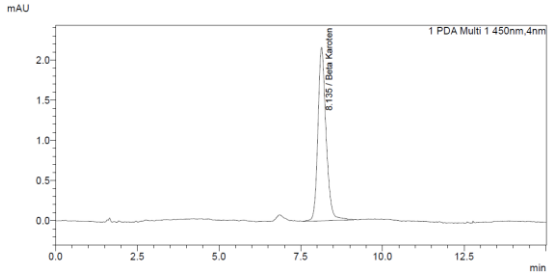

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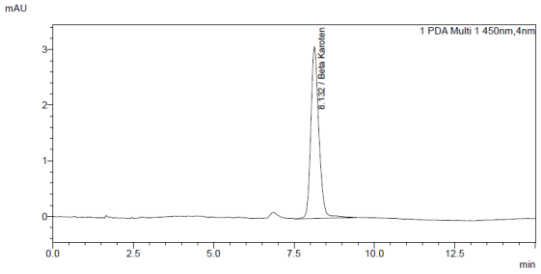

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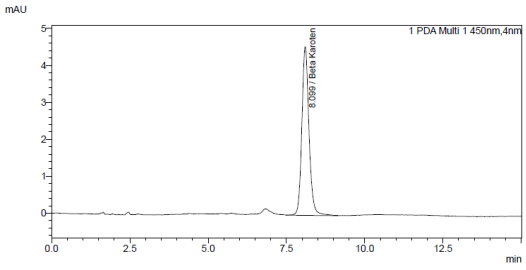

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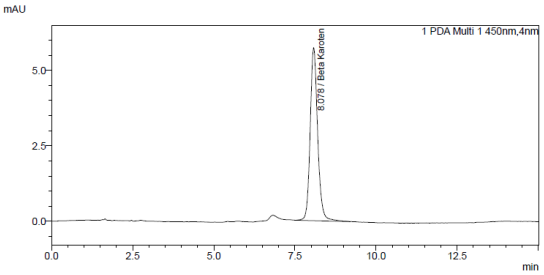

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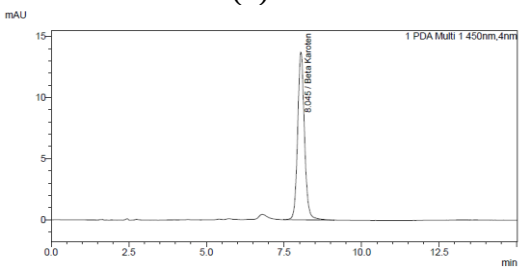

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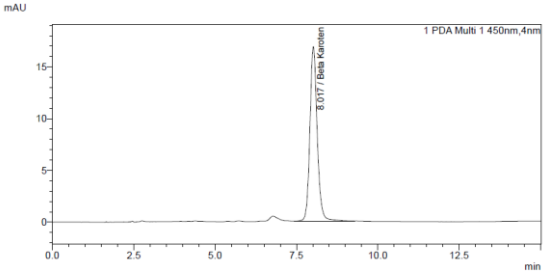

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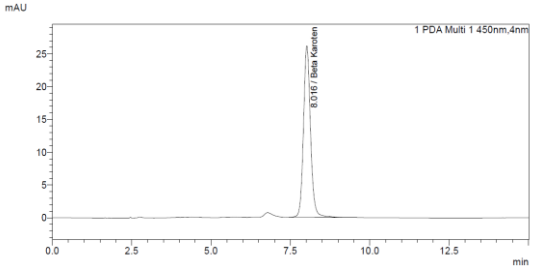

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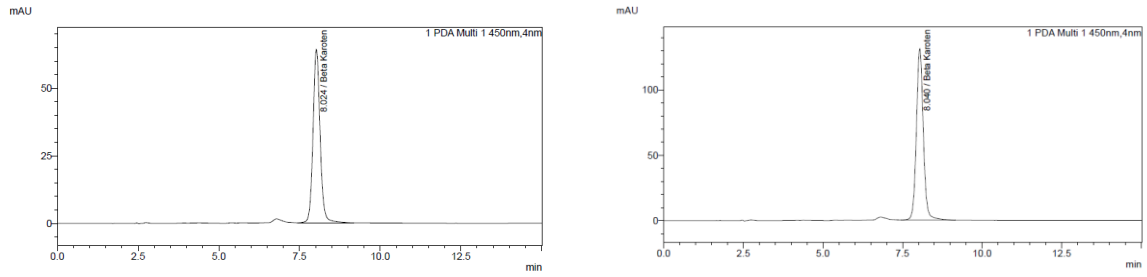


(i) (j)

Figure 1. Raw Beta Carotene Chromatograph using HPLC (a) Concentration 0.08 ppm; (b) Concentration 0.1 ppm; (c) Concentration 0.15 ppm (d) Concentration 0.2 ppm (e) Concentration 0.25 ppm; (f) Concentration 0.5 ppm; (g) Concentration 0.75 ppm; (h) Concentration of 1 ppm; (i) Concentration 2.5 ppm; (j) Concentration 5 ppm

Tabel 1. Hasil Kurva Kalibrasi Standar

C. Standar Antara (mg/L)	V. Standar (mL)	V. Akhir (mL)	C. Deret Standar (mg/L)	Retention Time (menit)	Area	C. Injeksi (mg/L)	Residual (%)
10,88	0,080	10,00	0,087	8,13	30232,94	0,10	14,91
10,88	0,100	10,00	0,109	8,14	37563,87	0,12	10,33
10,88	0,150	10,00	0,163	8,13	53761,29	0,16	0,66
10,88	0,200	10,00	0,218	8,10	72245,16	0,21	-1,31
10,88	0,250	10,00	0,272	8,08	91557,78	0,27	-1,65
108,84	0,050	10,00	0,544	8,05	209945,67	0,59	8,61
108,84	0,075	10,00	0,816	8,02	262957,53	0,74	-9,85
108,84	0,100	10,00	1,088	8,02	402430,76	1,12	2,62
108,84	0,250	10,00	2,721	8,02	978957,50	2,69	-1,06
108,84	0,500	10,00	5,442	8,04	1991336,37	5,46	0,29
Slope					**366031,98**		
Intercept					**-6387,99**		
R^2					**0,9996**		
R					**0,9998**		

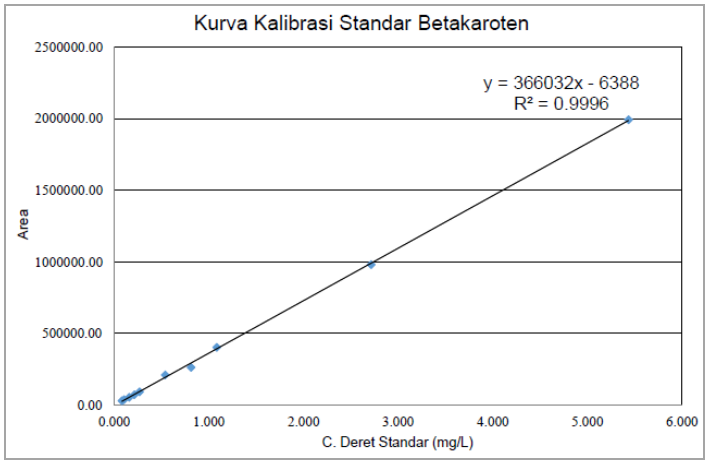
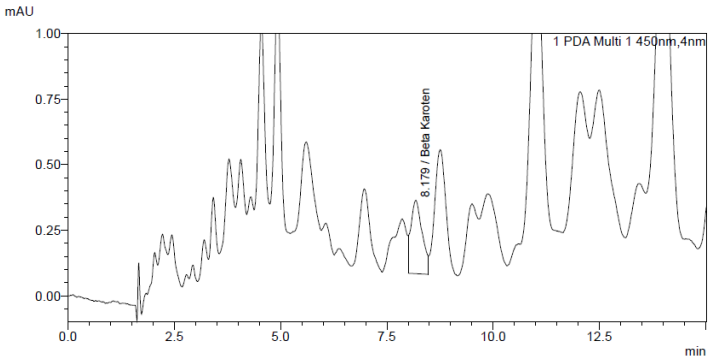
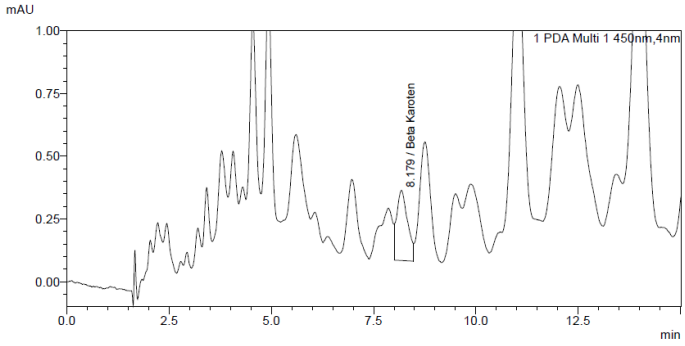


Figure 2. Standard Solution Calibration Curve Beta Carotene

The measurement of beta carotene levels of campolay fruit samples was carried out 2 times at a maximum wavelength of 446 nm injected as much as 20 µl into the KCKT device using the motion phase of chloroform:methanol (95:5) and a flow rate of 1.0 ml/min with a visible light detector at a wavelength of 446 nm so that the area was obtained.



(a)



(b)

Figure 3. Beta Carotene Chromatography of Campolay Fruit Samples Using HPLC Chromatograph Connected 1; (b) Chromatograph Connected 2

Based on the measurement of beta carotene levels of campolay fruit samples carried out at a maximum wavelength of 446 nm, chromatographs were obtained as shown in Figure 2 and the results are presented in the following table.

Table 2. Results of Measurement of Beta Carotene Area Area in Fruit Campolay

No	Sampel	Bobot (g); Volume (mL)	FP	V. Akhir (mL)	RT (menit)	Area	C. Injeksi (mg/L)	Kadar (mg/Kg : mg/L)
1	Ulangan 1	2,0848	1	10	8,18	5219,19	0,03	0,15
2	Ulangan 2	2,0192	1	10	8,18	5220,68	0,03	0,15

Beta carotene is a red-orange pigment that is very abundant in plants and fruits. Beta carotene is one of the antioxidants that can prevent diseases, especially degenerative diseases (Anjo et al., 2021). In this study, the flesh of campolay fruit *(Pouteria campechiana*) which is extracted with ethyl acetate solvent. Naturally, beta carotene is abundant in orange, red to dark green fruits and vegetables. The physical color indicator is found in campolay fruit. Based on various sources and research results, beta carotene can prevent cancer and reduce the risk of lung cancer because it is the main compound that attacks cancer (Awang-Kanak & Abu Bakar, 2018)

The quantitative analysis process begins with maceration extraction. Extraction is carried out by maceration because the sample to be studied is the pulp. The flesh of the fruit was chosen because the beta-carotene to be studied is found in the pulp. In addition, this extraction method was chosen because it is very simple, and can be used to extract simplicia with content that does not withstand heating, such as beta carotene. In this process, ethyl acetate is added to attract the carotenoid compounds. Ethyl acetate is a semi-polar solvent so that it can attract both polar and nonpolar compounds, has low toxicity, and is easily volatile so that it can be used for the extraction of carotenoids including beta carotene.

The testing process was carried out with a *High Performance Liquid Chromatography* (KCKT) instrument using a mixed chloroform: methanol (95:5) motion phase, flow rate of 1.0 ml/min with a visible light detector at a wavelength of 446 nm. Each sample and as a benchmark for synthetic beta-carotene made in the form of a solution with a concentration of 0.08 ppm; 0.1 ppm; 0.15 ppm; 0.2 ppm; 0.25 ppm; 0.5 ppm; 0.75 ppm; 1 ppm; 2.5 ppm; and 5 ppm is injected as much as 20 µl into the KCKT device. From the analysis with KCKT, the regression equation y = 366032x - 6388 was obtained. From the standard solution of beta carotene, a linear relationship between absorbance and concentration in absorbance measurement with a correlation coefficient (r) of 0.9996 was obtained. An r value close to 1 indicates that the regression equation is linear (Mangunsong et al., 2019). From the campolay fruit extract, the area of the peak area is obtained. The sample content was calculated by comparing the area of the peak area of the sample with the area of the peak area at the standard beta carotene, so that the beta carotene content was obtained from the pulp extract of campolay by high-performance liquid chromatography method.

Based on the results of the beta carotene content of campolay fruit obtained from this study, it can be concluded that the beta carotene content of campolay fruit is 0.15 mg/L. The carotenoid content in the flesh of campolay fruit is 278.24 µg/g (Puspita, Kurniawan, & Aiboi, 2019). Puspita, Kurniawan, Aiboi, et al., (2019), menyebutkan jika di dalam sawo mentega terdapat beragam fraksi-faksi karotenoid seperti; β-karoten, β-kriptosantin, violasantin, neosantin, ζ-karotenoid. Senyawa-senyawa biokatif tersebut berpotensial sebagai sumber pro vitamina A (Anjo et al., 2021).

The difference in yield can be caused by various factors, namely differences in soil conditions, temperature, weather, air humidity from the area of origin of the fruit used. Then another factor is the difference in the solvent used and the tool to analyze (Mangunsong, Puspita, Simamora, & Taswin, 2023).

CONCLUSION

Based on the results of the study, it was concluded that the synthetic beta-carotene comparator raw solution was made in the form of a solution with a concentration of 0.08 ppm; 0.1 ppm; 0.15 ppm; 0.2 ppm; 0.25 ppm; 0.5 ppm; 0.75 ppm; 1 ppm; 2.5 ppm; and 5 ppm is injected as much as 20 µl into the KCKT device. From the analysis with KCKT, the regression equation y = 366032x - 6388 was obtained. From the standard solution of beta carotene, a linear relationship between absorbance and concentration in absorbance measurement with a correlation coefficient (r) of 0.9996 was obtained. Measurement of beta carotene levels of campolay fruit samples was performed at a maximum wavelength of 446 nm. Campolay fruit beta carotene level of 0.15 mg/L.

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