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Research Article

β-Carotene Levels in Local Carrot (*Daucus Carota* L.) Extract from Two Different Planting Areas

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ABSTRACT

Carrots or *Daucus carota* L. are vegetables that have many benefits. β-carotene as the main content in carrots can act as provitamin A, antioxidants, and skin and facial care. Two types of local carrots, namely carrots grown in Citeko, Bogor Regency and Sukatani, Cianjur Regency, have the potential to be developed as a source of natural β-carotene for drug and cosmetic preparations. Determination of β-carotene levels in local carrots needs to be done before being used as drug and cosmetic preparations. The purpose of this study was to determine the β-carotene levels in the extracts of two types of local carrots, namely carrots from Citeko, Bogor Regency and Sukatani, Cianjur Regency. The sample powder was extracted by the maceration method using n-hexane solvent (1:10). Qualitative β-carotene testing was carried out by the TLC method using a silica gel 60 F254 stationary phase and a petroleum ether: benzene mobile phase (9:1). Determination of β-carotene levels using a validated method, namely the visible light spectrophotometry method with n-hexane solvent at a wavelength of 450 nm. The results of this study indicate that the β-carotene levels in Citeko carrot extract are 86.94 ± 5.53 mg/g and 104.09 ± 4.21 mg/g for Sukatani carrot extract. Based on the results of the ANOVA test, the β-carotene levels in the two types of carrot extracts did not show significant differences. Both types of local carrot extracts, Citeko and Sukatani, have the potential as sources of β-carotene for drug and cosmetic preparations.

Keywords: carrots; β-carotene; maceration; TLC; visible spectrophotometry

INTRODUCTION

Carrots or *Daucus carota* L., are known as one of the vegetables that have many benefits. The main content of carrots is β-carotene, which can act as a source of vitamin A (Harahap et al., 2020; Mangunsong et al., 2019). β-carotene also plays an important role in maintaining body immunity, warding off free radicals, helping vision, accelerating wound healing, and eliminating toxins in the body (Silalahi, 2022; Triastuti et al., 2013). In addition, β-carotene functions as an anticancer, antiobesity and bone-forming stimulant (Syukri, 2021). Carrots can also be used for facial and skin care, and to nourish hair (Samadi, 2014).

Local carrots have the potential to be a source of natural β-carotene that can be developed into medicinal and cosmetic preparations, in addition to processed food products. Several studies related to the use of carrots have been reported, but are still limited. Dewi & Wirahmi, (2019) made a lotion from carrots from Pasar Panorama Bengkulu. Meanwhile Ermawati et al. (2022) made a serum spray preparation as an anti-aging agent from carrots from the Puncak Arfak mountain area, West Papua. Sari and Zulfa (2022) reported the use of carrot extract in the formulation of an antioxidant feel-off gel mask, but the type of carrot used was not explained. Meanwhile, Fajriati et al. (2022) utilized carrots in the encapsulation of carotenoid pigments for halal natural pigments, but the type of carrot was not explained.Triastuti et al. (2013) reported the use of local Lampung carrots for mixed carrot drink products with various fruits. Cornelia and Nathania (2020) reported the use of Berastagi carrots for jelly candy. Meanwhile Ningtyas and Erwiyani (2023) made jelly candy with unmentioned carrot ingredients.

West Java is one of the contributors to local carrot production in Indonesia. The highest carrot production in Indonesia is in four provinces, namely Central Java, West Java, East Java and North Sumatra (Kementan, 2024). Carrot production in West Java during 2019-2023, decreased each year (Kementan, 2024). The highest carrot production in West Java is in Cianjur, Bandung, Garut, Sukabumi and Bogor Regencies. One of the carrot-producing villages in Cianjur Regency is Sukatani Village, Pacet District, and in Bogor Regency, Citeko Village, Cisarua District. This type of local carrot has advantages over other areas, namely that it does not rot easily. Firmansyah (2016) reported that based on the weight of the tubers, the low level of leaf rot attacks, and the sweetness level of the tuber flesh, Cisarua carrots have very good adaptation.

Local carrots have the potential to play a role in supporting the maintenance and improvement of public health. Local carrots from Citeko and Sukatani were selected to be developed into medicinal preparations, cosmetics and processed food products. Identification and determination of β-carotene levels in local carrot extracts need to be determined before the carrot extract is made into medicinal and cosmetic preparations. The purpose of this study was to determine the β-carotene levels in local carrot extracts based on different planting areas, namely Citeko Cisarua, Bogor Regency and Sukatani Pacet, Cianjur Regency, West Java, Indonesia –The results of the study are expected to provide information on the β-carotene content of local carrots, to be further developed into medicinal preparations, cosmetics and processed food products. In addition, the results of the study are expected to encourage the sustainability of local carrot farmers.

METHODS

Materials and Equipments

The materials used in this study were two types of local carrots, standard β-carotene (Sigma-Aldrich), n-hexane (Merck), petroleum ether (Merck), and benzene (Merck). The tools used in this study were an oven (Memmert®), analytical balance (LabPRO DT224C), rotary evaporator, UV-Vis spectrophotometer (Jasco V-730), cuvette, chamber, silica gel 60 F254 TLC plate, capillary tube, UV chamber, furnace (Daihan Scientific), crucible, blender, maceration bottle, desiccator, 40 mesh sieve and a set of glassware (Pyrex®).

Methods

Raw Material Collection

The samples used in this study were two types of local carrots from West Java. One type of sample was obtained from a carrot plantation in Citeko Village, Cisarua District, Bogor Regency and one type from a carrot plantation in Sukatani Village, Pacet District, Cianjur Regency.

Making of Carrot Powder and Testing of Powder Characteristics

The making of carrot powder refers to Sirait et al. (2016). Furthermore, the testing of powder characteristics was carried out including testing of water content and ash content (Kemenkes, 2017).

Extract Preparation and Extract Characteristic Testing

The extraction process refers to Cornelia & Nathania (2020) with a modification of the soaking time from 3 days to 6 days with n-hexane solvent. Furthermore, the extract is tested for characteristics which include testing the water content and ash content (Kemenkes, 2017).

Qualitative Test of β-Carotene on Carrot Extract with TLC Method

A qualitative test of β-carotene on carrot extract was carried out using the TLC method (Fajriati et al., 2022). Stationary phase using silica gel 60 F254 TLC plates. Optimization of the mobile phase was carried out with a combination of acetone: diethyl ether: hexane (2:3:6), acetone: diethyl ether (8:2), and petroleum ether: benzene (9:1). The TLC plate containing the reference solution and sample was observed under visible light and UV 366 nm. A good Retention factor (Rf) value according to Wulandari (2011) is 0.2-0.8.

Determination of β-carotene Levels in Sample Extracts

Making β-Carotene Stock Solution and Determining the Maximum Wavelength

Preparation of β-carotene standard solution refers to Agustina et al. (2019) with modification of the solvent type and concentration. Measure the maximum absorbance with a visible spectrophotometer at a wavelength of 400-500 nm (Syukri, 2021).

Validation Parameter Testing

The validation parameter testing of the analysis method for determining β-carotene levels in carrot extract includes. testing linearity, accuracy, precision, Limit of Detection (LoD) and Limit of Quantification (LoQ) (Ashokrao et al., 2022; Chandra et al., 2017a; Saiya & Caroles, 2022).

Measurement of β-Carotene Levels in Samples

A series of standard solutions of β-carotene with concentrations of 5, 7, 10, 11, and 15 ppm were made from a stock solution of β-carotene of 100 ppm and the absorbance was measured using a visible spectrophotometer at the maximum wavelength of β-carotene. Weighed 50 mg of thick carrot extract and then dissolved it with n-hexane in a 25 mL measuring flask. Pipetted as much as 2.5 mL and made up to the volume using n-hexane in a 50 mL measuring flask. Absorbance was measured using a visible spectrophotometer at the maximum wavelength of β-carotene with n-hexane as a blank.

Data Analysis

The concentration of the carrot extract sample solution was calculated based on the calibration curve of the standard solution. The content of β-carotene in the carrot sample was calculated through the regression equation y = bx + a. The content of β-carotene in the carrot sample was calculated using equation 1.

$$\beta\text{-carotene content (mg/g)} = \frac{C \times V \times Fp \times 10^{-3}}{W} \quad ......(1)$$

Description:
C= Concentration (µg/mL),
V= Total volume of sample (mL),
Fp= Dilution factor,
W= Sample weight (g)

Statistical analysis to determine the level of β-carotene in carrots with SPSS 24 using the OneWay Analysis of Variance (ANOVA) test.

RESULTS AND DISCUSSION

Quality of Carrot Powder

The raw materials used are two local carrots. Local carrots were obtained from Citeko Village, Cisarua District, Bogor Regency and Sukatani Village, Pacet District, Cianjur Regency. Each was taken from two farmers. The initial carrot material weight was 3 kg each, and the results of the sieving obtained the weight of the carrot powder were 241.50 g (Citeko carrots) and 244.50 g (Sukatani carrots) respectively (Table 1). The yield value of Sukatani carrots was slightly higher (8.15%) than Citeko (8.05%). The results of this yield value were slightly lower than the results of the study by Sari & Zulfa (2022) with a yield value of 8.28%. Organoleptically, these two types of carrot powder give almost the same appearance. All types of powder give fine powder, orange carrot color, and distinctive carrot odor (Figure 1).

The quality testing of both samples includes water and ash content. The results of determining the water content of both types of carrot powder gave almost the same value. The water content value of the Citeko sample was only slightly higher (5.11 %) than the Sukatani sample (5.08 %) (Table 1).

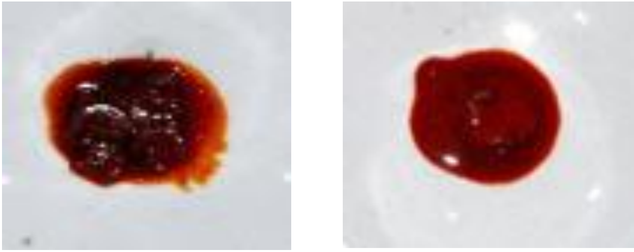
These results meet the water content requirements according to Marjoni (2020) which states that the water content requirement for powder is ≤ 10 %. Water content that meets the requirements can guarantee the quality of the preparation to avoid the growth of microorganisms.The results of determining the ash content of the two types of carrot powder gave slightly different values. The ash content value of the Citeko sample was higher (1.79 %) than the Sukatani sample (1.49 %) (Table 1.). These results meet the requirements for total ash content, which is ≤16.6 % according to Marjoni (2020). The ash content of Citeko carrot extract is higher than Sukatani carrot extract. This shows that Citeko carrot extract contains a higher mineral content than Sukatani carrot extract. Minerals in carrot tubers can come from soil that contains various minerals including potassium, calcium and magnesium. These results indicate that Citeko soil likely contains higher minerals than Sukatani.

a b

Figure 1. Carrot powder
Description: (a) Citeko carrot powder; (b) sukatani carrot powder

Table 1. Results of Water and Ash Content of The Carrot Powder

Carrot Sample	Water Content (%)	Total Ash Content (%)
Citeko	5.11 ± 0.33	1.79 ± 0.06
Sukatani	5.08 ± 0.03	1.49 ± 0.03
Requirements	≤10 (Marjoni, 2020)	≤16.6 (Marjoni, 2020)



a B

Figure 2. Carrot thick extract

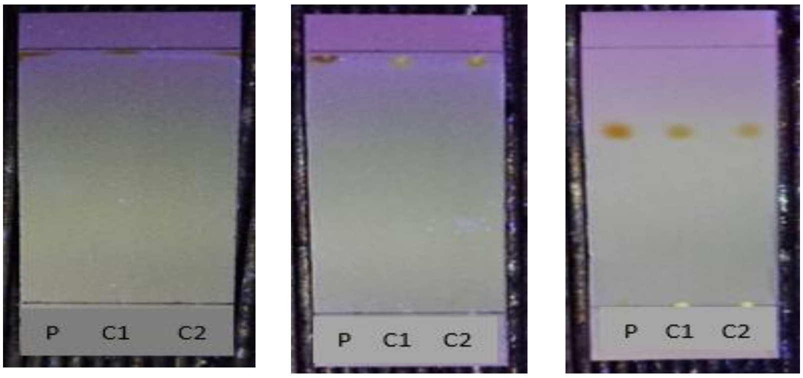
Description: (a) Citeko carrot ; (b) Sukatani carrot

Tabel 2. Yield of Dry Powder and Extract of Carrot

Carrot Sample	Powder Weight(g)	Average Extract Weight (g)	Average Extract Yield (%)
Citeko	50	0.95 ± 0.03	1.90 ± 0.04
Sukatani	50	0.92 ± 0.02	1.84 ± 0.05

Tabel 3. Results of Extract Water and Ash Content

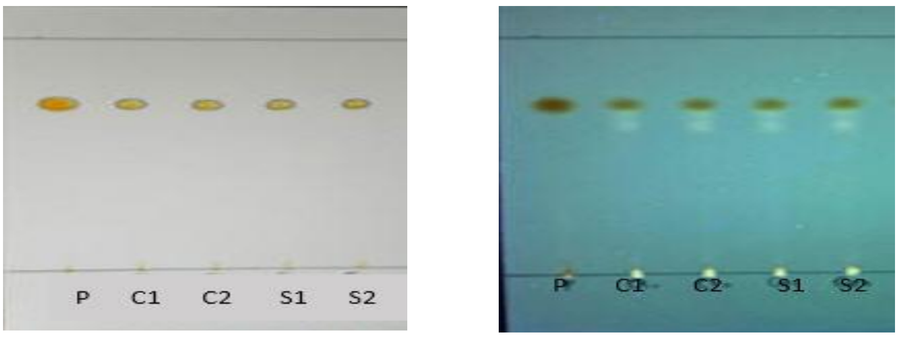
Carrot Sample	Water Content (%)	Total Ash Content (%)
Citeko	6.10 ± 0.20	1.97 ± 0.11
Sukatani	5.53 ± 0.46	1.85 ± 0.17
Requirements	± <10 % (Marjoni, 2020)	± <16.6 % (Marjoni, 2020)



a b c

Figure 3. TLC chromatogram of mobile phase optimization results

Description:Mobil phase: a. acetone: diethyl ether: hexane (2:3:6), b. acetone: diethyl ether (8:2), c. petroleum ether: benzene (9:1). P=Standard, C1,2= Sample of Citeko carrot extract.



a b

Figure 4. TLC chromatogram with the best mobile phase

Description: a. Under visible light, b. under UV light 366 nm, P= Standard β-carotene, C1-2=Sample of Citeko carrot extract, S1-2=Sample of Sukatani carrot extract

Quality of Carrot Extract

The preparation of thick extract from carrots was carried out using the maceration method using hexane solvent, with three repetitions. The maceration method was chosen because this method is relatively simple and does not use heating. This method is suitable for β-carotene compounds, which have heat-resistant properties. The hexane solvent is used because the extracted β-carotene compound is nonpolar. Cornelia & Nathania (2020) reported that β-carotene compounds are more soluble in n-hexane, compared to ethyl acetate and ethanol. The extraction results of the two samples were thick extracts that were slightly reddish-orange (Figure 2.).

The yield of the two simple extracts gave values that were only slightly different. The yield value of the Citeko sample was 1.90 ± 0.042 %, while the Sukatani sample was 1.84 ± 0.049 % (Table 2.). The yield values of these two samples were higher than the yield reported by Syakri et al. (2020) with a yield value of 1.06 %.

Extract quality testing includes water and ash content. The test results are presented in Table 3. The water content and ash content of the Citeko sample gave a water content value of 6.10 % and ash content of 1.97 %, this value is higher than the Sukatani sample with a water content value of 5.53 % and ash content of 1.85 %.

The water content of both samples meets the requirements based on Marjoni (2020), which is less than 10 %. The water content that meets the requirements can maintain the quality of the extract from the growth of microorganisms. Likewise, the ash content of both samples meets the requirements based on Marjoni (2020), which the ash content in the extract is less than 16.6 %. The ash content that meets the requirements can maintain the quality of the extract, avoiding the influence of excessive minerals.

Chromatogram Profile of β-carotene on Carrot Extract by TLC Method

A qualitative test of β-carotene on extracts was carried out using the TLC method using silica gel 60 F254 plates. At the beginning of identification, optimization of the mobile phase was carried out using three combinations of solvents, namely acetone: diethyl ether: hexane (2:3:6), acetone: diethyl ether (8:2), and petroleum ether: benzene (9:1). Based on the TLC chromatogram obtained (Figure 3.), the combination of mobile phases that provided the best separation was the mobile phase with a combination of petroleum ether: benzene (9:1) with an Rf value of 0.72 for standard and sample (Figure 3 c).

The best mobile phase is then used for the complete identification of β-carotene in the extracted sample. As a stain detector, observations were made under visible light and UV light at 366 nm. The complete TLC chromatogram results are presented in Figure 4.

Observation of the spots on the side under visible light (Figure 4a), was also observed under UV light 366 nm (Figure 4b.) because β-carotene has a chromophore group consisting of long conjugated double bonds. The β-carotene standard on the TLC chromatogram produced an orange-yellow color (Figure 4) and gave a Retention factor (Rf) value of 0.74. Likewise, all samples gave orange-yellow spots and had an Rf value of 0.74, which was identical to the β-carotene standard. Thus, it was concluded that all samples contained β-carotene. The Rf in the results of this study has a good value, because it is between 0.2-0.8 (Wulandari, 2011). The Rf of the β-carotene stain from this study is lower than the Rf value reported by Ningtyas & Erwiyani (2023), with the Rf value of β-carotene in carrot extract being 0.88. However, the Rf value of β-carotene from this study is higher, when compared to the Rf value reported by Agustina et al. (2019) with the Rf value of β-carotene carrot extract of 0.54.

Validation of Analysis Method

Before the validation of the method, the maximum wavelength was determined first. The maximum wavelength of β-carotene obtained in this study was 450 nm, with an absorbance value of 0.67. This result is the same as the maximum wavelength of β-carotene reported by Kassaye et al. (2023).

The validation parameters of the analytical method tested include linearity, accuracy and precision (standard addition method), LoD, LoQ, robustness and ruggedness. The results of the method validation are presented in Table 4. The results of the linearity obtained the value of the linear regression equation $y = 0.0411x + 0.0717$ with a correlation coefficient (R^2) value of 0.9928. This value indicates that the regression equation is linear and measurements at that concentration range can be used for analysis.

The results of the accuracy test obtained a % recovery value with a range of 98.0839-101.0106 %. Based on these results, it is stated that the % recovery value has met the requirements, namely 80-110 % based on AOAC (2023). Thus, it can be concluded that the visible spectrophotometric analysis method to be tested is a method that can be used to determine the levels of β-carotene in carrot extract. The results of the precision test obtained % RSD values in standard solutions of 8, 11, and 13 ppm of 1.17935 %, 0.13323 %, and 0.07719 %, respectively. Based on these results, it is stated that the % RSD values at the three concentrations meet the requirements based on AOAC (2023), namely <7.3 %.

Table 4. Results of Validation Methods

Parameter	Results	Requirements,
Linearity	y = 0.441x + 0.0717 R^2 = 0.9928	R^{2} => 0.99 (Saiya & Caroles, 2022) (Chandra et al., 2017)
Limit of Detection (LoD) *Limit of Quantification* (LoQ)	LoD = 1.1345 ppm LoQ = 3.7815 ppm	
Accuracy	99.4221-100.9358 (%)	80-110 % (AOAC, 2023)
Precision	0.07719-1.17935 (%)	<7.3 %. (AOAC, 2023)
Robustness and *Ruggedness*	*Robustness* • λ 449 nm RSD = 0.0261 % • λ 451 nm RSD = 0.0690 %	< 2 % (Ashokrao et al., 2022)
	Ruggedness • Analyst 1 RSD = 0.0619 % • Analyst 2 RSD = 0.0796 %	

The LoD (Limit of Detection) and LoQ (Limit of Quantification) values obtained were 1.1345 ppm and 3.7815 ppm, respectively. The *Robustness* test gave a % RSD value of 0.0261 and 0.0690, while the *Ruggedness* test gave a % RSD value of 0.0620 and 0.0795. The results of the *Robustness* and *Ruggedness* tests gave an RSD value of <2%. This shows that the method is strong and quite robust. The results of the *Robustness* and *Ruggedness* tests meet the requirements based on Ashokrao et al. (2022).

β-Carotene Contents in Carrot Extract

Measurement of β-carotene levels in two types of local carrot extract samples was carried out using the visible light spectrophotometry method at a maximum wavelength of 450 nm. β-carotene levels were determined by making a calibration curve. The results of making the calibration curve provide a linear regression equation of y = 0.0372 x + 0.0404 and a correlation coefficient (R^2) value of 0.9963.

The results of the measurement of β-carotene levels in sample extracts based on the regression equation above are presented in Table 5. The calculation results obtained β-carotene levels in Citeko carrot extracts lower levels compared to Sukatani samples. The β-carotene levels in Citeko carrot extracts were 86.9395 mg/gram, while the β-carotene levels in Sukatani extracts gave levels of 104.0853 mg/gram. This difference in levels can be caused by, among other things, differences in the height of the growing place. Sukatani Village is located higher than Citeko Village. The height of Sukatani Village is 1200-1500 m above sea level with an average temperature of 18-25 ºC, while Citeko has an altitude of 1000-1025 m above sea level with an average temperature of 20-29 ºC. Although there are differences in the height of the growing place. However, the conditions of Citeko and Sukatani Villages are still considered good conditions for carrot growth. According to Firmansyah (2016), the optimal temperature for carrot plants is 18-21 ºC with an altitude of >500-1000 m above sea level.

Table 5. β-carotene Content in Carrot from Different Planting Area

Sample type	β-karoten content (mg/gram)		Average β-carotene content (mg/gram)		Total average β-carotene content (mg/gram)
	P1	P2	P1	P2	
Citeko	83.06 83.01 83.01	90.86 90.89 90.81	83.03 ± 0.03	90.85 ± 0.04	86.94 ± 5.53
Sukatani	101.16 101.18 100.99	107.07 107.02 107.10	101.11 ± 0.10	107.06 ± 0.04	104.09 ± 4.21

Description: **P1**= Sample from farmer-1; **P2**= Sample from farmer-2

The β-carotene content in carrot extract in this study was higher than the results of previous studies, which used extraction with heating. Hiranvarachat et al. (2013) reported that the MAE method gave a content value of 0.233 mg/g, while Basuony et al. (2022) using the soxhlet method gave a content value of 49.75 mg/g. This shows that the maceration method produces a higher content value. The use of hexane solvent in this study resulted in a higher carotene content value than more polar solvents. The results of this study were higher than ethanol solvents which gave a β-carotene content value of 1.258 mg/g (Mirheli & Dinani, 2018), and also higher than the use of ethyl acetate solvent which gave a β-carotene content of 0.109 mg/g (Cornelia & Nathania, 2020). The high content of β-carotene in both carrot extracts makes both types of extracts potentially be used as pharmaceutical preparations, both for health purposes and for cosmetics.

Analysis of Variance (ANOVA Test) was used to test whether there was a difference in β-carotene levels in two types of carrot sample extracts. The test results obtained a Sig value of 0.073. This value is > 0.05. This indicates that there is no significant difference between the β-carotene levels in Citeko and Sukatani carrot extracts. Thus, both types of carrot extracts have the same potential to be used as ingredients for medicines and cosmetics.

CONCLUSION

The results of this study indicate that the levels of β-carotene in Citeko carrot extract were 86.94 mg/g ± 5.53 and 104.09 ± 4.21 mg/g in Sukatani carrot extract. The results of the ANOVA test showed that the β-carotene levels in these two types of extracts did not show significant differences. Thus, both types of local carrot extracts, Citeko and Sukatani, have the potential to be used as sources of β-carotene for medicinal and cosmetic preparations.

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CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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