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Analysis of β-carotene Content in Plants using Some Methods: A Review of Current Research

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ABSTRACT: β-carotene is a type of carotenoid functioning as provitamin A in the formation of vitamin A and also as an antidote to free radicals. β-carotene is a non-polar compound that is very well soluble in non-polar solvents such as n-hexane, petroleum ether, and acetone. β-carotene functions as an antioxidant in the body. β-carotene can be found in several plants such as vegetables, fruits, and flowers. This review discusses β-carotene analysis using a UV-Vis Spectrophotometer, High-Performance Liquid Chromatography (HPLC), and TLC-Densitometry. Analysis of β-carotene in various plants such as red spinach (Amaranthushydridus L.), bitter melon (Momordicacharantia L.), red chilies (Capsicum annuum L. var. Abreviatumfingerhuth), curly red chilies (Capsicum annuum L. var. Longum) sendt), cayenne pepper (Capsicum frutescens L.), moringa (Moringaoleifera), cherry (muntingiacalabura L), pineapple (Ananascomosus L), plantain (Musa Paradisiacal L), kepok banana (Musa paradisiaca forma typica), mustard greens green (Brassica juncea L coss), chicory (Brassica pekinensia L.), Fuji Beni shogun apples, citrus fruits (Citrus sinensis L.), Papuan red fruit (Pandanusconoideus), yellow pumpkin (CucurbitamoschataDuch), Tepurang (Momordicacochinchinesis, Spreng), tomatoes (Lycopersicumesculentum L.) Cassava (ManihotesculentaCrantz), Carrots (Daucuscarota), Ziziphus jujube Miller, sweet orange (Citrus sinensis L), green melon (Cucumismelo L. Var Sky rock), and orange melon (CucumisMelo L. Var cantaloupe).

Keywords: β-carotene, Spectrophotometry UV-Vis, HPLC, TLC-Densitometry

I. INTRODUCTION

Carotenoids are tetraterpenoid compounds consisting of C-40 chains which have beneficial effects on human health. [1] Carotenoids are yellow, orange, and red lipophilic compounds found in plants, algae and microorganisms. Carotenoids can also be used as dyes in food, pharmaceutical products, cosmetic products, and can reduce the risk of various diseases such as eye diseases, degenerative diseases, and antioxidants.[2][3]

The role of carotenoids is provitaminA converted into retinol by the enzymes oxygenase and reductase. The efficiency of vitamin A and intestinal carotenoid absorption is determined by the regulation of the proteinnumber.[4] Provitamin A carotenoids become a vitamin A conversion factor that can be used in the development of foods in well-nourished populations and is used to help combat vitamin A deficiency worldwide. [5] Carotenoids are classified into two main groups of substances, namely carotene (without oxygen) and xanthophyll (containing oxygen). [6]

Carotene is a vitamin A-related substance that is produced by plants but cannot be synthesized by animals. Carotene is a photosynthetic orange-yellow pigment found in fruits and vegetables. The carotene groups are α-carotene, β-carotene, y-carotene, and β-cryptoxanthin. Whereas those belonging to the xanthopyhill group include zeaxanthin and lutein. Carotenoids and β-carotene are precursors of vitamin A and important for human health. β-carotene is a carotenoid compound. β-carotene has the structural formula $C_{40}H_{56}$ which contains 40 carbons with 15 conjugated double bonds and 2 β-ionone rings at the end of the molecule. This structure makes β-carotene highly hydrophobic and non-polar in nature. β-carotene is a red-orange pigment found in many fruits, vegetables, and green plants. [6] [7] [8]

β-carotene in the body is converted into retinol (vitamin A). Retinol is very beneficial for the retina of the eyes, skin, and mucous membranes. β-carotene also has properties such as reducing the risk of cancer, infectious diseases, antioxidants and also protecting the skin, mucous membranes from the effects of UV radiation on plants, humans, and animals. [9]

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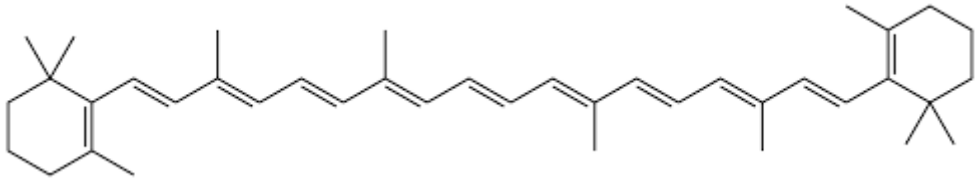

Figure1. β-carotene structure[10]

β-carotene is used as an orange-red dye in various products of the food industry. β-carotene is used as a coloring agent for tablets in the pharmaceutical industry, while in the cosmetic industry it is used as a bioactive cream ingredient that protects skin lesions from oxidation and UV exposure.[11]

β-carotene analysis was performed using UV-Vis spectrophotometry, High-Performance Liquid Chromatography (HPLC), and TLC-Densitometry methods. The spectrophotometer can measure the ultraviolet (UV) and visible spectrum. In general, the ultraviolet and visible light spectra of a substance do not have a high degree of specification. However, the spectrum is suitable for both quantitative and qualitative analysis. The spectrum is useful as additional identification. The wavelength range for measurements in ultraviolet (UV) light is 190-380 nm and visible light (Vis) is 380-780 nm. [12]

Liquid chromatography is a physical separation technique that is carried out between solid and liquid phases. The sample is separated into component constituents (analytes) by distributing (via partition, adsorption, or other interactions) between the mobile phase (fluid flowing) and stationary phase (sorbent packed in columns). For example, the flowing liquid can be an organic solvent such as hexane and the stationary phase is a porous silica particle packed in a column. HPLC is a modern form of liquid chromatography that uses a column of small particles and the mobile phase is in a pump at high pressure.[13] High-performance liquid chromatography (HPLC) is a method used to separate, detect, and measure a variety of drugs and related regulators.[14]

Densitometry is an instrumental analysis method for determining qualitative and quantitative based on the interaction of electromagnetic radiation (REM) with analyte stains in the TLC stationary phase. This method is called TLC-Densitometry. The qualitative determination of TLC-Densitometry analytes was carried out by comparing the Rf value of the analyte and the standard. The purity of the analyte was identified by comparing the densitometric spectrum of the analyte and the standard with the same Rf spot. Meanwhile, the quantitative analysis of the analyte was carried out by comparing the area of the analyte with the known concentration of standard at the stationary phase. Another way is by calculating theanalyte spot density with the standard spot density. Electromagnetic radiation interaction (REM) is the intensity of light hitting the compound molecules in the spot. The interaction of electromagnetic radiation with a spot in the TLC stationary phase determines the intensity of light absorbed, transmitted, reflected (reflected) by the analyte spot from the original REM intensity.[15] This review article discusses several plants containing β-carotene which were analyzed by several methods such as UV-Vis spectrophotometry, high-performance liquid chromatography (HPLC), and TLC-densitometry.

II. COLLECTING THE DATA

This review article was prepared using literature study techniques from sources or literature as primary data such as official books and international journals in the last 10 years (2010-2020). The main reference searches are taken from trusted online websites such as ScienceDirect, NCBI, Researchgate, Google Scholar, and other trusted and published journals.

III. ANALYSIS METHODS

Spektrophotometry UV-Vis

Table 1. β-karoten analysis by using Spektrophotometry UV-Vis

No	Plants	Solavent	λ Max	β-caroten concentration	Ref
1	Red spinach (Amaranthushyd	Petroleum eter:acetone (1:4)	451 nm	14.6 ±0.00575 mg/kg	[16]

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	ridus L.)				
2	Pare (Momordica charantia L.)	Acetone	450 nm	0.7822 mg/100 g	[17]
3	Big red chili (Capsicum annuum L. var. abreviatumfinger huth)	Hexane:acetone:ethanol (2:1:1 v/v)	452.4 nm	10.54 mg/100g	[18]
4	Curly red chili (Capsicum annuum L. var. Longumsendt)	Hexane:acetone:ethanol (2:1:1 v/v)	452.4 nm	5.57 mg/100g	[18]
5	Cayenne Pepper (Capsicum frutescensL.)	Hexane:acetone:ethanol (2:1:1 v/v)	452.4 nm	0.36 mg/100g	[18]
6	Moringa (Moringa oleifera L.)	Acetone	450 nm	3.31 mg / g	[19]
7	Cherry (Muntingia calabura L.)	Acetone: n-hexane (4:6)	473 nm	1.4831 mg/100 g	[20]
8	Pineapple (Ananas comosus L. (Merr) variety queen)	Hexane:acetone:ethanol(2:1: 1 v/v)	450 nm	11.72 µg/g	[21]
9	Pineapple (Ananas comosus L. (Merr) variety Cayenne)	Hexane:acetone:ethanol(2:1: 1 v/v)	450 nm	9.92 µg/g	[21]
10	Raja banana (Musa paradisiacal L.)	Petroleum eter:acetone (1:4)	450 nm	0.222 mg/100 g	[22]
11	Kepok banana (Musa paradisiaca Forma typica)	Petroleum eter:acetone (1:4)	450 nm	0.261 mg/100 g	[22]
12	Mustard green (Brassicajuncae L.)	Acetone	449.6 nm	99.23 mg/g	[23]
13	Chinese Cabbage (Brassica pekinensia L.)	Acetone	449.6 nm	40.91 mg / g	[23]
14	Sweet potato	Acetone	449 nm	68.48 µg/g	[24]
15	Carrot	Acetone	449 nm	74.06 µg/g	[24]

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Analysis of β-carotene content in red spinach leaves (Amaranthushybridus L.) was carried out using visible spectrophotometry. Spinach leaves tested consisted of fresh red spinach leaves and boiled red spinach leaves. Each sample was extracted using petroleum ether: acetone solvent with a ratio of 1:4, where the acetone solvent was first dissolved with 0.01% BHT. This analysis was performed qualitatively using thin-layer chromatography with petroleum ether: acetone (9:1) as a solvent. The qualitative analysis used TLC plate silica gel 60 F_{254}. The results obtained that the spinach leaf sample contained β-carotene with an Rf value of 0.56. The qualitative analysis of red spinach leaves using visible spectrophotometry at a wavelength of 451 nm and obtained β-carotene levels of 14.6 ± 0.00575 mg/kg for fresh spinach leaves, 8.50 ± 0.001703 mg/kg for boiled red spinach leaves. [16]

β-carotene in bitter melon (Momordicacharantia L.) was extracted using a soxhlet flask with 100 ml acetone as solvent. The acetone extract was re-extracted using petroleum ether as much as 3 times 25 ml, then saponified with 15% KOH solution, washed with distilled water until free alkaline, then anhydrous $NaSO_4$ was added and filtered. The use of anhydrous $NaSO_4$ aims to attract water in the extract to be free from water. The qualitative analysis of bitter melon was carried out using thin-layer chromatography with petroleum ether: benzene (9:1) as eluent. The analysis of the bitter melon fruit samples resulted yellow spots and Rf value of 0.4. The quantitative analysis was performed using UV-Vis spectrophotometry at a wavelength of 450 nm and obtained β-carotene levels of 0.7822 mg/100 g. [17]

Analysis of β-carotene levels was carried out on various types of chilies such as large red chilies (Capsicum annuum L. var. Abreviatumfingerhuth), curly red chilies (Capsicum annuum L. var. Longumsendt) and cayenne pepper. (Capsicum frutescens L). Several types of chilies are extracted using hexane: acetone: ethanol solvent to separate β-carotene compounds contained in the sample from other compounds. The results of the quantitative analysis were carried out at a wavelength of 452.4 nm and obtained carotene concentration of 10.54 mg/100 g in a sample of large red chilies (Capsicum annuum L. var. Abreviatumfingerhuth), 5.57 mg/100 g of chili red curly (Capsicum annuum L. var. Longumsendt) and 0.36 mg/100 g in cayenne pepper (Capsicum frutescens L.). The difference in β-carotene levels in each type of chili is caused by several factors such as the color and level of maturity of the chilies. The redder the chili, the higher the β-carotene content obtained. [18]

Moringaoleifra L. leaves were extracted using acetone solvent, collected, and evaporated. The acetone extract was re-extracted using ether as much as 3 times 25 ml, then evaporated and saponified with the addition of 15% KOH solution in methanol and left to stand overnight. The saponification results were extracted again with ether 3 times 25 ml and measured the pH value = 7 then the extract was evaporated until a thick extractwas obtained. The qualitative testing was performed using thin layer chromatography with ether: benzene (9:1) eluent. The analysis was carried out by comparing the samples of Moringa leaf extract and the standard. The Rf value of the sample was 0.781 cm and the plate was yellow. Quantitative analysis was performed by comparing the standard carotene with moringa leaf extract samples and measured at a wavelength of 450 nm. The average β-carotene content obtained was 3.31 mg/g. [19]

Karsen fruit (Muntingiacalabura L) was extracted using acetone: n-hexane (4:6) solvent and added with 0.1 g of magnesium carbonate then stirred using a shaker for 30 minutes 350 rpm. Samples were filtered and washed with 30 ml acetone: n-hexane (1:1) and 10 ml aquadest. The extract was collected to form two phases and transferred to a separating funnel. The organic phase obtained was put into Erlenmeyer and vacuumed at 30^{o}C to remove the remaining solvent to obtain a greenish-yellow extract. The extract obtained was put into a column eluted with acetone: n-hexane solvent with a ratio of 1:9. The β-carotene elution was put into Erlenmeyer 100 ml and the extract obtained was measured. The absorbance of the extract was measured using UV-Vis spectrophotometry at a wavelength of 473 nm and theβ-carotene level was 1.4831 mg/100 g. [20]

Analysis of β-carotene content in Ananascomosus L. (Merr) queen and cayenne varieties was carried out using the UV-Vis spectrophotometric method. Extraction of Ananascomosus L. (Merr) was carried out to separate β-carotene compounds from other compounds contained in pineapple. Ananascomosum L. (Merr) varieties queen and cayenne were extracted using a solvent hexane: acetone: ethanol (2:1:1, v/v/v) and stirred using a magnetic stirrer for 30 minutes and filtered using a Buchner funnel. The results of β-carotene analysis obtained by the UV-Vis spectrophotometric

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method of Ananascomosus L. (Merr) were 11.72 µg/g for the queen variety and 9.92 µg/g for the cayenne variety. These results indicate that the highest levels of β-carotene are found in Ananascomosum L. (Merr) queen variety. [21]

β-carotene in plantain (Musa paradisiacal L.) and kepok banana (Musa paradisiaca forma typica) was extracted with petroleum ether:acetone (ratio 1:4) containing 100 ml BHT. Extraction is carried out to separate the compounds contained in the sample from other compounds. The choice of petroleum ether as a solvent is to attract hydrophobic carotenoids compounds, while the use of acetone as a solvent is to attract hydrophilic organic compounds. The extraction process is carried out using a centrifuge. The pulp obtained is washed 3 times. The filtrate was mixed with 250 ml acetone then put into a separating funnel and 250 ml of aquadest is added and 2 ml of saturated NaCl is slowly added. The mixture was shaken and allowed to stand until it formed 2 phases. Qualitative analysis was carried out by thin-layer chromatography using a comparison of pure β-carotene and petroleum ether: benzene (9:1) used as eluent. The quantitative analysis of plantain and kapok banana fruit extracts was carried out using the UV-Vis spectrophotometric method at a wavelength of 450 nm. The qualitative results of each plantain and kapok banana showed yellow spots with Rf values of 0.96 cm and 0.97 cm, respectively. The β-carotene levels obtained from quantitative analysis were 0.222 mg/100 g for plantains and 0.261 mg/ 00 g for kapok bananas.[22]

Analysis of β-carotene in mustard greens (Brassica juncea L coss) and chicory (Brassica pekinensiaL.) was carried out using the UV-Vis spectrophotometric method. Samples of mustard greens and chicory were extracted by maceration using acetone and re-extracted with petroleum ether as much as 3 x 25 ml. The extract was saponified by adding 15% KOH and washed with distilled water until alkaline free. The petroleum ether extract obtained was dried over anhydrous Na_2SO_4. Qualitative analysis was performed using Thin Layer Chromatography (TLC) with comparison of pure β-carotene and petroleum ether: benzene (9:1) eluting fluid. The results obtained are yellow spots with an Rf value of 0.4. The quantitative analysis was performed using the UV-Vis spectrophotometric method at a wavelength of 449.6 nm. The β-carotene content obtained was 99.23 mg/100g in mustard greens and 40.91 mg/100g in chicory. The difference in the results obtained is caused by several factors such as differences in species, color, and climate.[23]

The β-carotene content in sweet potatoes and raw carrots was extracted using acetone solvent and the absorption was measured at a maximum wavelength of 449 nm. The analysis showed that carotene content obtained was 68.48 ± 4.1 µg/g in sweet potatoes and was 74.06 ± 3.8 µg/m in fresh carrots.[24]

High-performance liquid chromatography (HPLC)

Table 2. β-caroteneanalysis using HPLC

No	Plants	Mobile phase	Column	Detector	Flow rate	β-karoten concentration	Ref
1	Fuji BeniShogunate Apple	Acetonitrile:methanol (80:20 v/v)	Epslise XDB-C_{18} column (4.6 × 150 mm)	The chromatogram was monitored at 450 nm	2.0 ml/min	390.41 µg/100g	[25]
2	Orange fruit (Citrus sinensis L.)	Acetonitrile:methanol (20:80)	Zorbax SB-C18 column (250 × 4.6 mm × mm and 5 µmas particle size)	Wavelength determination at 466 nm	1 ml/minute	28.80 µg/g	[26]
3	Papua red fruit (Pandanus conoideus)	(A) acetonitrile and water(80:20 v/v) and (B)	Develosilcombi RP-5 C_{30}- column (50 × 4.6 mm, 5 µm)	Shimadzu SPD-10 AV UV-Vis detector	1 ml/min	10.8 – 118.0 nm/mg	[27]

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		acetonitrile: methanol: ethyl acetate (68:5:27 v/v/v)					
4	Summer squash (Cucurbita moschata Duch)	Metanol:t-butyl methyl ether (8:2 v/v)	C_{30} (YMC carotenoid S-3, 4.6 mm × 250 mm reversed phase)	UV/Visible photodiode array detector	0.8 mL/min	244.22 µg/g	[28]
5	Tepurang (Momordi cacochinc hinesis, Spreng)	Acetonitrile (6:4) (eluent A) andMeOH (eluent B)	Inertsil ODS-3 (250 × 4.6 mm ID, 5 µm) column	Photodiode array detector (PDA)	1.5 ml/min	0.257-0.379 mg/g	[29]
6	Tomat (Lycopersicumesculentum L.)	Acetonitrile:dicloromethane (75:25v/v)	XDB-C18 column (5µm; 4.6 mm×150 mm)	UV-Vis spectrofotometer detektor (SPD-GAV)	1.5 ml/menit	27.94±0.06 mg/kg	[30]
6	Cassava (Manihote sculentaCrantz)	80% methanol dan 20% methyl tert-butyl ether	C_{30} YMC®carotenoid S-3 (4.6 × 250 mm; particle size 5µm)	UV/Visible photodiode array detector	0.8 ml/min	1-37-7.66 µg/g	[31]
7	Carrot (Daucuscarota)	980 ml methanol dan 20 ml trichloromethane	C18 (5 µm, 250,0 × 4.6 mm)	Two-wave line detector ultraviolet at wavelength2489 nm	0.9 mL/min	41.06 ± 0.02 µg/g	[32]
8	Ziziphus jujube Miller	Methanol:acetoneitril:tetrahydrofuran (73:20:7 v/v)	YMC-Pack ODS-AM column (250 mm × 4,.60 mm, 5 µm)	Adiode array detector (SPD-M10Avp)	1.0 ml/min	35.0 µg/100g	[33]

Determination of β-carotene content in apples by comparing the β-carotene content in apple peel and pulp. Meat and apple samples were extracted with acetone: petroleum ether (20:80, v: v) in Erlenmeyer at 25°C and re-extracted by ultrasonic for 20 minutes. The extract was filtered over a Buchner funnel, filtered, and washed with acetone solvent until it was colorless. The petroleum ether phase was separated by adding 10% NaCl 3-4 times to the volumetric flask and in the rotary evaporator. Determination of β-carotene was carried out using HPLC under acetonitrile: methanol (80:20, v/v) mobile phase, Eplise XDB-C18 column (4.6 × 150 mm), flow rate 2.0 ml/minute with column temperature 40°C. The yield of β-carotene content in apple peel was higher than that of apple flesh, namely 390.41 µg/100g and 326.75 µg/100g. [25]

Determination of β-carotene in the citrus fruit (Citrus sinensis L.) peel was carried out using RP-HPLC. The flavedo and albedo orange peel samples were extracted by the sokletation method using 3 kinds of solvents such as ethanol, benzene, and petroleum ether. Of the three types of solvents, only petroleum ether (non-polar) solvent is very good for extracting carotenoids. Quantitative

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analysis was performed using the HPLC Agilent 1100 system, SB-C18 zorbax column (250 × 4.6 mm × mm and 5 µm particle size, with acetonitrile: methanol (20:80) eluent, flow capacity 1 ml/minute, column temperature 30℃ with a wavelength of 466 nm. The β-carotene levels obtained were 28.80 µg/g in flavedo oranges and 28.00 µg/g in albedo oranges.[26]

Analysis of β-carotene in Papua red fruit ((Pandanusconoideus) was carried out using high-performance liquid chromatography or HPLC with mobile phase conditions (A) acetonitrile and water (80:20, v/v) and (B) acetonitrile: methanol: ethyl acetate (68:5:27, v/v/v), Develosilcombi RP-5 C30- column (50 × 4.6 mm, 5 µm), Shimadzu SPD-10 AV UV-Vis detector with a flow rate of 1 ml/min and obtained β-carotene content ranges from 10.8 to 118.0 nm/mg. [27]

Analysis of β-carotene in pumpkin (Cucurbitamoschatav Duch) was carried out using high-performance liquid chromatography (HPLC) with the mobile phase conditions of Methanol: t-butyl methyl ether (8: 2, v/v), C30 (YMC carotenoid S-3, 4.6 mm × 250 mm reversed-phase), UV/Visible array detector, flow rate 0.8 ml/min. The analysis was carried out for 60 minutes and the total β-carotene was 244.22 µg/g. [28]

Analysis of β-carotene in tepurang (Momordicacochinchinesis, Spreng) was performed using high-performance liquid chromatography (HPLC) using several mobile phase components such as Acetonitrile (6: 4) (eluent A), MeOH (eluent B), Inertsil column ODS-3 (250 × 4.6 mm ID, 5 µm), Photodiode array detector (PDA) with a flow rate of 1.5 ml/min and injection volume of 20µl with a maximum wavelength at 472 nm. Analysis results obtained β-carotene content of 0.257-0.379 mg/g. [29]

Tomatoes (Lycopersicumesculentum L.) were extracted by sokletation with hexane for 12 hours. The extract was analyzed using HPLC with acetonitrile: dichloromethane (75:25, v/v) mobile phase conditions, XDB-C18 column (5µm; 4.6 mm × 150 mm), UV-Vis spectrophotometer detector (SPD-GAV), flow rate 1.5 ml/min ata wavelength of 470 nm. The β-carotene content obtained in tomato peels was 27.94 ± 0.06 mg/kg. [30]

β-carotene in Cassava (ManihotesculentaCrantz) was analyzed using high-performance liquid chromatography (HPLC) with the mobile phase used was 80% methanol and 20% methyl tert-butyl ether, C_{30} YMC® carotenoid S-3 (4.6 × 250 mm; particle size 5µm), UV/Visible photodiode array detector, flow rate 0.8 ml/min. 25 µL of the extract was injected at a temperature of 30°C with an analysis time of 60 minutes and the total β-carotene was obtained around 1.37-7.66 µg/g.[31]

β-carotene analysis on Carrots (Daucuscarota) was performed using high-performance liquid chromatography or HPLC with 980 ml methanol and 20 ml trichloromethane, C_{18} (5 µm, 250.0 × 4.6 mm) mobile phase conditions, two-wave ultraviolet channel detector. The variable wavelength of 2489 with a flow rate of 0.9 ml/min. The β-carotene content obtained was 41.06 ± 0.02 µg/g.[32]

β-carotene in Ziziphus jujube Miller was analyzed by high-performance liquid chromatography (HPLC) with the mobile phase methanol: acetonitrile: tetrahydrofuran (73:20:7), YMC-Pack ODS-AM column (250 mm × 4.60 mm, 5 µm), Adiode array detector (SPD-M10Avp) with a flow rate of 1.0 ml/min and injection volume of 20 µl at a maximum wavelength of 450 nm. The β-carotene content obtained was 35.0 µg/100g. [33]

TLC-Densitometry

Table 3. β-carotene analysis using TLC-Densitometry

No	Plants	Mobile phase	Rf Value	β-carotene concentration	Ref
1	Sweet orange (Citrus sinensisL.)	Chloroform:ethyl acetate (7:3)	0.20	30.5610 ppm	[34]
2	Green melon (CucumismeloL. var. Sky Rock)	n-hexane:ethyl acetate (8:2)	0.725	0.22 mg/100 g	[35]

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3	Orange melon (CucumismeloL. var. Cantaloupe)	n-hexane:ethyl acetate (8:2)	0.73	2.05 mg/100 g	[35]

β-carotene levels of sweet orange peel(Citrus sinensis L) were carried out using TLC-Densitometry. Sweet orange (Citrus sinensis L.) is one of the organic wastes having many benefits. Syahfitri et al. (2020) validated the method and determined the carotene levels in extracts of sweet orange peel samples without washing and by washing with aquadest. The extraction process was carried out by abusing it in the stationary phase of silica gel and in the mobile phase of chloroform: ethyl acetate (7:3). The Rf value obtained is 0.20. The β-carotene content obtained from sweet orange peel from n-hexane extract was 30.1938 ppm without washing and 30.5610 ppm by washing. Method validation using TLC-Densitometry obtained linearity of 0.999, precision 0.0777%, LOD 0.30951µg/ ml and LOQ 1.0317µg/ml. [34]

β-carotene analysis on samples of green melon and orange melon was carried out using TLC-Densitometry which was extracted using chloroform as a solvent. The extraction process was carried out 4 times with 20 ml of chloroform. The chloroform phase was carried out by a rotary evaporator and produced a concentrated melon extract. The quantitative-carotene testing was carried out by comparing the on samples with β-carotenestandard and the mobile phaseused was n-hexane: ethyl acetate (8: 2). Each 20 µl sample solution was spotted on the TLC plate then eluted using the mobile phase. The β-carotenepeak area was measured with a density at a maximum wavelength of 457 nm. The Rf value obtained was 0.725 forthe green melon sample and 0.73 for the orang melon. In the results of quantitative analysis of each melon, theβ-carotene content was 0.22 mg/100 g in green melon, 98.76% accuracy test, 1.50% precision test, and 2.05 β-carotene content mg/100 g on orange melon, accuracy test 98.81%, precision test 1.14%. [35]

IV. CONCLUSION

β-carotene is a carotenoid belonging to a tetraterpenoid compound and has 2 β-ionone rings. β-carotene is a provitamin A compound obtained from various plants and functions as an antidote to free radicals. β-carotene is found in many-colored vegetables and fruits. β-carotene acts as an antioxidant. The higher the β-carotene level, the better it is to ward off free radicals in the body. This review discusses the β-carotene content of several plants analyzed using SpectrophotometryUV-Vis, High-Performance Liquid Chromatography (HPLC), and TLC-Densitometry.

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