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How to Extract and Examine β-Carotene in Carrot (*Daucus carota*)

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Abstract-- **Carrots (Daucus carota), is the main sources of beta carotene besides tomatoes and palm oil. At present, β-carotene has been used extensively in food, industry, and medicine, so that beta carotene is of high economic value. The β-carotene in carrot can be obtained by chemical preparation. The researchers, usually to have β-carotene mainly done with organic solvents. It is very rarely done without a chemical solvent. This study aims to extract β-carotene without chemical solvent and also to determine β- carotene using UHPLC and GC-MS. Approximately 300 gram carrots, cleaned, cut into small pieces and mashed in a blender, given aqua 20 mL. separate the filtrate from that matrix. To the filtrate was then, added some chemical salt, centrifuged 5000 x g for 30 minutes to have pellet. The pellets are then removed, evaporated with the vacuum to dry at a temperature of 40o C for 48 hours. β-carotene pellets are measured with UV-vis, HPLC and GC-MS. Using column UHPLC and the mobile phase was chloroform-methanol (95: 5) with a flow rate of 1 ml /minute at a UV detector wavelength of 460 nm. The retention time examination of β-carotene and standard (sigma beta carotene) using HPLC and GC-MS was 3.75 and 9,80 minutes respectively. GC-MS examination showed ions and molecules of carotenoid. Research showed that this method can be used to be developed for the withdrawal of β-carotene in carrots and others, without any need for organic solvents.**

Keywords*: *β-carotene, Carrot, HPLC, GC-MS, Carotenoid.

I. INTRODUCTION

β-carotene is the most abundant provitamin A carotenoid in human diet. It exerts a number of beneficial functions in mammals, including humans, owing to its ability to generate vitamin A as well as to emerging crucial signaling functions of its metabolites. Carotenoids are C40 tetraterpenoid pigments that are found in plants, fungi and bacteria. In plants, these compounds accumulate in the plastids giving the characteristic bright yellow, red and orange color to many fruits and vegetables .They function as structural and functional accessories of the photosynthetic apparatus, specifically to serve as light-harvesting pigments and protect against photooxidative stress. Fruits and vegetable containing vitamin C, vitamin E, tocoferol, carotenoids have been suggested as a natural sources of natural antioxidant. Antioxidant function are associated with decresed DNA damage, diminished lipid peroxidation, maintained immune function and inhibit malignat transformation [1]. Antioxidants have recently been discussed not only among scientists but also in societies that are increasingly aware of their benefits.1 Besides being known as a powerful weapon to ward off a variety of diseases, antioxidants are also believed to be able to keep young [2]. Along with the times, causing changes in lifestyle people who tend to live an unhealthy lifestyle such as smoking, drinking liquor, consuming junk food and being exposed to excessive ultraviolet light. As a result, there are so many free radicals is obtained, can lead to harm to the body. These free radical compounds can be formed as a result of the chemical processes, that occur in the body, such as oxidation, cataracts, metabolism, and inflammation.

In certain the number of free radicals is needed as part of the body's defenses. But in reality, free radicals often form beyond their needs, so that their role changes wild or destructive (damaging) which can cause various diseases such as hardening of the arteries, coronary heart disease, stroke, cancer and premature aging

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To protect the body from attacks by free radicals, the body needs antioxidants, including consisting of β-carotene, vitamin E, vitamin C and selenium [3]. The β-carotene is one of the antioxidants that can prevent from disease. This antioxidant compound is able to neutralize free radicals in the body which is a trigger source for various diseases, like as, metabolic degenerative, as well as cancer diseases. Naturally, β-carotene is abundant in fruits such as pumpkin, palm oils, red fruit, watermelon, mangoes, tomatoes, melons, and carrot. One of the main sources of β-carotene is carrots, as the high-value compound. In recent years carotenoids have represented a good alternative for the pharmaceutical and food industries and especially for human health, they prevent different diseases, such as cancer, macular degradation, and cataract.

Carrots are classified as a group of vegetables, contain carotenoid groups. The level of β-carotene that found in carrots is also varying [3]. Differences in the content of β-carotene in carrots remembering that β-carotene is a use of the antioxidant compound, Therefore, to pursuit the content of β-carotene and to develop the methods of extraction of beta carotene is running by the researcher and needed right know to the future [4], [5]. In this study, the isolation of β-carotene was carried out using ultra high-performance liquid chromatography (UHPLC) and Gas Chromatograph (GC-MS). This study also to got the right and careful method in determining β-carotene, and let to know the possibility of this method to determine β-carotene in carrots.

II. METHODS

A. *Material*

Carrots, which are obtained from the Super market in Palembang, β-carotene ingredients, chloroform (Sigma), tetrahidrofuran (THF), methanol (Sigma) , aquabidest (Sigma), acetonitrile Sigma grade HPLC. Standard Beta carotene Sigma Aldrich

High performance liquid chromatography using (Ultimate U-HPLC Thermo 3000 series), GC-MS series, Ultraviolet-visible spectrophotometer (Analytic Jena specord 200), Analytical scales (Sartorius Dragon), Micro scales (Sartorius Dragon), Blender (Philips), Rotavapor (Buchi V -800), Separating funnels, Glassware, Whatmann Filter paper number.40.

B. *Methods*

1. *Determination of optimum Ultra-HPLC conditions and GC-MS*

a. Determination of the maximum absorption wave length of β-carotene in UV-vis detector.

A total of 20 mg β-carotene of isolated, is inserted into a 50-mL measuring flask dissolved and diluted with chloroform until the mark. Then piped 2.5 mL into 10-mL pumpkin measuring, diluted with chloroform until the mark. Then the spectrum was made using a UV-Vis spectrophotometer at a wavelength of 420-500 nm [1].

b. Selection of the mobile phase and flow rate.

A total of 20 mg β-carotene of isolated is put into a 50-mL measuring flask dissolved and diluted with chloroform to the mark line. Then piped 2.5 mL, was put into a pumpkin measuring 10 mL diluted with chloroform until the mark. Inject 20 µl into the HPLC tool using the mobile phase of methanol-chloroform (95: 5); methanoltetrahydrofuran–water (67: 27: 6); chloroform-tetrahidrofuran-water (67: 27: 6); acetonitrile-chloroform (92: 8); chloroform -methanol (95: 5) and chloroform-tetrahidrofuran-methanol (70: 25: 5) with a flow rate of 0.5 mL / minute and 1 mL / minute. The mobile phase and the selected flow rate provide the best separation with a not too long retention time.

2. *Identification of β-carotene in carrots.*

a. Extracts/Isolates:

Fresh carrots that have been cut and mashed, weighed 300 g (carrots), 300 g of blended using 15 mL water solvents, then filtered. The bulk is seperated. The filtrate was then rinsed with the calcium salts, centrifuged 3000-5000 rpm 15 minutes. The residue as a pellet is seperated from the filtrate. The pellet was then removed from the filtrate. Rinse the pellet to removed the calsium salt. The pellet was then drying with nitrogen vacuum , or rotary evaporator at temperature 40° C. Then, Stored the pellet in cold temperatures.

b. Standard solution Standard solutions:

A total of 10 mg β-carotene is put into a 50-mL measuring flask dissolved and diluted with chloroform until the mark. Then pipette 2.5 mL was put into a 10-mL measuring flask diluted with chloroform to the mark line (Standard beta carotene).[7]

c. How to identify:

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

A number of test solutions were injected as much as 50µl into the GC-MS tool, set the GC MS 310 ^{0}C, Start set from 60^{0}C, injector temperature 205°C, then

compared the retention time with the standard retention time of reference.

3. *Quantitative analysis by high performance liquid chromatography*

a. System suitability test.

System suitability tests are conducted to find out whether tools, methods and conditions form a single analysis system. 20 µl of the standard β-carotene solution is injected 5 times into the HPLC tool, then the peak area is measured with the optimum HPLC condition, then the relative standard deviation values are calculated.

b. Determination of levels of β-carotene in HPC carrot extract.

The test solution was sonicated for 10 minutes. Each injected 20 µl into the HPLC tool and measured its peak area with optimum HPLC conditions. Levels of β-carotene isolates can be calculated then.

III. RESULTS

The standard β-carotene identification doing by [6]. can be seen in Figures, 1, 2 and 3. The retention time of beta carotene showed 1,309 minutes and 2, 86 minutes respectively with UV vis recorder at 450 nm wave length. It means that any difference of the retention time between low and high of velocity rate of the mobile fase. The identification of raw material of beta carotene in carrots of this study that obtained from the measurement testing using HPLC and GC MS is depicted in figured, (Figure 4, 5 and 6). The standard chromatogram of β-carotene with a retention time of 1,392 minutes show in Figure 1, with flow rate 1 ml /minute. In Figure 2 depicted the standard β-carotene chromatogram with a retention time of 2,86 minutes with flow rate of mobile phase 0.5 mL / minute. Figure 3 is the standard material for UV-vis beta carotene chromatograph [6]. Figure 4 is a UV –Vis beta carotene of carrots. Figure 5 is a chromatogram of beta carotene showed retention time 1,903 minute produced by U-HPLC.

Analytical conditions: GC/MS (EI) was carried out on a Perkin Elmer (AutoSystem XL) gas chromatograph coupled to the MS detector (Perkin Elmer TurboMass) in the electron impact mode (70 eV). The column was PE-5HT (30 m × 0.25 mm × 0.1 µm); carrier gas: helium (1 mL/min); the inlet mode was split: 50:1; and the injector temperature was 250◦C. The initial column temperature was 60◦C, and after 1 min the temperature was raised to 310◦C with 4◦C per min. Thereafter, the conditions were held for 6 min. The mass spectrometer measurement was scanned from 40 to 600 m/z. According to the literature, the GC/MS method was successfully applied in many cases to analyze products with lower molecular weight than retinol). The major metathesis products were hexadecanoic acid, methyl ester, Pentadecanoic acid, methyl ester, Hexadecanoic acid, methyl ester as determined by GC/MS.

Figure 1. Chromatograph of standard beta carotene produced: RT 1,309 minutes inflow rate 1 mL/min with phase mobile chloroform: THF:Methanol=70:20:5).

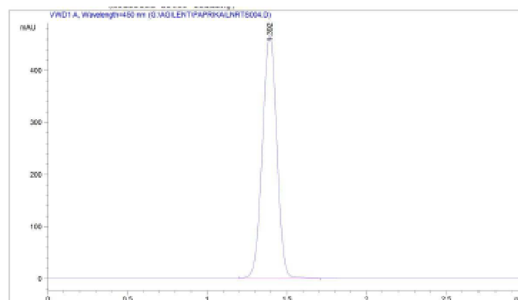


Figure 2. Chromatograph of standard and beta carotene produced: Retention time RT 2,86 minute flow rate 0,5 mL/min with phase mobile Chloroform: THF: Methanol=70:20:5)

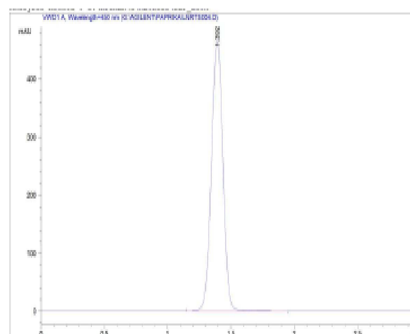


Figure 3. Chromatograph of beta carotene UV-vis In wavelength 450 nm.

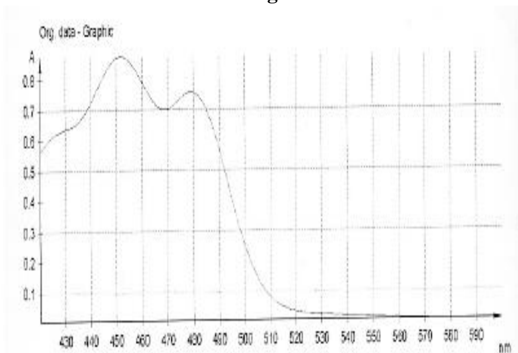
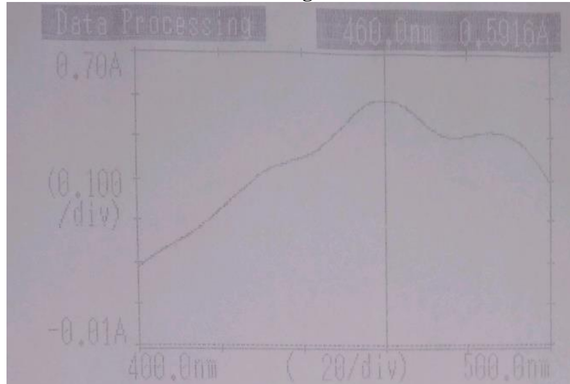


Figure 4. Chromatographspectra of beta carotenesample tested on UV-visat 460 nm wavelength in chloroform solvent .



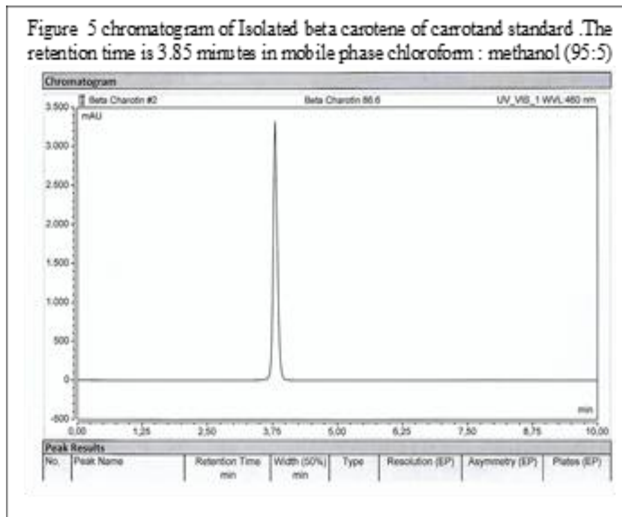
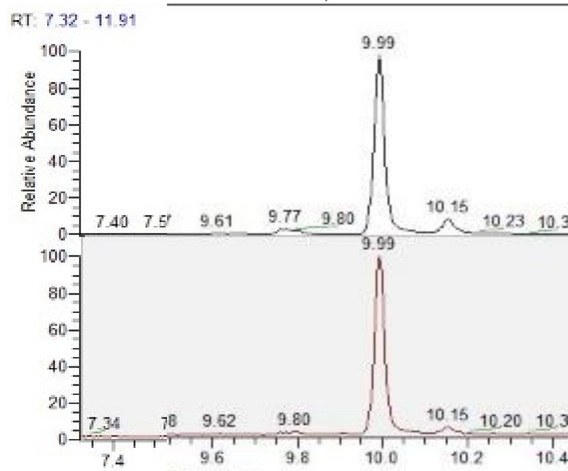


Figure 5 chromatogram of Isolated beta carotene of carrotand standard .The retention time is 3.85 minutes in mobile phase chloroform : methanol (95:5)

Figure 6. Cromatograph of beta caroten in carrots using GC-MS to produced Hexadecanoic acid, methyl ester; Pentadecanoic acid, methyl ester and Hexadecanoic acid, methyl ester , with retention time in 9,9 minutes.



IV. DISCUSSION

Beta carotene is a groups of carotenoids, isoprenoid, hydrophobic, so it is called soluble in organic solvents. The chemical properties of beta carotene are mainly due to the multiple containing double bon [1], [5]. Organic solvents that often used to extracted beta carotene are mainly chloroform, petroleum ether, dichloromethane, and hexane. The isolation of beta carotene from carrots with organic solvents has been carried out, one of them is the Fikselova study [8]. investigating the relationship of solvent combinations and temperature of loss. In this study the isolation of beta carotene from carrots was used without organic solvents, the principle of go to green. The double bond in beta carotene can settle with calcium salts so this principle is used in research to get beta carotene from carrot juice. The separation of precipitate is used by by centrifuge. The pellets obtained are as orange beta-carotene. Mostly in references, doing to extract of beta carotene almost with organic solvents. Reversely, in this study, did not used of organic solvents, but we can sure that with the principle of precipitation according to the nature and principles of the reaction of calcium salt with in beta carotene. The subsequent beta carotene as standard beta carotene (Sigma Aldrich) is treated by using Ultra HPLC. The result is compared any difference from the standard beta carotene and beta carotene. It is not fully complete to understand because of the limitation of material and time.

From the results of the optimum conditions, it was shown that β-carotene provided maximum absorption at a wavelength of 460 nm with chloroform solvents (Figure 4). The wavelength shift from 450-460 is possible influenced by the solvent factors used that are different when measuring than the reference [6], [8]. Based on the experimental results, the best comparison is chloroform-methanol (95: 5) with a flow rate of 1 mL / minute, according with retention times obtained faster and the resolution is good. The mobile phase commonly used according to conditions is chloroform, alcohol, hexane, methanol, acetonitrile, test grade HPLC [9], [10]. Furthermore, it can be given a supporting solution such as THF [11]. The results of wavelength determination can be seen in Figure 5; The results of the selection of the mobile phase and flow rate are shown in Figure 5. Although several studies have shown different beta carotene (retention time) results from this study, the mobile phases performed are different, different flow rates and different HPLC devices [6], [12]. So that each RT measurement is an optimal condition carried out by researchers in producing a chromatogram of beta carotene to produce

a relatively fast RT and clear peak images. [1], [13]. The content of carotenoids in plants is influenced by several factors, these may be genetic, environmental, or strategies used to manage the crop during its growth. The latter can result in an increase in the concentration of carotenoids [1], [6], [8].

In this study, the retention time of beta carotene was 1,903 minutes using a flow rate of 1 mL / minute with the mobile phase of chloroform: methanol (95: 5)in certainly condition. In the determination method developed a β-carotene peak was detected between only 2-3 minutes. The sample was further observed at various wavelengths thus detect impurities with absorbance maximum different from 440 nm, no considerable disturbances in the β-carotene region were observed. Peaks indicating other substance (e.g. impurities) was eluted and thus detected earlier in the chromatogram (Figure 4). Using a comparator flow rate of 1 ml / minute standard β-carotene can be separated at retention time 1,392 minutes. Improvement of peak chromatogram can be continued by adding a combination of mobile phase solution with THF combination to obtain a sharper peak. From the results of the linearity test (Figure 1), the value for the standard solution of β-carotene is 0.9971 (not showed). The r value shows the ideal value because it is close to 1, so the correlation coefficient between the solution concentration and the peak area detected by HPLC is good and can be used for research. The research of [6]. and [8]. has provided inspiration in this study, especially in the use of raw data on beta carotene. Further research is being carried out for the preparation of formulations and preparation of standard beta carotene.

V. CONCLUSION

The used of method isolation, has produced beta carotene. The proof was carried out by ultra high performance liquid chromatography (UHPLC) and GC MS compare to standard beta carotene. Suggest to produced beta carotene in general.

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