Open Journal of Applied Sciences, 2024, 14, 2996-3003
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Isolation and Identification of Beta-Carotene from Carrots

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How to cite this paper: Smajlagić, A., Srabović, M., Huremović, M., Pehlić, E., Ademović, Z., Kozarević, E.-Č., Ibišević, M. and Siočić, J. (2024) Isolation and Identification of Beta-Carotene from Carrots. *Open Journal of Applied Sciences*, **14**, 2996-3003.
https://doi.org/10.4236/ojapps.2024.1411196

Received: October 2, 2024
Accepted: November 2, 2024
Published: November 5, 2024

Abstract

Carotenoids are a group of natural pigments that are isolated from plants and are known for their great importance in maintaining human health. Beta-carotene is an organic compound, from yellow to red-orange colour, which is found in many plants, fruits and vegetables. All carotenoids, especially beta-carotene, which is the subject of this research, are used in the pharmaceutical, food and cosmetic industries. It is also important in medicine as it prevents the occurrence of many diseases. Beta-carotene is a precursor of vitamin A and is used in various research due to its strong antioxidant effect. The highest amount of beta-carotene is found in carrots, followed by apricots, tomatoes, asparagus, broccoli, pumpkins, plums, etc. This plant pigment was isolated from carrots using a reflux extraction method using the organic solvents methanol and methylene chloride. The isolated carotenoid was confirmed by UV, TLC, FTIR, and HPLC methods. The absorption maxima of the UV spectrum of the isolated compound (β-carotene) range from 200 to 280 nm and 400 to 500 nm. The identification of beta-carotene was done by analysis using the FTIR method, where functional groups that are present indicate the isolated compound. HPLC method is rapid, effective and sensitive for carotenoid analysis. Analysis of the HPLC method confirms the isolated compound with an absorption maximum of 448 nm, which was compared with the standard.

Keywords

Carotenoids, Isolation, Beta-Carotene, UV, TLC, FTIR, HPLC

1. Introduction

Carotenoids are the most widespread pigments in nature [1] that have been interesting for research in many scientific areas for centuries and are still intensively researched. These are the compounds that are present in yellow, orange and red plants, unsaturated hydrocarbons that are derived from isoprene units and consist entirely of carbon and hydrogen (carotenes) or carbon, hydrogen and oxygen (xanthophylls). Fruits and vegetables that contain vitamin C, vitamin E, tocopherol, and carotenoids are suggested as natural sources of antioxidants [2]. Antioxidants are molecules that can prevent various diseases and are believed to slow down aging process. Carrot (*Daucus carota* L.) is a biennial herb that belongs to the Apiaceae family. Carrot root contains 88% water, 1% protein, 7% carbohydrates, 0.2% fat and 3% fibre, along with vitamins (antioxidants) and minerals [3]. Tetraterpenoid pigments (C_{40}) carotenoids appear in several isomeric forms, of which α and β-carotene are two primary forms of carotene. Among carotenoids, β-carotene is the substance most often found in food supplements [4]. The β-carotene molecule found in carrots, pumpkins, apricots, plums, tomatoes, asparagus and other plants is a precursor of vitamin A. In Latin, the word "carotene" means carrot and that is the origin of the name carotene itself. The biggest source of beta-carotene is precisely from carrots. In the human body, β-carotene is broken down by β-carotene dioxygenase in the mucosa of the small intestine into two molecules of retinyl, which is later reduced to vitamin A (retinol) [5]. Beta-carotene is considered the main carotenoid that also has antioxidant properties [6] [7] and therefore reduces the risk of many diseases [8] [9] (cancer, cardiovascular diseases and old age-related diseases). The structure of beta-carotene was derived in 1930 [10] and is shown in Figure 1. The molecular formula of beta-carotene is $C_{40}H_{56}$ and the molar mass is 536.9 g/mol. The carotenoid molecule (β-carotene) is present in the form of red to red-brown crystals with a melting point of 176˚C - 184˚C [11]. Beta-carotene is practically insoluble in water and hardly soluble even in methanol, acetonitrile (ACN) or dimethylsulfoxide (DMSO). Solubility was improved in hexane, chloroform, methylene chloride and tetrahydrofuran (THF) as the best solvent tested [12]-[14].

The IUPAC name of beta-carotene is 1,3,3-Trimethyl-2-[3,7,12,16-tetrametil-18-(2,6,6-trimethylciclohex1en1il)-octadeca-1,3,5,7,9,11,13,15,17-nonaen-1-il]ciclohex-1-ene.

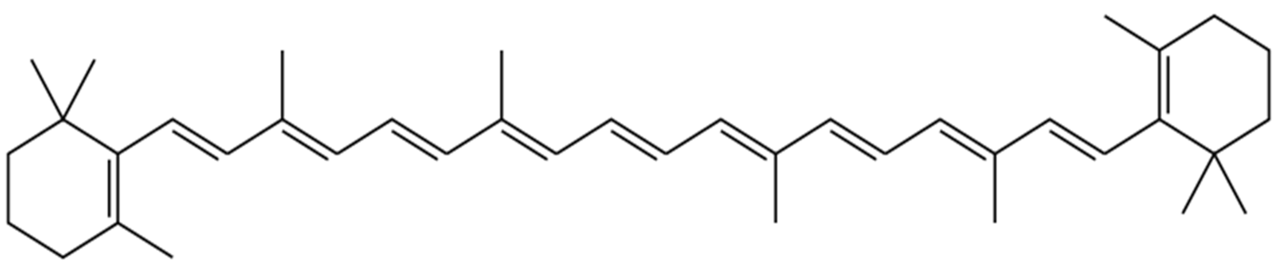

Figure 1. Structure of β-carotene.

Beta-carotene is very important primarily because of its action, the potential of provitamin A and as a compound used as a dye. The purpose of the research is the isolation of the selected carotenoid utilizing the extraction method, which has shown good results. Therefore, the aim of the research is to isolate and identify beta-carotene.

2. Material and Methods

2.1. Material

- Carrot
- Methylene chloride, CH_2Cl_2
- Methanol, Sigma Aldrich
- Distilled water
- Anhydrous sodium sulphate, Na_2SO_4
- Hexane, Sigma Aldrich
- Acetone, Sigma Aldrich
- Isopropyl alcohol, Sigma Aldrich

Add 10 grams of carrots, then reflux with 12.5 ml of methanol and 25 ml of dichloromethane under reflux in a round-bottomed flask over a water bath at a temperature of 50˚C - 55˚C for five minutes with occasional shaking. Polar organic substances are extracted in methanol, and non-polar carotenoids in dichloromethane. The cooled mixture is filtered through a Büchner funnel in a vacuum flask. The procedure is repeated with the remaining carrot sample and 25 ml of methylene chloride. Both filtrates were combined and gently shaken three times in a separatory funnel with 25 ml of water to remove the remaining polar substances from the dichloromethane. The lower, orange dichloromethane layer containing the dissolved carotenoids is dropped into an Erlenmeyer flask and dried in anhydrous Na_2SO_4. The upper aqueous layer, which contains polar substances, is discarded.

2.2. Methods

The identification of beta-carotene was done by the following methods:

FTIR analysis:

- The analysis of isolated beta-carotene was performed on a Thermo Scientific Nicolet I S10 spectrometer in the ratio 4000 to 450 cm^{-1}, with a resolution of 2 cm^{-1}.

UV/VIS spectroscopy:

- The isolated sample was analysed with a UV/VIS spectrometer in the wavelength range of 200 - 500 nm.
- TLC chromatography: Mobile phase is hexane:acetone (9:1) and stationary phase of silica gel plates. The analysis was done on the Camag UV cabinet with the lamp at 254 nm.
- HPLC analysis of isolated molecule was on the HPLC LC-40, column Luna NH_2, 5 μm, 250 × 4.6 mm, flow rate 0.5 mL/min.

3. Discussion

Beta-carotene, an isoprenoid, is hydrophobic in nature and as such is soluble in organic solvents. Usually, non-polar solvents, such as hexane, petroleum ether or tetrahydrofuran (THF), are an excellent choice for extraction of non-polar carotenes or esterified xanthophylls, whereas polar solvents such as acetone, ethanol, and ethyl acetate are more appropriate for extraction of polar carotenoids [15]. In this research, organic solvents such as methanol, methylene chloride, hexane, acetone and isopropanol were used. During the extraction of beta-carotene from carrots, methanol as a solvent in which beta-carotene is not soluble separates polar organic substances. Methylene chloride as the second solvent in this research extracts non-polar organic substances. After solvent extraction, the obtained carotenoid was analysed. Figure 2 shows the FTIR spectra of standard and isolated *β*-carotene, obtained in the range from 450 to 4000 cm^{-1}. The spectrum of the isolated carotenoid shows the presence of CH_3 stretching at 2921.12 cm^{-1} and 2850.72 cm^{-1}. Wavelengths at 755.37 cm^{-1} and 696.46 cm^{-1} of fingerprints indicate the presence of an aromatic alkene. The region at the wavelength of 1452.37 cm^{-1} indicates vibrations of antisymmetric deformation of CH_3 and CH_2 groups [16]. The spectrum of the isolated compound has a characteristic peak at 1092.63 cm^{-1} corresponding to C-O stretching vibrations (1). Comparing with the spectrum of the *β*-carotene standard, we can say that the isolation of the chosen carotenoid has been confirmed (2).

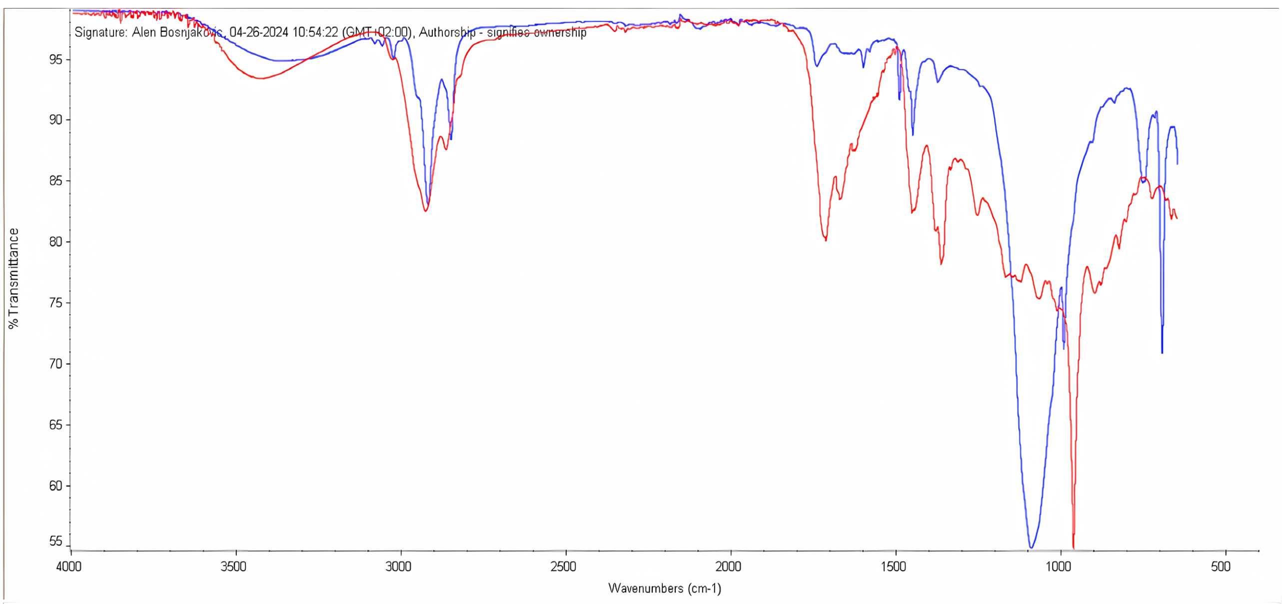

Figure 2. Spectrum of isolated (2) (blue) and standard *β*-carotene (1) (red).

Analysis of the UV/Vis spectrum of isolated beta-carotene is shown in Figure 3. The spectrum shows the wavelengths at Λmax = 247.97 nm and Λmax = 450 nm and comparing it with the spectrum of the standard carotenoid (1) confirms that the isolated molecule has been identified (2).

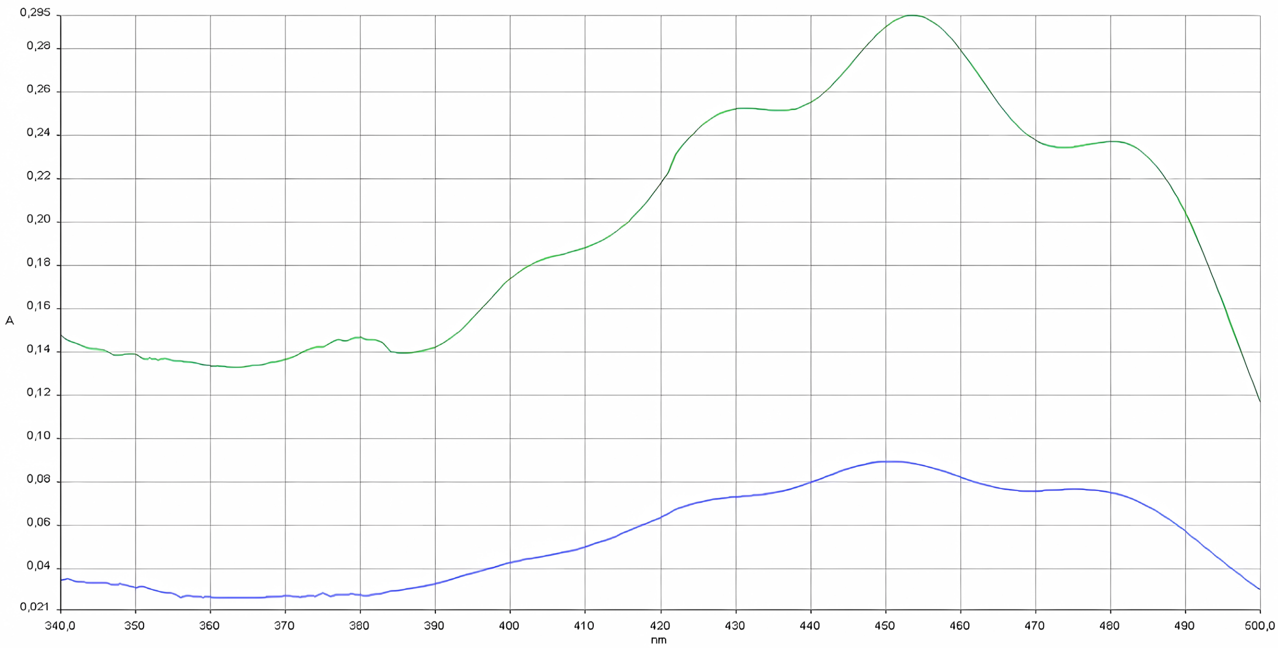

Figure 3. UV/VIS spectrum of isolated (2) (blue) and standard β-carotene (1) (green).

Figure 4 shows the TLC chromatography of the isolated beta-carotene compound compared to the beta-carotene standard. The Rf values of the isolated carotenoid are 0.90 compared to the standard, which is 0.91. With this analysis, we can see that beta-carotene was successfully isolated with the appropriate mobile phase hexane:acetone (9:1) and it is confirmed by the literature data of other studies [17].

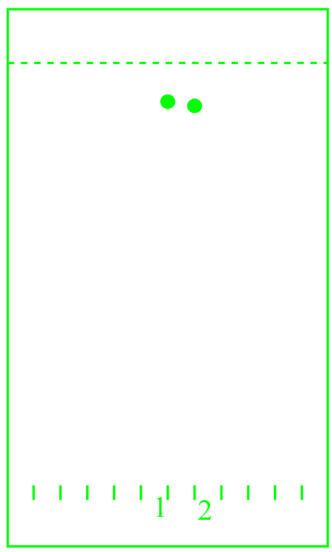

Figure 4. TLC chromatography of standards (1) and isolated β-carotene (2).

By HPLC analysis, the isolated beta-carotene was confirmed with an absorption maximum of 448 nm compared to the β-carotene standard of 450 nm (**Figure 5**).

Small impurities are present in the sample compared to the standard. In this study, the retention time of β–carotene was 3.8 min with the mobile phase of hexane:isopropanol (9:1).

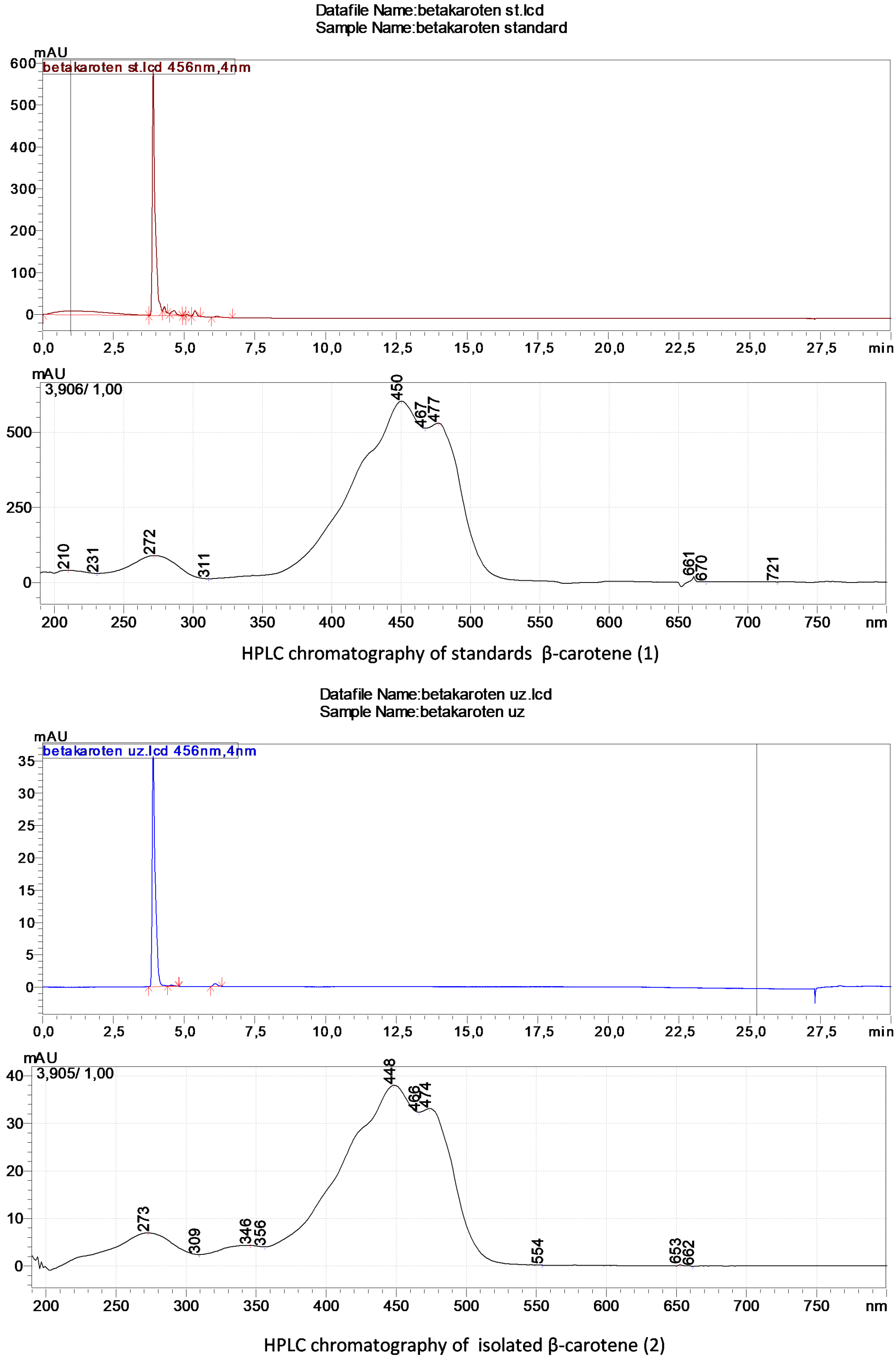

Figure 5. HPLC chromatography of standards (1) and isolated β-carotene (2).

4. Conclusions

Carotenoids are classified as a group of micronutrients that have significant antioxidant, anticancer and anti-inflammatory properties. Some of these carotenoids are converted by the body into vitamin A, which is necessary for eyesight, growth and proper development. This is precisely why the beta-carotene molecule is important because it acts as provitamin A.

The growing demand for beta-carotene, *i.e.* natural beta-carotene, has led to research and consideration of extraction methods from natural sources. Beta-carotene is widely used in the food, pharmaceutical and cosmetic industries. In this research, a selected organic compound (carotenoid) was identified from carrots. The selected reflux method for beta-carotene extraction showed good results. The results of FTIR, UV and TLC analysis indicate a successfully isolated molecule. HPLC analysis also confirms the isolated carotenoid, which is compared with standard beta-carotene.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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