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Short Communication

Beta Carotene Determination in Different Vegetables by High Performance Liquid Chromatography

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Abstract

β-carotene is an enriched source of vitamin A and is frequently present in vegetables. The vegetables rich in β-carotene should be used on the daily menu due to their dietary importance. The deficiency of vitamin A may cause severe health issues like premature deaths of children. So, the present study was conducted for the evaluation of vegetables containing high amounts of β-carotene. Six vegetables were selected from the local market of Lahore, Pakistan, and beta carotene was analyzed by high performance liquid chromatography. It was concluded that carrot was rich in beta carotene contents, i.e., 12950±5.0 µg/100g. The sequence of beta carotene amount in the selected vegetables was carrot>spinach>brinjal>tomato>bitter gourd>cabbage. It is suggested that vegetables like carrot, and spinach should be used on a daily basis for good health maintenance.

Keywords: Beta carotene, Vitamin A, HPLC, Vegetables

Introduction

β-carotene, a member of the carotenoids family, has been known as an essential dietary source of vitamin A for many years [1]. Vitamin A dearth has unsympathetic effects on reproduction, growth, and resistance to infection [2]. Vitamin A has an important role in many biological processes like cellular differentiation, cartilage and bone development, and growth [3]. Carotenoids are commonly produced by photosynthetic organisms like plants, animals, and some species of bacteria, where they are involved in metabolic and biosynthetic processes. The yellow, orange and red colors of different fruits, vegetables, fishes, birds, and flowers are due to carotenoids, therefore, they are also called as colorants [4]. In the food industry, they are used as a natural coloring agent. Some carotenoids are a part of the western diet, e.g., lycopene, lutein, zeaxanthin, and cryptoxanthin [5]. The importance of carotenoids lies in their beneficial properties regarding human health. For instance, the antioxidant property of carotenoids is proven to be significant for human health in various ways [6]. Research on carotenoids has shown that it is involved in the regulation of cells, gene expression system, boosting up the immune response, and enzymes of drug metabolism [7]. Nowadays living habits and lifestyle are bit changed. Prevalent use of cigarettes and irregular diet has led to the production of free radicals that can cause serious damage to macromolecules like DNA, proteins, fatty acids of low density lipoproteins and cholesterol, etc. [8]. The increase in the production of free radicals elevates oxidative stress, which increases the risk of lethal diseases, e.g., cardiovascular diseases, cancer, rheumatoid arthritis, diabetes, and chronic inflammation [9].

β-carotene is an important carotenoid known for its pro-vitamin activity, which is then metabolized into active vitamin A by the human body [10]. β-carotenoid present in fruits and vegetables is regarded as a rich source of antioxidants. It is proven effective for all chronic diseases as it reduces oxidative stress by eradicating the production of free radicals [1, 11]. Studies and investigations on carotenoids, especially β-carotene, showed that they combat the effect of certain diseases, most commonly cancer [12], light sensitivity [13] disorders, problems regarding age [14], and cardiovascular diseases. It is broadly assumed that β-carotene in leafy vegetables and orange-colored plants is a rich source of antioxidants and helps to eliminate oxidative damage [15, 16]. Based on the antioxidant property and pro-vitamin A activity of β-carotene, it is naturally considered to extend the human life span. Experimental in vivo animals showed that β-carotene reduces free radicals [17], thus preventing oxidative damage that is considered the main cause of chronic diseases. Observations showed that a high intake of beta carotene through diet reduces the mortality rate [18], and results may vary [19]. Moreover, unsatisfactory results were found with the β-carotene supplementation [20]. The reason behind it is that different sources of β-carotene have different effects on metabolism rate [21]. For instance, β-carotene is naturally in food or supplements, and both have an impact on human health.

Advancement in the methods for the determination of carotenoids in food is for two main reasons. Firstly, it was assumed that previously reported values of carotenoids in different vegetables and fruits were inaccurate because methodologies were unreliable and insufficient to report and discriminate the carotenoids and vitamin A of supreme importance for human health [22, 23]. Secondly, carotenoids without vitamin A activity are assumed to play other important roles than nutrition and eyesight. Due to conjugated double bonds, carotenoids may act as a trap or antioxidant and play a significant role in cancer causatives and elimination [24]. Subsequently, from the 1970's HPLC (high performance liquid chromatography) has become the common source of carotenoid determination in various vegetables and fruits because of its rapid separation technique moreover it achieves better resolution and hence better results [25]. Some of the studies deal with characterizing many carotenoids, but the processes were difficult and not suitable for routine examination. Developments are being made as the determination of carotenoids is a complicated procedure. The present study entails the determination of β-carotene in different vegetables.

Materials and Methods

Different vegetables collected from the local market of Allama Iqbal Town, Lahore were selected for the determination of β-carotene by using HPLC. By taking edible parts of the collected samples, a composite sample was made by mixing each vegetable in a minute quantity. A 100 g of composite sample was taken, and a subsample of 10 g was separated for the extraction procedure. After that, the pre-analyzed sample was simply washed with tap water and placed in inert condition at a temperature of -4 ^{o} C to avoid any spoilage [26]. The further analysis was conducted at the Food and Biotechnology Research Centre, PCSIR Laboratories Complex Lahore.

Beta Carotene Extraction

Beta carotene was extracted from the collected samples and was analyzed by using the High performance Liquid chromatography technique [27].

10 g of inert sample (kept at -4°C) was mixed with 30 mL of acetone, a standard solvent for the extraction of carotene from vegetables. The resulting mixture was then filtered through Whatman filter paper. The residue was washed twice with acetone to obtain a colorless extract. After it, the residue was disposed of, and the remaining filtrate was mixed with 20 g of anhydrous sodium sulphate. The removal of anhydrous sodium sulphate was done by filtration and the volume of extract was lessened by a rotary evaporator that works on the principle of evaporation. The extract was then transferred to a 100 mL volumetric flask and the volume was made up to the mark by adding in it acetone and water, making the final extract with 80% of acetone.

Standard Preparation of Beta Carotene

1 g standard of beta carotene enclosed in a vial (kept at -4°C) was brought from Merck. 10 mg was dissolved in 100 mL n-hexane to prepare a stock solution of beta carotene. The concentration of standard was made equal to 10 ppm. The stock solution of beta carotene was dissolved in different known concentrations [26]. For instance, 2, 4, 6, and 8 ppm dilutions were attained in 5 mL of each n-hexane solution. Every working standard solution was introduced into the HPLC system present at the Laboratory of PCSIR Lahore. Chromatographic conditions were according to the Perkin Elmer HPLC software containing series 200 HPLC pump (isocratic gradient), having a C-18 column coupled with C series 200 detector was used. "TCWS 4.0 software" was used for the quantification and peak identification in the HPLC system. Analysis was made by running the mobile phase (acetonitrile, dichloromethane, and methanol by the ratio of 70:20:10, respectively) at a flow rate of 1 mL per minute. The wavelength was set at 452 nm, and the pressure of the column was maintained at 1000-1200 psi. The standard solution of beta carotene (20 µL) was introduced when the injector was in the load phase.

Sample Evaluation

A sample of beta carotene in 80% acetone was used as a standard for HPLC analysis. 20 µL of each vegetable sample was taken by using a microsyringe. Peak identification and quantification were obtained by comparing the sample retention time with the standard retention time of β-carotene.

Results and Discussion

Beta carotene is of peak interest because of its antioxidant properties and various health benefits like protecting vision, improving respiratory health, promote brain health, help to remove dandruff due to its hydrating property, and curing diabetes [28]. The amount of beta carotene varied from trace amounts in sweet potatoes, onion, mushrooms, and mint to thousands µg/100g in cabbage, bitter gourd, tomato, brinjal, spinach, and carrot. From the present data, it was observed that dark green vegetables are considered to have more beta carotene content in comparison with other vegetables, e.g., spinach contained 7824±0.5 µg/100g, followed by cabbage containing 1050±2.0 µg/100g and bitter gourd 1232±2.0 µg/100g were all vegetables that tend to have more carotenoids, apart from these vegetable carrots contained maximum amount of β-carotene 12950 ±5.0 µg/100g and the name β-carotene is also due to this reason [29]. Some of the vegetables were selected from the local market of Lahore specifically to determine the amount of β-carotene. In this research, six vegetables were chosen, and their results are shown in Table 1.

Table 1. **Amount of β-carotene (µg/100g) in different vegetables.**

Vegetable	Local Name	Botanical Name	Beta Carotene (µg/100g) ±SD
Bitter Gourd	Karaila	*Momordica charantia*	1232±2.0
Brinjal	Baingan	*Solanum melongena*	2450±1.0
Cabbage	Gobi	*Brasicca capitate*	1050± 2.0
Carrot	Gajar	*Daucasa carota*	12950±5.0
Spinach	Palak	*Spanacia oleracea*	7824±0.5
Tomato	Timater	*Lycopersicum esculentum*	1590±1.0

This data was in correspondence with earlier data and proved that among root vegetables, carrots tend to have the highest content of carotenoids and β-carotene in carrots is beneficial for improved night vision [30]. The comparison Table 2 for carotene in other vegetables reported worldwide is given below.

The comparison showed that the amount of beta carotene is highest in carrot followed by spinach and paprika (Table 2). Chromatograms and β-carotene content in different vegetables are shown in Fig. 1. Whereas, Fig. 2 represents the β-carotene in standard solution. Fig. 3 shows that the concentration of beta carotene was highest in carrot followed by spinach, brinjal, tomato, bitter gourd, and cabbage. The concentration of beta carotene in green leafy vegetables was given in the range of 80-9204 µg/100g [36].

Table 2. **Amount of β-carotene in different vegetables/food items reported worldwide.**

Vegetable	Amount of β-carotene	Reference
Cabbage	2441± 9.8 µg/100g	[1]
Tomato	508071.6 µg/100g	[1]
Spinach	12850± 3.4 µg/100g	[1]
Carrot	6400±10.05 µg/100g	[31]
Paprika	8000± 12.02µg/100g	[31]
Pumpkin	3168 ±10.08µg/100g	[31]
Corn	8460± 5.01µg/100g	[31]
Carrot	20300± 2.0 µg/100g	[31]
Green Pepper	125.87 ±10.98 µg/g	[32]
Red Pepper	1060.24 ±15.67 µg/g	[32]
Yellow Pepper	611.54 ±09.45 µg/g	[32]
White rice	68.00±0.33 µg/g	[33]
Fried rice	379.00±0.01 µg/g	[33]
Beans porridge	4257.00±0.98 µg/g	[33]
Green Pepper	116.08±0.21mg/100g	[34]
Red pepper	126.05±0.15 mg/100g	[34]
Tomato	54.12±0.08 mg/100g	[34]
Bitter gourd	1078±9.64 µg/100g	[35]
Brinjal	2100±11.35 µg/100g	[35]
Cabbage	910±10.81 µg/100g	[35]
Tomato	1610±8.66 µg/100g	[35]
Spinach	9940±23.06 µg/100g	[35]
Red Chilli	3290±8.54 µg/100g	[35]
Lady Finger	3220±29.81 µg/100g	[35]
Cucumber	280±11.68 µg/100g	[35]
Carrot	11210±72.62 µg/100g	[35]
Bottle guard	140±4.58 µg/100g	[35]

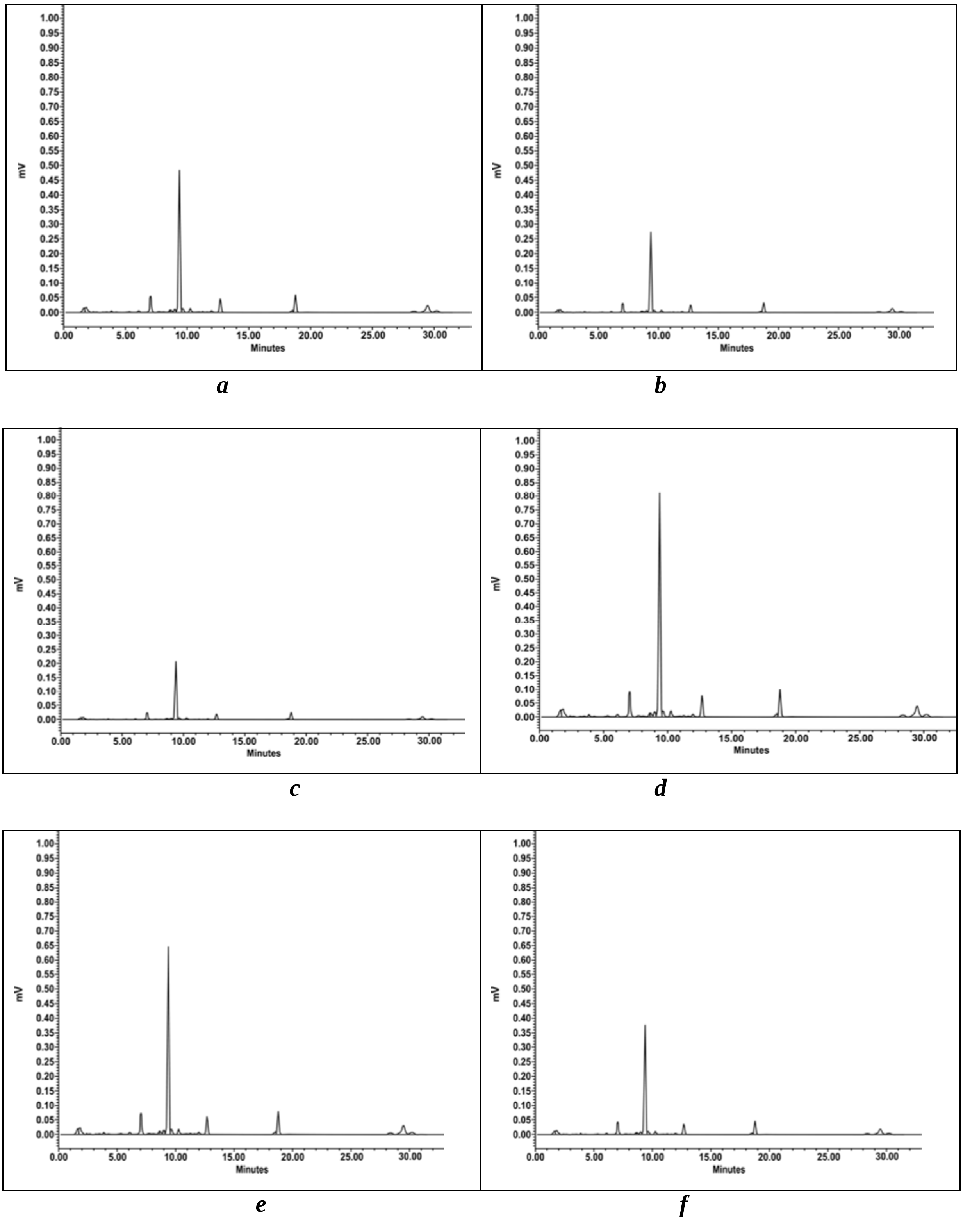

Figure 1. **The chromatogram of beta carotene in brinjal, bitter gourd, cabbage, carrot, spinach and tomato (a-f)**

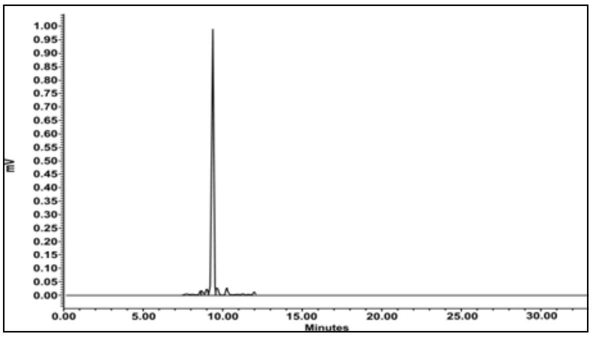

Figure 2. **The chromatogram of Beta carotene in standard solution**

In Fig. 3, it was observed that the cabbage has the lowest concentration of β-carotene while the carrot has the highest concentration of β-carotene.

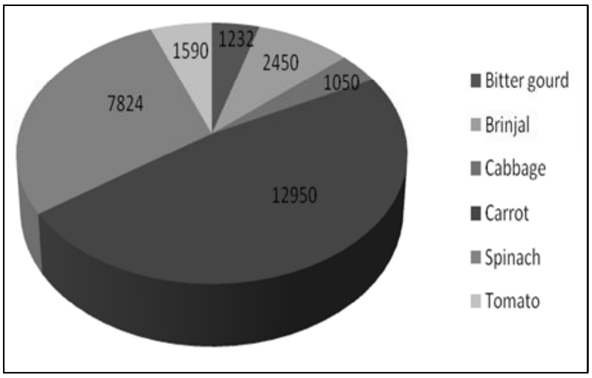

Figure 3. **Concentration of beta carotene (µg/100g) in different vegetables**

Among leafy vegetables, spinach has the highest amount of carotenoids and is closed to the reported value [36]. Tomatoes have a mean value of 1590 µg/100g. However, this value is lower than that reported [37]. The carotenoid content varying in different vegetables [38-39] may be due to the reason that carotenoids are highly sensitive to air, temperature [40], and other climatic conditions, so their determination needs much proficiency and experimental processes such as extraction, temperature variation or solvent used in the mobile phase of HPLC must be carefully handled to avoid inaccuracies and variation in results.

Conclusion

HPLC is considered the most efficient and sensitive technique for carotenoid analysis. Vegetables, especially dark green vegetables, are an excellent source of carotenoids and other vitamins, i.e., vitamin A. Present research has shown that vegetables can fulfill 50% of our daily need for micro and macronutrients. Vegetables are a good source that justifies daily nutrient requirements and helps to induce the immune response against several diseases. β-carotene in vegetables is popular for its antioxidant activity and helps to fight chronic diseases. To alleviate vitamin A deficiency, vegetables are cost effective and a better source to replace expensive animal food resources, which are not affordable for the low income population of any country. More vegetables containing β-carotene must be explored to completely eliminate the vitamin A deficiency in our population.

Conflict of Interest

There is no conflict of interest in this research.

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