DETERMINATION OF BETA CAROTENE CONTENT IN FRESH VEGETABLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A study was conducted during 2005, at Department of Agricultural Chemistry, NWFP Agricultural University, Peshawar Pakistan. Composite samples of twenty different kinds of vegetables available were collected from local markets of Peshawar and analyzed for their β-carotene content using high performance liquid chromatography (HPLC). The result indicated that the minimum content (>80 µg/100g) was found in potato, mushroom and mountain ebony and the highest amount (<80 µg/100g) in lettuce, spinach and carrot. The result in conformity with other workers clearly reflected the food value of vegetables. It was concluded that besides, major nutrients, vegetables also contributes fairly good amount of micro nutrients (vitamin A from carotenes). Hence it is suggested that green leafy vegetables and carrot should be used in our daily food menu.

INTRODUCTION

Carotenoids are synthesized exclusively by photosynthetic organisms including crop plants, algae, few fungi and certain bacteria where they play a vital role in plant metabolism and bio-synthesis of other bio-molecules. Aside from providing aesthetic qualities as colourants in the plant and the animal kingdoms, these pigments also play significant role in photo system-I and photo system-II. Most importantly, they acts as light harvesters and protect chlorophylls from photo oxidation.

The carotenoids derived their name from a major pigment in carrot root, Daucus carota, which is undoubtedly among the most widespread and important pigments in living organisms. This group of pigments is found throughout the plant kingdom (although their presence is often masked by chlorophyll). Thus, a wide variety of foods and feeds-yellow vegetables, tomatoes, apricots, oranges, egg yolk, chicken, butter, shrimp, lobsters, salmon, trout, yellow corn, etc. owe their colour principally to carotenoids, as do certain food colour extracts from natural sources such as palm oil, paprika, annatto, and saffron (Williams, 1984). Carotenoids are defined by their chemical structure having 40-carbon chain. The hydrocarbon carotenoids are known as carotenes, while oxygenated derivatives of these hydrocarbons are known as xanthophylls (Britton, 1995).

For many communities in developing countries, the major source of vitamin A in the diet is carotenoids especially beta carotene. Major advances have occurred in understanding the role and mechanisms of action of carotenoids. Carotenoids, with their highly reactive conjugated double bonds, act as free radical traps or antioxidants and may play an important role in quenching of toxic radicals. In view of the vital physiological functions of carotenoids, much attention has been focus on the determination of these pigments in foods as well as in human blood sera.

In recent years, there has been particular emphasis on understanding the types and concentrations of various carotenoids in foods. It is thought that previously reported values of vitamin A activity in food composition tables may have been unreliable since methodologies were not standardized since reliable methods were not used. Advances in studies into the structure and properties of various carotenoids have shown that only a handful of the hundreds of carotenoids occurring in nature possess vitamin A activity. Some of these may occur in higher concentrations than β-carotene, the most potent precursor of vitamin A (Bauernfeind, 1972).

Fresh new data are needed to update the old food composition table for South Asia. In this regard the present research study was initiated to determine the beta carotene content consumed in vegetables and vitamin A activity in human blood samples. Since no work has been done to determine β-carotene in Pakistani vegetables by HPLC and the determination is important to know its nutritional and therapeutic significance. The present study was undertaken to determine the beta carotene content in fresh vegetables.

MATERIALS AND METHODS

Sample Collection for Beta Carotene

Twenty different kinds of vegetables available at local markets in Peshawar were purchased. Each vegetable samples were taken and mixed together to get a composite sample. From composite sample,

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100g of lab sample was taken and from that, 10g of sub sample were selected for extraction. The pre analyzed samples were washed with tap water and were kept in inert condition, at -4°C temperature (Khalil and Varananis, 1996). The sample was then cut into small pieces and was mixed well. Analysis was carried out in the laboratory of Department of Agricultural Chemistry NWFP Agricultural University, Peshawar.

Pigment Extraction

Carotene was extracted from vegetables for "Reversed phased HPLC system" by the method of Khalil and Varananis, (1996).

10g of sample was homogenized in 30ml of acetone and then 0.1% (BHT) solution in acetone was added as an antioxidant. The resulting extract was filtered through Buchnar's funnel. The residue was washed twice with acetone till it become colorless. The residue was discarded and the filtrate was combined with 20gm of anhydrous sodium sulphate. The anhydrous sodium suphate was removed through filtration and the volume of extract was reduced by rotatory evaporator. The extract was transferred quantitatively to 100ml volumetric flask and the volume was made up to the mark with acetone and water, so that the final extract contain 80% of acetone.

Standard Preparation of Beta Carotene

Standard of beta carotene (1g enclosed in vial) was obtained from Merck. Stock solution of bets carotene was prepared by taking 10mg in 100ml n-hexane. The concentration of stock solution was equal to 100 ppm.

The stock solution was diluted to different known concentration e.g. 20, 40 and 60ppm, dilutions were obtained in 5 ml of each n-hexane solutions.

Each working standard solution was injected into HPLC system installed at the Laboratory of Department of Agricultural Chemistry, Peshawar and chromatographic condition was Perkin Elmer HPLC programme containing LC-1000 pump (Isocratic), having C18 column and connected with LC 250 UV/VIS detector was used. Peak identification and quantification was made by "CSW 32 software" for HPLC system. HPLC was calibrated by running mobile phase (Acetonitrile, dichloromethane and methanol by the ratio of 70:20:10, respectively) at the rate of 2ml per minute. Wave length was fixed at 452 nm. The pressure of the column was kept 1800-2000 PSI. Each standard solution (20µl) of beta carotene was injected when the injector was in load mode. The standard beta carotene peak was achieved at the retention time of 4.7 minutes (Rt = 4.7). The concentrations of the beta carotene standards were plotted against the peak area to obtain a straight line.

Sample Assay

Each sample of beta carotene extract in 80% acetone was used for HPLC assay like standard; each vegetables sample (20µl) was taken by micro liter syringe. The peak was automatically identified and quantified by comparing its retention time of the sample with the standard retention time.

RESULTS AND DISCUSSION

Vegetables were analyzed for their beta carotene content because it is the precursor of vitamin A and eaten in both raw and cooked form by humans in daily life (Bendich and Higdon, 2004). The beta carotene content (Table-1) varied from trace amount in potato, mushroom and mountain ebony to thousands µg/100g in carrot, spinach, mint and lettuce. From the data it was evident that dark green vegetables contained more beta carotene as compared to other vegetables e.g. spinach contained 9940 µg/100g, followed by mint, kulfa, lettuce and lady finger that were all dark green in appearance, apart from carrot that contain maximum amount of beta carotene (11210 µg/100g). These observations are in fair agreement to those reported by Higdon and Norman (1996) in which beta carotene content in carrot was 10110µg/100g, spinach 8230µg/100g, lettuce 3945 µg/100g and tomato 1930µg/100g and trace amount of beta carotene content in onion potato and mushroom. The beta carotene content reported in this study was higher than that of Williams *et al.* (1984), who found 520µg/100g in lady finger. This variation in beta carotene content of lady finger may be due to varietals difference. The result of this study was also supported by Agte *et al.* (2000), who analyzed 24 green vegetables for different micro nutrients contents including beta carotene. They reported that beta carotene content in green vegetables ranges from 80-9204 µg/100gm. Like wise, beta carotene content of these vegetables also corresponds to those reported by Robinson *et al.* (1986). They analyzed summer vegetables for their micronutrient content.

The data of this study concerning green vegetables was some what close to Patricia *et al.* (2004). However, they reported much lower content of beta carotene in carrot (5340 µg/100g). In contrast, they reported much higher content of beta carotene in tomato (3500 µg/100g). It appeared that their sample of carrot may have higher moisture content as compared to those analyzed in the present study and the variation in tomato content of beta carotene may be due to the use of immature samples, because

content of beta carotene drops by 77% during the ripening process (Rigo *et al*. 1999).

The slight variation in the data compared to others may be due to difference in experimental condition, extraction procedures, column length, diameter, temperature and different solvents used as mobile phase in HPLC etc. Temperature during analytical process, the storage time of vegetable and extraction process of the samples must be kept in control because all these things has a tremendous effect on the results (Marcela and Amaya, 2003). Variation in ecological growth conditions like variety and environmental aspects may also be contributing factors.

CONCLUSION

High Performance Liquid Chromatography (HPLC) is a rapid, efficient and sensitive technique for carotenoid analysis. Vegetables (especially dark green vegetables) are good source of carotenoid and micro nutrients (i.e. vitamin A). The result of the present study suggested that vegetables can fulfill our daily nutrient requirements, and 50% of our daily diet should be composed of vegetables. They are fairly good sources of micro nutrient (vitamin A) and can alleviate vitamin A deficiency in mass population of the country. Vegetables are cost effective and can substitute the animal food sources, which are expensive and beyond the purchasing power of the low income groups of population, especially in rural areas. More vegetables particularly wild vegetables should be explore as a source of beta carotene content.

Table-I: ***Beta carotene content (µg/100g and IU) in different fresh vegetables***

Sample	**Local name**	**Botanical name**	**µg/100g**	**IU**	**SDV**
Bath spongy	Tori	Lufa sagyptice	966	579	28.54
Bitter Guard	Karila	Monordicacharentia	1078	646	9.64
Bottle Guard	Khadu	Legennaria vulgarus	140	84	4.58
Bringal	Bangan	Solanum melongena	2100	1200	11.35
Cabbage	Bhand Gobi	Brasicca capitata	910	546	10.81
Carrot	Gajar	Daucasa carota	11210	7266	72.62
Cucumber	Khira	Cucumus sativus	280	168	11.78
French beans	Kacha Lobia	Vicia faba	882	529	7.54
Green Chili	Sabaz Mirch	Citrulus fistulosus	1750	291	7.00
Khulfa	Sag	Capsicum annum	6580	3948	45.29
Lady finger	Bhindi	Hibiscus esculentus	3220	1932	29.81
Lattuce	Salad	Lactuca sativum	3220	1932	52.57
Mashroom	Khubi	A. Compampestris	Tr	-	-
Mint	Podina	Menthe viridus	4550	2730	48.77
Mountain ebony	Kachnar	Bauhinia variegate	Tr	-	-
Onoin	Piyz	Allium capa	Tr	-	-
Potato	Alu	Solunum tuberosum	Tr	-	-
Red chelli	Surkh Mirch	Citrulus fistulosus	3290	1974	8.54
Spanich	Palik Sag	Spanacia oleracea	9940	5940	23.06
Tomato	Timater	Lycopersicum esculentum	1610	966	8.66
Mean			**1610**		

REFERENCES

Agte, V.V., K.V. Tarwadi, S. Mengale and S.A. Chiplonkar. 2000. Potential of traditionally green leafy vegetables as natural Sources for supplementation of eight micronutrients in vegetarian diets. J. Chro. Bio. Med. 6(13): 885-891.

Bauernfeind, J.C. 1972. Carotenoid vitamin A precursors and analogs in foods and feeds. J. Agric. Food Chem. 20:456-473.

Bendich, A. and G.S. Higdon. 2004. Biological actions of Beta-Carotene. Am. J. Nut. 32(2):225-230.

Borensfein, J.C. and G.B. Bunnell. 1966. Carotene, major pigment in carrot roots. J. Mal. Nut. 763(2):1-8.

Britton, G. 1995. Carotenes by their chemical structure. Amer. J. Nut. 47(2):1990-2001.

Khalil, I.A. and F.R. Varananis. 1996. Carotiniod extraction and analysis by reversed phase HPLC system. Sarhad J. Agric. 105(67): 15-21.

Marcela, C. and R.B.D. Amaya. 2003. Carotenoid composition of cooked green vegetables from restaurants. J. Food Chem. (83): 595-600.

Patricia, Y., H. Niizu, B. Delia and A. Rodriguez. 2004. New data on the carotenoid composition of raw salad vegetables. J. Food. Comp. Anal. 18(8): 739-749.

Rigo, E.R., F.L. Finger, V.W.D. Casali and A.A. Cardoso.1999. Inheritance of fruit color and pigment changes in a yellow tomato nutrient. J. Mol. Bio. 47(1):101-104.

Robinson, C.H., R.L. Marlilyn, L.C. Wanda and A.E. Garwick. 1986. Analyzed summer vegetables for their micronutrient content. J. Nut. 45(8)497-502.

Williams, E.R. and M.A. Caliendo. 1984. Book of nutrition: principles, issues and applications. McGraw Hill Book Co. pp. 311-321.

One type of liquid chromatography is HPLC (High Performance Liquid Chromatography), a universal analytical method utilised mainly in analysis of complex samples with non-volatile compounds, especially biologically active substances.Â Measurements of carotenoid and beta-carotene content). The above methods have been appropriately modified to determine the Î²-carotene content in cosmetic preparations. Keywords: Î²-Carotene; Lycopene; High Performance Chromatography; Emulsion Stability. Introduction. Go to.Â In the study, determination of Î²-carotene content in commercial creams with use of Determination of beta carotene in fresh vegetables â€¦ 768.Â Sample Assay Each sample of beta carotene extract in 80% acetone was used for HPLC assay like standard; each vegetables sample (20Âµl) was taken by micro liter syringe. The peak was automatically identified and quantified by comparing its retention time of the sample with the standard retention time.Â Like wise, beta carotene content of these vegetables also corresponds to those reported by Robinson et al. (1986). They analyzed summer vegetables for their micronutrient content.Â CONCLUSION High Performance Liquid Chromatography (HPLC) is a rapid, efficient and sensitive technique for carotenoid analysis. Since no study on carotenoids determination in vegetables by High Performance Liquid Chromatography has Toluene (BHT) @ 0.05 g into 50 ml solution in been done in North West Frontier Province (NWFP), acetone was added as an antioxidant. The resulting the present study was designed to determine beta- extract was filtered through Buchnarâ€™s funnel.Â Muller, H. The determination of the beta-carotene Development and evaluation of a new method content in selected vegetables and fruits by for rapoid caroteniod determination in HPLC and photodiode array detection. J. Food vegetables. J. Chromatogr.