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Effect of Boiling and Storage on Beta-Carotene Content of Different Vegetables

Farida Anjum, Barkat Ali Khan, Nadia Noreen, Tariq Masood and Sulaiman Faisal[1]
Agricultural Research Institute, Tarnab, Peshawar-Pakistan
[1]Department of Biosciences, COMSATS, Islamabad-Pakistan

Abstract

Sixteen different fresh vegetables collected from local market in Peshawar were analyzed for their beta-carotene content using HPLC. The effect of storage (one week at room temperature) and boiling (one hour) on beta-carotene were also assessed. It was observed that carrot contained the highest amount of beta- carotene (14000 µg/100g), followed by spinach (9000 µg/100g), mint (8618.4 µg/100g) and kulfa (6580 µg/100g). Mushroom and potato contained the least amount of beta-carotene. Maximum loss of beta-carotene occurred by boiling peas (62%) while, minimum loss was observed in spinach (4.4%). Highest loss due to storage occurred in tomato (76.32%) while, lowest in carrot (19.9%). Based on the results of this study it is suggested that vegetables must be kept in cold storage or refrigerator to preserve their nutrient content. Different form of vegetables should be used in the daily food menu to fulfill the partial nutrients requirements.

Key words: Boiling, storage, beta-carotene, vegetables

Introduction

The health benefits of eating generous amounts of vegetables, whether fresh, frozen, canned, juiced or dried, are unquestionable. Research shows that it may be the combination of nutrients and other substances rather than the individual nutrients themselves, which provides the health enhancing effects of vegetables (Agte *et al*. 2000). Among these important nutrients the most important and beneficial are carotenoids. The predominant carotenoids found in human tissues are beta-carotene, alpha-carotene, lycopene, lutein, zeaxanthin, and beta-cryptoxanthin; their relative abundance depends on dietary intake (Khalil and Saleemullah, 2004).

The most widely studied and well-understood nutritional role for carotenoids is their provitamin A activity (Barua and Olson, 2000, Evangelina *et al.* 2001) notably beta-carotene. Dietary beta-carotene is obtained from a number of fruits and vegetables, among the vegetables the most important sources of carotenoids are carrots, spinach, peaches, apricots, and sweet potatoes (Gimeno *et al*, 2001). Carotenoids also play an important potential role in human health by acting as biological antioxidants (Khalil and Saleemullah, 2004). Beta-carotene similarly enhanced both humeral and cell-mediated immune responses (Chew *et al.* 1991, Britton, 1995). Epidemiologic studies have shown inverse correlation between the consumption of carotenoid-rich vegetables and the incidence of cancer (Huck *et al*. 2000).

It is suggested that we should take one dose (5 to 6 mg about twice the average daily American intake) daily mixed-carotenoid supplement that provides 25,000 IU vitamin A, However individual dietary carotenoid consumption is quite variable (Jaarsveld *et al*. 2000).

Vegetables are either consumed in the raw or cooked form. In the raw state, most of the nutrients are retained, whereas any degree of cooking generally results in partial loss of nutrients and considerable changes in its sensory characteristics (Kala and Prakash, 2004). The carotenoid composition may also varies with variety, culture, cultivation conditions, the state of maturity, the post-harvest and storage handling, the climate and the geographical localization, the type of sample and the part of plant (Mercadante and Amaya, 1990, Yamini *et al.* 2001). Loss of carotenoids during processing and storage of food has been reported in numerous papers. (Amaya, 1997, Rajyalakshmi *et al.*2001, Mercela *et al.* 2003). It is also recommended that processing and storage of foods should be optimized to prevent carotenoid losses (Amaya, 2001, Çinar 2005).

Keeping in view the importance of carotenoids and their considerable looses due to different parameters especially due to boiling and storage at home scale, reliable assessments of carotenoids in local vegetables are necessary for accurate quantification

Corresponding author: Farida Anjum
Agricultural Research Institute,
Tarnab, Peshawar-Pakistan
E.mail: freedaanjum@yahoo.com

of dietary vitamin A intake. Since no study on by High Performance Liquid Chromatography has been done in North West Frontier Province (NWFP), the present study was designed to determine beta-carotene contents in vegetables commonly available in NWFP, Pakistan.

Materials and Methods

Sample collection

Beta-carotene determination was carried out in some common vegetables (spinach, lettuce, green pepper, carrot, mint, fresh bean, bitter gourd, etc) grown in agro ecological conditions of Peshawar. These vegetables were purchased from three different shops in three different localities of Peshawar. Mixing these vegetables made a composite sample of about one kg. From composite sample, 100 gm of lab sample was taken and from that, 10 gm of sub sample were drawn 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. The analysis was carried out in the laboratory of Agricultural Chemistry, NWFP Agricultural University, Peshawar.

Extraction of sample

Carotene was extracted from vegetables for "Reversed Phased HPLC System" by the method of Khalil and Varananis, 1996.

To determine the effect of boiling, the sample, were boiled for one hour. For storage effect, sample was stored for about one week at room temperature during June-September. Sample from each fresh, boiled and stored samples was homogenized in 30 mL of acetone and then 0.1% Butyrated Hydroxyl carotenoids determination in vegetables Toluene (BHT) @ 0.05 g into 50 ml 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 colourless. About 20 g anhydrous sodium sulphate (as hygroscopic material) was added, and then remove through filtration. Volume of extract was reduced on rotary evaporator. The sample were further washed with 10 mL of acetone and refiltered. Filtration was repeated thrice to confirm purity. The extract was transferred to 100 mL volumetric flask and the volume was made up to the mark with acetone and water, so that the final extract contains 80% of acetone. (Khalil and Varananis, 1996).

Standard Preparation of Beta Carotene

A 100 ppm stock solution of beta-carotene was prepared. Different concentration were prepared and run on HPLC (isocratic system using a Shimadzu chromatograph including LC-10 AS pumps. 20-ul Reodyne injector, SPD-10A UV detector operating at 190-370 nm and a supelco C_{18} anlytical column (25 cm*4.6 mm i.d)) with 2 mL/min. flow rate mobile phase (Acetonitrile, Dichloromethane and Methanol by the ratio of 70:20:10 v/v, respectively). The wavelength was fixed at 452 nm. The pressure of the column was kept at 1800-2000 psi.

The standard beta-carotene peaks were integrated by computer software programmed "CSW 32" to quantify the content of beta-carotene. The concentrations of the standards were plotted against the peak area to obtain a straight line (Fig. 1). The sample peaks were compared to the standards for estimation of the beta-carotene concentration.

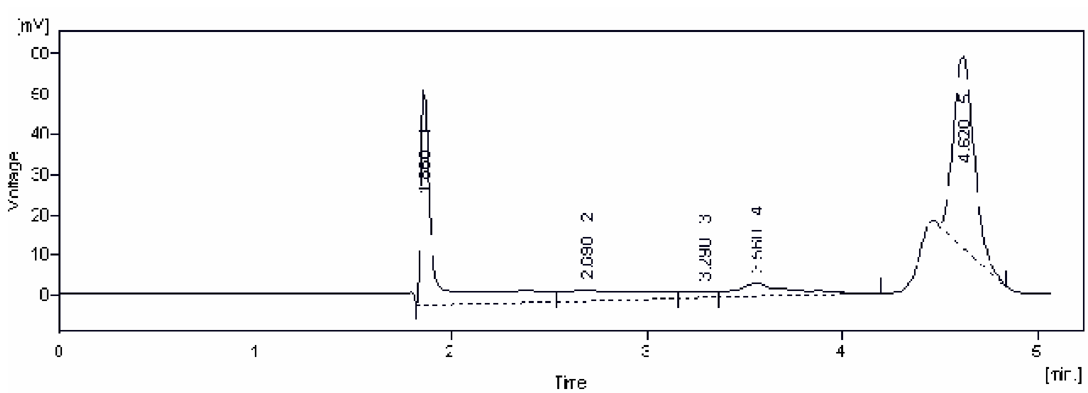

Fig. 1 Liquid chromatogram of beta-carotene standard

Results and Discussion

The effects of boiling and storage on beta carotene in 16 vegetables were determined using HPLC. Data are summarized in Table-1 and Table-2 respectively.

Effect of boiling

It is hypothetically believed that boiling causes rapid reduction in carotene content. Many scientists also proved it by their research. But most of this work was done in foreign countries. Since experimental evidence of this was lacking in our country it was of interest to determine the effect of boiling on beta-carotene in vegetables. The data presented in Table-2, indicate that the boiling caused reduction in beta-carotene content of carrot from 14000 µg/100g to 7900 µg/100g (43.6%). A pronounced reduction in beta-carotene content of Lettuce, mint, green chilli, bringal, fresh been, bath sponge and bitter gourd was found to be 47.6%, 35%, 55.3%, 35.3%, 24%, 22% and19.3% respectively. About 62.7% and 14.9% of losses was found in peas and capsicum respectively.

The loss in beta-carotene during processing is because trans form change to cis form Hachett *et al.* (2002) that are not biologically active. The present result is in favour with those of USDA Agricultural Research Service (1998) that indicated the beta-carotene content of boiled tomato as 620 µg/100g.

Present results are in fair agreement to that of Jane *et al.* (1988) who reported 53 and 40 % loss of beta-carotene due to boiling in lettuce and carrot, respectively. The results are in agreement with that of the Santana *et al.* (1998) who reported 7000-µg/100g beta-carotene in boiled carrot. However, the results deviate from that of Yadav and Sehgal (1995) who reported 0.0 to 1.3 and 1.5 to 2.1% losses in spinach due to boiling.

Table-1: Beta-carotene content (µg/100g) of vegetables as affected by boiling

Vegetables	Local Name	Botanical Name	Fresh (µg/100g)	Boiled (µg/100g)	%Loss
Bath spongy	Tori	*Lufa sagyptice*	900[efg]	700[d]	22.2
Bitter Gourd	Karila	*Monordicacharentia*	1078[defg]	870[d]	19.2
Bringal	Bangan	*Solanum melongena*	1465.8[def]	947.8[d]	35.3
Cabbage	Bhand Gobi	*Brasicca capitata*	80[g]	56[d]	30
Carrot	Moli	*Daucasa carota*	14000[a]	7900[b]	43.6
French beans	Kacha Lobia	*Vicia faba*	882[efg]	670[d]	24
Green Chilli	Sabaz Mirch	*Citrulus fistulosus*	1772[de]	791.7[d]	55.3
Kulfa	Sag	*Portulaca oleracea*	6580[c]	6000[c]	8.8
Capsicum	Shimla	*Capsicum annum*	990[defg]	842.8[d]	14.8
Lettuce	Salad	*Lactuca sativum*	2100[d]	1100[d]	47.6
Mushroom	Khumbi	*Agricus compampestris*	Trg	Trd	-
Mint	Podina	*Menthe viridus*	8618.4[b]	5600[c]	35
Potato	Aalu	*Solunum tuberosum*	Trg	Trd	-
Spinach	Palik Sag	*Spanacia oleracea*	9000[b]	8600[a]	4.4
Peas	Matar	*Pissum satavum*	290[fg]	108[d]	62.7
Tomato	Tamatar	*Lycopersicum esculentum*	680[efg]	566[d]	16.7

Means followed by different letter(s) in the column are statistically significantly different at 5% probability.

Effect of storage

It has been found that storing the vegetables for longer time at room temperature gradually decreases beta-carotene content of vegetables. In order to confirm this idea the vegetables were stored for one week at room temperature ($37C^0$) and were analysed for beta-carotene content. The results in Table-3 revealed that storage of vegetables for one week significantly reduced beta-carotene content. In carrot, storage caused reduction of beta-carotene content from 14000µg/100g to11210 µg/100g (about 19.9% losses). Likewise, in lettuce, spinach, mint, kulfa, green chilli, bringal, french been, bath sponge storage caused 59.5%, 23.3%, 46.8%, 31.5%, 65.1%, 72%, 44.5% and 61.6% reduction in beta carotene content, respectively. Beta-carotene content in bitter gourd declined to about 45.3% while, in capsicum, tomato and peas it was reduced by 27.3%, 76% and 68% respectively.

Carotenoid losses during post harvest storage were reported in some vegetables, particularly leaves (Negi and Roy, 2000). The present results agree to that of Li *et al.* (2000), who reported 17% loss of beta-carotene due to storage in carrot. The results also correspond to that of Craft *et al.* (1988) who reported 60 %loss in beta-carotene in peas due to storage. The present result matched to that of (Yadav and Seghal (1995) who reported 55 to 63 % loss in lettuce due to storage. However it deviates from that of Muller (1998) who reported 27% loss of beta-carotene due to storage in carrot as 27%.

Table-2: Beta-carotene content (µg/100g) of vegetables affected by storage

Vegetables	Local Name	Botanical Name	Fresh (µg/100g)	Stored (µg/100g)	% Loss
Bath spongy	Tori	*Lufa sagyptice*	900^{efg}	345^{d}	61.6
Bitter Gourd	Karila	*Monordicacharentia*	1078^{defg}	590^{d}	45.3
Bringal	Bangan	*Solanum melongena*	1465.8^{def}	409.5^{d}	72.8
Cabbage	Bhand Gobi	*Brasicca capitata*	80^{g}	35^{d}	56.3
Carrot	Gajar	*Daucasa carota*	14000^{a}	11210^{g}	19.9
French beans	Kacha Lobia	*Vicia faba*	882^{efg}	489^{d}	44.6
Green Chili	Sabaz Mirch	*Citrulus fistulosus*	1772^{de}	617^{d}	65.1
Kulfa	Sag	*Portulaca oleracea*	6580^{c}	4506^{c}	31.5
Capsicum	Shimla	*Capsicum annum*	990^{defg}	720^{d}	27.27
Lettuce	Salad	*Lactuca sativum*	2100^{d}	850^{d}	59.5
Mushroom	Khumbi	*Agricus compampestris*	Trg	Trd	-
Mint	Podina	*Menthe viridus*	8618.4^{b}	4586^{c}	46.8
Potato	Aalu	*Solunum tuberosum*	Trg	Trd	-
Spinach	Palik Sag	*Spanacia oleracea*	9000^{b}	6898^{b}	23.35
Peas	Matar	*Pissum sattivum*	290^{fg}	90^{d}	68.9
Tomato	Tamatar	*Lycopersicum esculentum*	680^{efg}	161^{d}	76.32

Means followed by different letter(s) in column are statistically significantly different at 5% probability.

Conclusion and Recommendations

From the present research work, it is concluded that dark green vegetables are good source of beta-carotene. Carrot is the richest source of beta-carotene. Boiling and storage caused significant decrease in beta-carotene of vegetables.

The result of the present study suggests that vegetables should be stored in cold storage in order to avoid loss of essential nutrients.

References

Agte,V. V., K. V. Tarwadi, S. Mengale and S. A. Chiplonkar. Potential of traditionally green leafy vegetables as natural sources for supplementation of eight micronutrients in vegetarian diets. J. Chromatogr., 2000, 13: 885-891.

Amaya, R. The retention of provitamin A carotenoids in prepared, processed, and stored foods. J. Biol. Sci., 1997, 3:345- 444.

Amaya, R. The effect of processing and storage on carotenoids content of vegetables. J. Food Sci., 2001, 7:2005-5802.

Barua, A. B and J. A. Olson. ß-carotene is converted primarily to retinoids in rats *in vivo.* Am. J. Nutr. Sci., 2000, 2:1996-2001.

Britton, G. UV/ visible spectroscopy. J. Food Sci., 1995, 4:13-63

Chew, B. P., T. S. Wong, J. J. Michal, F. E. Standaert and L. R. Heirman. Subcellular distribution of ß-carotene, retinol and α-tocopherol in porcine lymphocytes after a single injection of ß-carotene. J. Ani. Sci., 1991, 69:4892-4897.

Çinar, I. Carotenoid pigment losses of freeze dried plant samples under different storage conditions. Kahramanmaraş Sütçü İmam Univ. Deptt. Food Sci. and Tech. Kahramanmaraş, Turk. 2005.

Craft, N., E. Brown and J. Smith. Effects of storage and handling conditions on concentrations of individual carotenoids, retinal, and tocopherol in plasma. Am. Assoc. Clin. Chem.,1988, 7:15-25.

Evangelina, G., M. A. Mariana, N. A. Mónica and A. Beatriz. Carotenoid composition and vitamin A value of an Argentinian squash. J. Nutr. Sci., 2001, 34: 54-62.

Gimeno, E., A. I. Castellote, R. M. Lamuela, M. Raventós, M. C. Boronat and M. C. Sabater. Rapid high-performance liquid chromatographic method for the simultaneous determination of retinol, α-tocopherol and beta -carotene in human plasma and low density lipoprotein. J. Chromatogr., 2001, 2: 315-352.

Hackett, M., J. Lee and S. Schwartz. Thermal Stability and Isomerization of Lycopene in Tomato Oleoresins from Different Varieties. J. Food Sci.,2002, 69: 536-541.

Huck, K. L., H. Popp, Scherz and G. K. Bonn. Development and evaluation of a new method for rapoid caroteniod determination in vegetables. J. Chromatogr. Sci., 2000, 43: 21-65.

Jane, M., J. Dietz, Sachi and J. John. Reversed phase HPLC analysis of alpha and beta-carotene from selected raw and cooked vegetables. J. Nutr. Sci., 1988, 38:333-341.

Kala, A and J. Prakash. Nutritional composition and sensory profile of microwave and conventional cooked vegetables. J. Food Sci.,2004, 15:1745-4506.

Khalil, I. A and Z. Varanani. Novel reversed phase HPLC technique for chloroplast pigment analysis of crops. Sarhad J. Agric.,1996, 12:569-574.

Khalil, I. A and Saleemullah. Book of Bio Analytical Chemistry 2nd Edt. National Book Foundation Islamabad: 26-29. 2004.

Li, X., F. Xiao, Q. Chen and B. Huang. The simultaneous determination of lycopene and beta-carotene in fruit vegetables and plasma by HPLC. J. Agric.,2000, 26: 444-446.

Mercandate, A and R. Amaya. Carotenoid composition and vitamin A value of some native Brazilian green leafy vegetables. J. Food Sci.,1990, 25: 213-219.

Mercela, C., B. Delia and R. Amaya. Carotenoid composition of cooked green vegetables from restaurants. Food Chem.,2003,83: 595-600.

Muller, H. The determination of the beta-carotene content in selected vegetables and fruits by HPLC and photodiode array detection. J. Food Nutr., 1998, 2: 88-94.

Negi, P and S. Roy. Effect of drying condition on quality of green leaves during long term storage. Food Res. Int.,2000, 34: 283-287.

Rajyalakshmi, P., K. Venkatalaxmi, K. Venkatalakshmamma, Y. Jyothsna, K. Devi and V. Suneetha. Total carotenoid and beta-carotene contents of forest green leafy vegetables consumed by tribals of south India. J. Food Sci., 2001,56: 225-238.

Santana, P., S. Paulo, B. Sebastião, P. Héctor and Q. Valéria. Evaluation of total carotenoids, alpha and beta-carotene in carrots during home processing. J. Nutr. Sci.,1998, 18: 101-206.

USDA Agricultural Research Service. Comparison of vitamin levels in raw vs. cooked foods**.** pp.23-42**.** 1998.

Yadav, S and K. Sehgal. Effect of home processing on ascorbic acid and beta-carotene content of spinach and amaranth leaves. J. Food Sci., 1995, 2:125-31.

Yamini, C., N. Ranjana, Y. Chaturvedi and R. Nagar. Levels of beta-carotene and effects of processing on selected fruits and vegetables of the arid zone of India. J. Food Sci.,2001,56: 127-132.