

## Identification and Quantification of Major Carotenoids in Some Vegetables

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**Abstract:** An HPLC study of 6 raw vegetables (Okra, green beans, eggplant zucchini, carrot and tomato) most frequently consumed worldwide was carried out to determine their carotenoid composition. The samples were purchased from supermarket in the city of Boston, USA. Neoxanthin, violaxanthin and lutein were contained in all samples except tomato for neoxanthin, carrot and tomato for violaxanthin and carrot for lutein.  $\beta$ -carotene was contained in all samples while  $\alpha$ -carotene was contained only in carrot. Lycopene was contained in okra, green beans and tomato. Carrot had  $\alpha$ -carotene and  $\beta$ -carotene as principal carotenoids and lutein as minor component. Relatively high ratios (9-cis to all-trans  $\beta$ -carotene) of above 0.2 g/g were noted in green beans, eggplant and okra.

**Key words:** carotenoids, vegetables, HPLC,  $\beta$ -carotene, lutein, Jordan

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### INTRODUCTION

Carotenoids are fat soluble compounds that are associated with the lipidic fractions. From a chemical point of view, carotenoids are polyisoprenoid compounds and can be divided into two main groups: (a) carotenes or hydrocarbon carotenoids only composed of carbon and hydrogen atoms and (b) xanthophylls that are oxygenated hydrocarbon derivatives that contain at least one oxygen function such as hydroxyl, keto, epoxy, methoxy or carboxylic acid groups. Their structural characteristic is a conjugated double bond system, which influences their chemical, biochemical and physical properties.

Carotenoids are a class of natural pigments occurs widely in nature. They are synthesized by plants and many microorganisms, so animals have to obtain them from food. Up to now, more than 600 carotenoids have so far been isolated from natural sources. However, only about 40 are present in a typical human diet. Of these 40 about 20 carotenoids have been identified in human blood and tissues<sup>[1]</sup>.

Fruits and vegetables containing vitamin C, vitamin E (tocopherols) and carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, zeaxanthin and lycopene) have been suggested as a natural source of antioxidants. Antioxidant functions are associated with decreased DNA damage, diminished lipid peroxidation, maintained immune function and inhibited malignant transformation or proliferation *in vitro* that are thought to prevent the development of some diseases<sup>[2,3]</sup>. Also,  $\alpha$ -carotene,  $\beta$ -carotene and  $\beta$ -cryptoxanthin are considered as provitamin A carotenoids<sup>[4]</sup>. More over, it

is generally accepted that the significance of vegetable consumption can play an important role in maintaining health and reducing the risk of illness<sup>[5]</sup>. In particular, increased vegetable consumption helps reduce the risk of cancer<sup>[6]</sup>. That is why vegetables as well as fruits are widely recommended as healthy food<sup>[7]</sup>. Vegetables are also a valuable part of the diet owing to their nutritive values<sup>[8]</sup>. They are low in energy containing limited amounts of carbohydrates and fats and high in dietary fiber, minerals and vitamins.

Carotenoids, provitamins A or not, have been credited with other beneficial effects to human health: enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and muscular degeneration<sup>[9-13]</sup>. The carotenoids' action against diseases has been attributed to antioxidant activity, specifically to their ability to quench singlet oxygen and interact with free radicals<sup>[14-16]</sup>. However, other mechanisms have been reported such as modulation of carcinogen metabolism, regulation of cell growth, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of cell-to-cell gap junctional communication, retinoid-dependent signaling and filtering of blue light<sup>[12,13,17-19]</sup>.

The vegetables analyzed in this paper are consumed worldwide. These vegetables also are grown, harvested and consumed widely by the Middle East population including Jordan. The carotenoid contents of Okra, green beans, zucchini, eggplant, tomato and carrot have been determined in several countries but the results are somewhat diverging. More data obtained by reliable methods are needed even for these vegetables

in order to differentiate natural compositional variation from analytical variability.

## MATERIALS AND METHODS

The experimental work was carried out at Carotenoids and Health Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA), Tufts University, Boston, USA.

**Reagent, chemicals and source of sample:** All-trans- $\beta$ -carotene, 9-cis- $\beta$ -carotene,  $\alpha$ -carotene, lutein, zeaxanthin, lycopene, Methyl-Tert-Butyl Ether (MTBE) and ammonium acetate (analytical grade) were purchased from Sigma Company (St Louis, MO, USA). Milli Q water was obtained from Water Purification Systems and used for all procedures using water. Tetra Hydro Furan (THF) and methanol were purchased from JT Baker Chemical (Philipsburg, NJ). Vegetables were purchased at a vegetable market from Boston, MA, USA.

**Chromatography and HPLC analysis:** Carotenoids were quantified using a high-performance liquid chromatographic (HPLC) system equipped with C30 column (3  $\mu$ m, 150 $\times$ 4.6 mm, YMC, Wilmington, NC, USA). Carotenoids were monitored at 455 nm, with a Waters 2996 photodiode array detector (Milford, MA, USA). The HPLC mobile phases were methanol: Methyl-Tert-Butyl Ether (MTBE): water (85:12:3 by volume, with 1.5% ammonium acetate in water) for solvent A and methanol: MTBE: water (8:90:2 by volume, with 1.0% ammonium acetate in water) for solvent B. The gradient procedure, was as follows: (1) start at 100% solvent A, (2) a 21 min linear gradient to 45% solvent A and 55% solvent B, (3) 1 min hold at 45% solvent A and 55% solvent B, (4) an 11 min linear gradient to 5% solvent A and 95% solvent B, (5) a 4 min hold at 5% solvent A and 95% solvent B, (6) a 2 min linear gradient back to 100% solvent A, (7) a 28 min hold at 100% solvent A. The flow rate was 0.4 ml min<sup>-1</sup> and the injection volume was 20  $\mu$ l. Carotenoids were quantified by determining peak areas under the curve in the high-performance liquid chromatograms calibrated against known amounts of standards. Each peak was confirmed by the retention time and characteristic spectra of the standards.

**Sample preparation:** The extraction of the vegetable, using a modified procedure from Riso and Porrini<sup>[20]</sup>, was performed by incubating 500 mg of the pureed vegetable together with 10 mL methanol for 1 h in a

shaking incubator at 120 rpm. Afterwards, the mixture was homogenized for 30 sec in an ice bath and the probe washed with methanol. The mixture was centrifuged at 3000 rpm for 5 min. The methanol layer was transferred into a 50 mL volumetric flask and the extraction repeated four times with 10 mL of Tetra Hydro Furan (THF), followed by vortexing and centrifugation. The THF layers were combined with the methanol layer and the volume brought up to 50 mL. One mL of the extract was taken, dried under nitrogen and re-suspended in 1 mL of ethanol. Triplet samples of each vegetable were extracted.

All the necessary precautions were taken to avoid losses of carotenoids and other errors during analysis, e.g., working under red lights, finishing the analysis within the shortest possible time, exclusion of oxygen, avoiding high temperature and contact with acids, using high purity solvents, exhaustive extraction.

## RESULTS AND DISCUSSION

Common English, Arabic and Scientific names of vegetables included in this study are given in Table 1. Fig. 1a, b, c, d, e and f shows chromatographic profiles of vegetables (okra, green beans, eggplant, zucchini, carrot and tomato) respectively. The principal carotenoids were lutein, neoxanthin, violaxanthin and  $\beta$ -carotene. Okra also had 9-cis- $\beta$ -carotene and 13-cis- $\beta$ -carotene as major carotenoids (Fig. 1a). Cis-lutein and zeaxanthin were detected in zucchini (Fig. 1d). Carrots had  $\alpha$ -carotene and  $\beta$ -carotene as the main carotenoids and neoxanthin as minor component (Fig. 1e). Lycopene predominated in tomato (Fig. 1f) and was detected also in okra and green beans (Fig. 1a and b) respectively.

The means of carotenoids contents of raw vegetables are given in Table 2, along with those nutrient contents from the USDA database<sup>[21]</sup> and other published databases<sup>[22-25]</sup> to enable comparisons of values obtained in the present study. In general, all the vegetables analyzed appeared to be considerable sources of carotenoids, with the exception of eggplant which had lower carotenoids content. All raw

Table 1: Common English, Arabic and scientific names of vegetables included in this study

Common English name	Arabic name	Scientific name
Okra	Bamia	<i>Abelmoschus esculentus</i>
Green beans	Fasolia	<i>Phaseolus coccineus</i>
Eggplant	Bazinjan	<i>Solanum melongena</i>
Zucchini	Kussa	<i>Cucurbita pepo</i>
Carrot	Jazar	<i>Daucus carota L.</i>
Tomato	Bandora	<i>Lycopersicum esculentus</i>

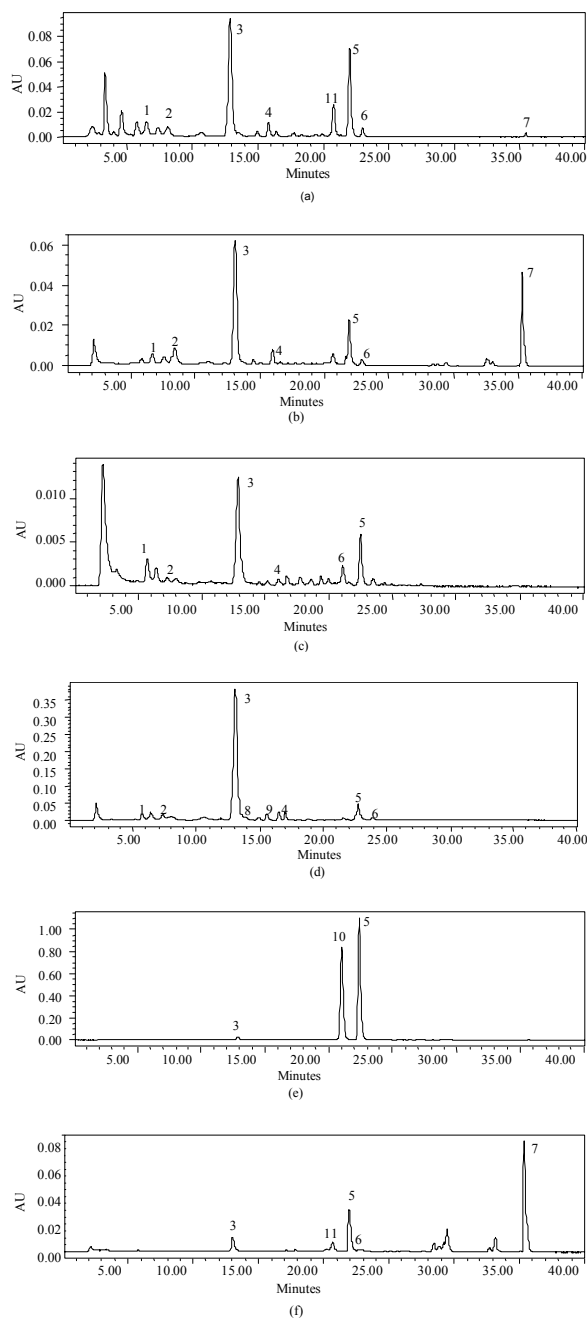


Fig. 1: Typical HPLC chromatograms of the carotenoids of (a) Okra, (b) Green beans, (c) Eggplant, (d) Zucchini, (e) Carrot and (f) Tomato. Peak identification: (1) neoxanthin, (2) violaxanthin, (3) lutein, (4) chlorophylls, (5)  $\beta$ -carotene, (6) 9-cis- $\beta$ -carotene, (7) lycopene, (8) zeaxanthin, (9) cis-lutein, (10)  $\alpha$ -carotene, (11) 13-cis- $\beta$ -carotene

vegetables in this study contained  $\beta$ -carotene and lutein with the exception of carrot which contained only  $\beta$ -carotene. Good sources of xanthophylls were zucchini, okra and green beans, in agreement with previously published data<sup>[22,23,26]</sup>. Although these carotenoids are not provitamins A, but the antioxidant property is linked with the conjugated double bond system, the maximum protection being giving by carotenoids having more than nine double bonds<sup>[27]</sup>. The  $\beta$ -carotene content of okra, green beans, carrot and tomato were much lower than that from both the USDA and other databases. These differences may be due to maturity of the vegetables, geographic or varietal differences.

All raw vegetables in the present study did not contain detectable levels of zeaxanthin, except zucchini which contained  $0.41 \mu\text{g g}^{-1}$ . Only carrot had  $\alpha$ -carotene  $47.1 \mu\text{g g}^{-1}$  edible portion and contained also the highest  $\beta$ -carotene ( $47.5 \mu\text{g g}^{-1}$ ). In contrast, 9-cis- $\beta$ -carotene was found in all vegetables, except carrot, while 13-cis- $\beta$ -carotene was found in both okra and tomato. All the  $\beta$ -carotene containing vegetables presented high ratios of 9-cis to all-trans  $\beta$ -carotene, ranging from 0.52 in green beans, 0.5 in eggplant, 0.22 in okra, 0.12 in zucchini down to 0.05 in tomato. The lack of literature information on the content of 9-cis  $\beta$ -carotene in plants and 13-cis- $\beta$ -carotene may be related to previous use of non-appropriate analytical conditions for identification of 9-cis- $\beta$ -carotene and 13-cis  $\beta$ -carotene and other stereoisomers of carotenoids<sup>[23]</sup>. Experimental nutritional medical studies with natural carotenoids, isomers of carotenes and carotenoids originating from different plant sources, have been limited. The present study and related previously published studies on natural carotenoids in plants, show that, even within the scope of  $\beta$ -carotene itself, more attention should be paid to the stereoisomeric configuration. The possible metabolic conversion of 9-cis  $\beta$ -carotene to 9-cis retinoic acid was recently reported and it is now considered as a potential medicine in certain types of tumours<sup>[23,28]</sup>.

The principal carotenoids found in foods are:  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein and violaxanthin. With the exception of violaxanthin, these carotenoids are also the most commonly found in the human plasma and have been, together with zeaxanthin, the most studied in terms of health promoting effects.

The experimental data showed large variations in carotenoid content of the foods assayed in this study. Prior and Cao<sup>[29]</sup> documented factors such as cultivars, maturity and other environmental factors such as

Table 2: Carotenoid contents, edible portion, of raw vegetables <sup>a</sup>

Vegetables	Concentration ( $\mu\text{g g}^{-1}$ )									
	Neox	Viol	Lutein	cis-lutein	$\alpha$ -Carot	$\beta$ -Carot	Zeax	9-cis $\beta$ -Carot	13-cis $\beta$ -Carot	Lycop
Okra										
This study <sup>b</sup>	2.08	1.58	8.98	- <sup>c</sup>	-	1.93	-	0.43	2.12	0.19
USDA <sup>d</sup>	-	-	5.16 <sup>e</sup>	-	0	2.25	-	-	-	0
Others <sup>f</sup>	-	-	-	-	-	0.69	-	-	-	-
Green beans										
This study	0.47	0.81	4.78	-	-	0.23	-	0.12	-	1.85
USDA	-	-	6.4	-	0.69	3.79	-	-	-	0
Others <sup>f</sup>	-	-	-	-	-	2.39	-	-	-	-
Eggplant										
This study	0.23	0.11	0.71	-	-	0.18	-	0.09	-	-
USDA	-	-	0	-	0	0.16	0	-	-	0
Others <sup>g</sup>	2	-	9.6	-	2.3	-	1.3	-	-	27.4
Zucchini										
This study	1.46	1.21	23.4	0.23	-	1.46	0.41	0.17	-	-
USDA	-	-	21.25	-	0	1.2	-	-	-	0
Others <sup>g</sup>	0.9	0.6	33.2	-	0.1	1.3	-	-	-	0.3
Others <sup>h</sup>	-	6.9	9.8	-	-	5.4	-	-	-	-
Carrot										
This study	1.33	-	-	-	47.1	47.5	-	-	-	-
USDA	-	-	3.58	-	37.67	63.91	-	-	-	0
Others <sup>g</sup>	0.5	0.1	7.8	-	425	1030	57.4	57.1	-	-
Others <sup>i</sup>	-	-	5.1	-	35	61	-	-	-	-
Tomato										
This study	-	-	0.39	-	-	0.79	-	0.04	0.32	2.11
USDA	-	-	1.23	-	1.01	4.49	-	-	-	25.73
Others <sup>g</sup>	7.6	9	14.3	-	-	14.5	-	-	-	243.1
Others <sup>i</sup>	-	-	1	-	-	3.2	-	-	-	35.4

<sup>a</sup>Neox, neoxanthin, Viol, violaxanthin,  $\alpha$ -Carot,  $\alpha$ -carotene,  $\beta$ -Carot,  $\beta$ -carotene, Zeax, zeaxanthin, Lycop, lycopene

<sup>b</sup>Values represent mean of three measurements

<sup>c</sup>Not determined

<sup>d</sup>USDA National Nutrient Databases for Standard Reference, Release 20(2007)

<sup>e</sup>Value of lutein and zeaxanthin

<sup>f</sup>Kandlakunte *et al.* (2008)

<sup>g</sup>Ben-Amotz and Fishler (1997). ( $\mu\text{g g}^{-1}$  dry weight)

<sup>h</sup>Rodriguez-Amaya *et al.* (2008)

<sup>i</sup>Niizu and Rodriguez-Amaya (2005)

sunlight exposure that may influence the antioxidant capacity of fruits and vegetables. The variability in the compositions and quantities of phytochemicals and antioxidants in different foods indicate the importance of eating a variety of food sources in particular, colored foods, in every meal.

### CONCLUSION

This study focused on the quantification of carotenoids of vegetables commonly consumed in the Middle East countries, including Jordan. In general, the results obtained from this study agree with those reported in the literature and the values fall within the wide ranges of data found in the literature. In any case, raw vegetables can provide high amounts of a variety of carotenoids that are important to human health.

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