Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 11 (23): 3797-3801, 2011
ISSN 1812-5654 / DOI: 10.3923/jas.2011.3797.3801

Mallow Carotenoids Determined by High-performance Liquid Chromatography

[1]Jafar Muhammad El-Qudah, [2]Jian Qin and [2]Guangwen Tang
[1]Department of Nutrition and Food Processing, Faculty of Agricultural Technology,
Al-Balqa Applied University, 19117, Al-Salt, Jordan
[2]Carotenoids and Health Laboratory, Jean Mayer USDA,
Human Nutrition Research Center on Aging and Friedman,
School of Nutrition Science and Policy, Tufts University, 711 Washington Street, Boston, MA, USA

Abstract: Mallow (*Corchorus olitorius*) is a green vegetable, which is widely consumed either fresh or dry by Middle East population. This study was carried out to determine the contents of major carotenoids quantitatively in mallow, by using a High Performance Liquid Chromatography (HPLC) equipped with a Bischoff C30 column. Neoxanthin and violaxanthin in mallow were characterized by Liquid Chromatography-Mass Spectrometry (LC-MS) with Atmospheric Pressure Chemical Ionization (APCI) as an ionization interphase. The total carotenoid content of mallow was estimated as 16.9 mg/100 g fresh weight. In which, major carotenoids in mallow are: lutein (5.3 mg/100 g), anhydrolutein (4.4 mg/100 g), β-carotene (3.7 mg/100 g), cis-anhydrolutein (1.0 mg/100 g), neoxanthin (1.6 mg/100 g), violaxanthin (0.3 mg/100 g), 9-cis-β- carotene (0.4 mg/100 g) and 13-cis-β-carotene (0.09 mg/100 g). Therefore, mallow is a rich source of carotenoids. As a popular vegetable consumed by the Middle East and surrounding populations, our finding of this study confirmed that mallow is an important source for carotenoids including provitamin A carotenoids.

Key words: Carotenoids, mallow, *Corchorus olitorius*, HPLC, mediterranean vegetable

INTRODUCTION

Mallow (*Corchorus olitorius*) is a green plant vegetable. The Latin name for this plant is Corchorus Olitorious. Its family name is Tiliaceae, and its Botanical references is 200 (Okusanya, 1979; Rahman, 1978; Verma and Arora, 1978). Mallow is grown in Mediterranean area, and popularly consumed by the Middle East population including Jordan, Syria, Egypt and many other countries in the Middle East region. It can make a mucilaginous, nutritious green soup with its crushed green leaves. The Arabic name of this dish is molokheya, and it is transliterated to several forms as, Moulokheyya, Mulukheyya, Mooookhieh, Mouloukhia, Mloukhia, Molohia, Molokhiya, Molukhyia, Mulukhia, Mulukhiya, Melokiyah, Meloukhia and other variations. Some people believe that Molokheya is originated in Egypt during the time of Pharaohs. It is also known as Egyptian Spinach. Others suggest that it was prepared first by ancient Jews. It is said that the original name for molokheya is Mulukeyya, which means of the kings, then the public turned the K to the present KH (Chen and Saad, 1981).

It is generally accepted that the significance of vegetable consumption can play an important role in maintaining health and reducing the risk of illness (Kalt, 2005). Further, consumption of vegetables and fruits high in α-carotene, β-carotene and lycopene such as carrots, spinach and tomatoes is associated with lower incidence of certain types of cancer, including lung, skin and digestive tract cancers (Franceschi *et al*., 1994; Mayne *et al*., 1994; Murakoshi *et al*., 1992). This is due to the facts that carotenoids are considered to be antioxidants in biological tissue as well as in food systems (Anguelova and Warthesen, 2000). Dietary carotenoids, or food rich in these colorful pigments, are considered to be beneficial in the prevention of a variety of major disease, including certain cancers and eye diseases (Krinsky and Johnson, 2005). Of all the carotenes, β-carotene is the most important one, because not only it can convert to Vitamin A in humans to provide Vitamin A nutrient but also it can function to prevent photosensitization and formation of skin tumors and potentially to increase immune response (Rao and Rao, 2007). In addition, it was proved that carotenoids have a favorable effect on influencing the cell cycle progression of the fibroblasts (Stivala *et al*., 1996).

Corresponding Author: Jafar Muhammad El-Qudah, Department of Nutrition and Food Processing, Faculty of Agricultural Technology, Al-Balqa Applied University, 19117, Al-Salt, Jordan
Tel: +962 795 639 227 Fax: +96253530469

In the Middle East, traditional vegetables, including mallow, are important source of nutrients and vitamins for many persons in the community. In Jordan, there are few researches on carotenoid pigments from natural products in human diets (Takrury, 1986; El-Qudah, 1997, 2008, 2009).

The aims of this study are to determine the carotenoid contents in mallow, that is, to identify and quantitatively determine mallow carotenoids by using HPLC and LC-MS.

MATERIALS AND METHODS

Sample preparation: Due to the light sensitivity of carotenoids, the analysis was performed under red lights. The extraction of the vegetable, using a modified procedure from Riso and Porrini (1997), 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 Tetrahydrofuran (THF), followed by vortexing and centrifugation. The THF layers were combined with the methanol layer and the volume brought up to 50 mL. One milliliter of the extract was taken, dried under nitrogen and re-suspended in 1 mL of ethanol to be used for further analysis.

Reagent, chemicals and source of sample: High liquid chromatography grade ethanol, 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. A Bischoff C30 column (3.0 μm, 3.0×150 mm) was purchased from Mac-mod Analytical Company (Chadds Ford, PA, USA). Lutein and zeaxanthin standards were gifts from DSM Nutritional Products (Basel, Switzerland) and were dissolved in ethanol for HPLC analysis. Mallow was purchased at a vegetable market from Boston, MA, USA.

Chromatography, HPLC and LC/APCI-MS analysis: High Performance Liquid Chromatography (HPLC) analysis was performed using the Waters Alliance 2695 (Milford, MA, USA) equipped with a Bischoff C30 column. Mobile phase A was composed of methanol, Methyl terbutyl ether (MTBE), water and NH_4Ac with the following ratios according to Li *et al.* (2007): 60:33:7 (v/v/v) (1.5% NH_4Ac in water). Mobile phase B contained (8%) methanol, (90%) MTBE, (2.0%) water and (1.0%) NH_4Ac in water.

The gradient procedure was as follows: (1) a 100% mobile phase A for 3 min; (2) a 15 min linear gradient to 80% mobile phase A; (3) a 17 min linear gradient to 55% mobile phase B; (4) a 15 min linear gradient to 95% mobile phase B and (5) a 2 min gradient back to 100% mobile phase A. Once the gradient was completed, the HPLC mobile phase was kept at 100% mobile phase A and the column was re-equilibrated for 15 min. The flow was 0.4 mL min^{-1} and the injection volume was 20 μL. Lutein, zeaxanthin and their isomers were measured at 445 nm.

To determine the mass of the two unidentified peaks, the LC-MS method with a positive ion mode was used. The LC was an Agilent 1100 and the mass spectrometers was a Bruker Esquire LC equipped with an Atmospheric Pressure Chemical Ionization interface (APCI). The extracted mallow samples were re-suspended in ethanol and injected into the on-line LC/APCI-MS. The mass spectrometer was set to scan the range of 530 - 630 Dalton and a temperature of the ionization chamber was set at 300°C. The voltage of the corona needle was optimized, resulting in a current of 4 to 8A. The drying and carrier gas was nitrogen at a temperature of 300°C.

Dietary intake: A cross-sectional dietary survey was carried out in a random sample of the adult population living in Amman, Jordan . Dietary intake was assessed by means of a Food-Frequency Questionnaire (FFQ), which was designed to estimate food intake over the past year (Nasreddine *et al.*, 2006). A self- reported questionnaire was administrated to 200 participants (100 males and 100 females), ranging in age from 18-70 years. The individual was asked to estimate the number of times per day, week, month or year he/she consumed this particular food product and the amount usually eaten per food item by making comparisons with the specified reference portion. The dietary intake of mallow was determined by using FFQ and data are expressed as serves per person per week. A serving of vegetable was defined as half a cup of cooked Mallow.

RESULTS

Table 1 shows dietary intake of cooked mallow by a sample of 200 adults residents in Jordan (100 men and 100 women) age 18-70 year. The average consumption is 1.27

Table 1: Dietary intake of mallow by men and women in Jordan

	Dietary intake (Mean±SD)		
	Men (n = 100)	Women (n = 100)	Total (n = 200)
Mallow (cooked)	1.26 ± 1.159	1.28 ± 0.98	1.27 ± 1.06

Data are expressed as serving/ person/ week

Table 2: Carotenoid contents of Mallow (μg/100 g)[a]

	Lutein	Anhydro-lutein	Cis-anhydro-lutein	Neo-xanthin	Viola-xanthin	13-cis-B-C	t-B-C	9-cis-B-C	Total
Mallow	5332.8±9.12	4417.3±5.96	1041.4±2.68	1612.6±1.56	2685.6±0.46	88.9±0.09	3707.5±7.1	438.9±1.34	16907.9

[a]Mean of eight replications±SD

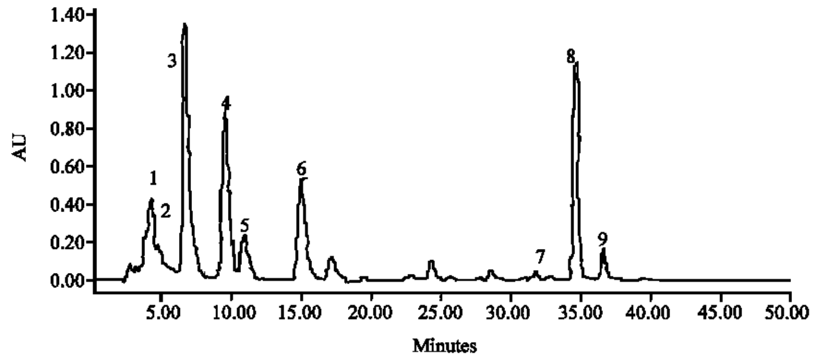

Fig. 1: Chromatographic profiles of mallow analyzed by HPLC. 1: Neoxanthin; 2: Violaxanthin; 3: Lutein; 4: Anhydrolutein; 5: Cis-anhydrolutein; 6: Chlorophyll; 7: 13-cis-β-carotene; 8: total β-carotene; 9: 9-cis-β-carotene

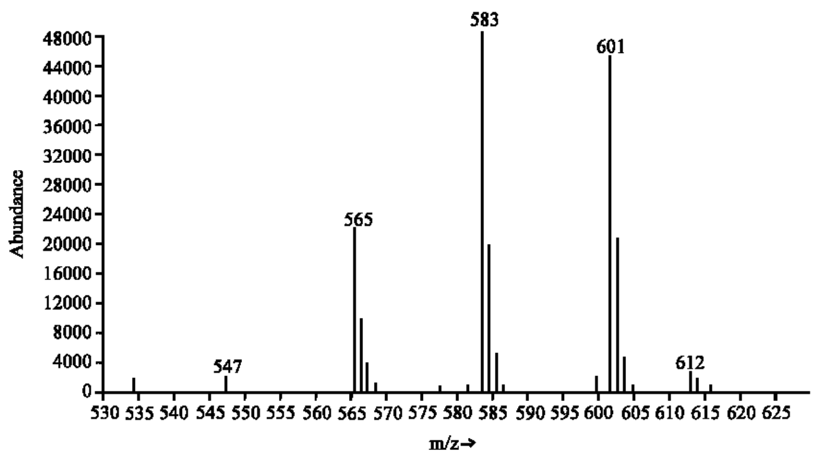

Fig. 2: Mass spectrum of mallow neoxanthin analyzed using APCI-LC/MS

cup per person per week. This indicates that its consumption is relatively high among Jordanian and it is well accepted meal. Mallow is usually consumed with rice and chicken, which increase the nutritive value of the whole meal.

Using standards, relative retention times and spectral characteristics carotenoids in mallow were identified. Figure 1 shows chromatographic profiles of mallow. Lutein, anhydrolutein and β-carotene were the major identified peaks. For further identification, neoxanthin and violaxanthin (Fig. 2, 3) were characterized by LC/APCI-MS in a positive mode.

The results for lutein, anhydrolutein (Budowski *et al*., 1963), cis-anhydrolutein, neoxanthin, violaxanthin, β-carotene, 13-cis-β-carotene and 9-cis-β-carotene content are presented in Table 2. The results presented in the table corresponding to the mean value of 8 replicates. The standard deviation for eight different samples is also included in the Table 2. The total carotenoids in mallow were estimated as a sum of lutein, anhydrolutein, cis-anhydrolutein, neoxanthin, violaxanthin, β-carotene, 13-cis-β-carotene and 9-cis-β-carotene content per unit weight. Lutein was found in highest amounts (5333±9 μg/ 100 g), representing about 31.4% of the total carotenoids content, followed by anhydrolutein (4417±6 μg/100 g) and β-carotene (3708±7 μg/100 g).

DISCUSSION

In this study we have identified appreciable levels of lutein and β-carotene in mallow, which is a green leafy vegetable commonly consumed as part of a Mediterranean diet. Various studies have shown a

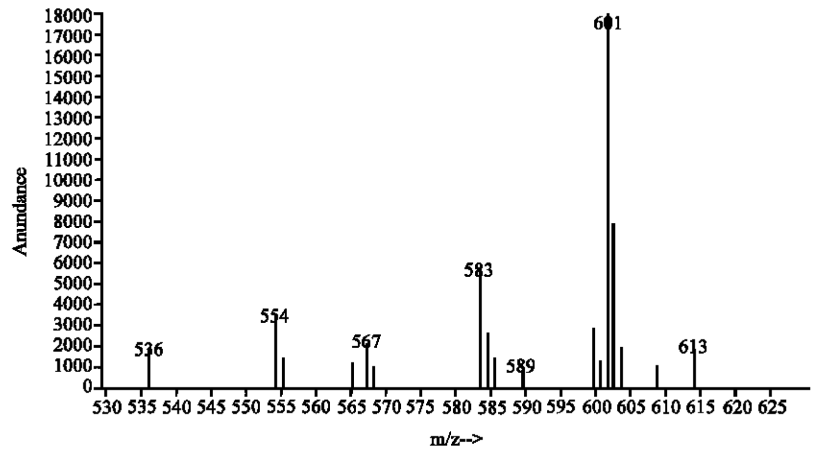

Fig. 3: Mass spectrum of mallow violaxanthin analyzed using APCI-LC/MS

significant correlation between habitual vegetable and fruit intake and plasma carotenoid concentrations (Martini *et al*., 1995; El-Sohemy *et al*., 2002; Agudo *et al*., 2002; Jansen *et al*., 2004).

Although there is no data on carotenoids content of mallow in the USDA National Nutrient database , the food composition tables for use in the Middle East show that the content of 100 g edible portion of mallow is 1260 retinol equivalent based on a conversion factor of 6 µg β-carotene or 12 µg other provitamin A carotenoid pigments to 1 µg retinol (Pellett and Shadarvian, 1970). FAO database indicates that the content of 100 g of raw leaves of mallow contain 6400 µg β-carotene (FAOUN, 1982). A recent study conducted in Tanzania, shows that the β-carotene content of Jute mallow in Singida distinct and Muheza district were 1.91 and 6.31 mg/100 g, respectively (Weinberger and Msuya, 2004).

The total carotenoid found in mallow by this study was about 16.9 mg/100 g and in which, β-carotene was about 3.7 mg/100 g. These values were higher than or approximately equal to those found in other commonly consumed vegetables. The β-carotene content in mallow is approximately two third of the value of spinach reported in the USDA database (U.S. Department of Agriculture, Agricultural Research Service, 2006). Similar contents of β-carotene are also seen in Korean database (National Rural Living Science Institute, 2001) and the Austrian database (Murkovic *et al*., 2000).

β-carotene is the most important provitamin A, having about twice as much activity as α-carotene. Lutein is the carotenoid implicated in the reduced risk of cataract and muscular degeneration (Moeller *et al*., 2000), together with zeaxanthin. It has also been reported to be inversely associated with colon cancer in both men and women (Slattery *et al*., 2000). Although possible health benefits of neoxanthin, violaxanthin, lactucaxanthin and other carotenoids have not been shown, their determination is important for future studies, as they have similar structures to those of carotenoids considered important to human health.

CONCLUSION

Vegetable mallow has lutein, anhydrolutein and β-carotene as principal carotenoids. The results of this study will provide basic distary carotenoids contents to update the Middle East food nutrition database. Thus, the information of dietary mallow carotenoids will help to better estimate dietary intakes and adequacies of vitamin A nutrition from food consumption surveys conducted in the region.

ACKNOWLEDGMENTS

The authors wish to thank USAID for their fund and Jean Mayer USDA-HNRCA, Tufts University for a fellowship given to the first author.

REFERENCES

Agudo, A., N. Slimani, M.C. Ocke, A. Naska and A.B. Miller *et al*., 2002. Consumption of vegetables, fruit and other plant foods in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts from 10 European countries. Public Health Nutr., 5: 1179-1196.

Anguelova, T. and J. Warthesen, 2000. Degradation of lycopene, α-carotene, β-carotene during lipid peroxidation. J. Food Sci., 6: 71-75.

Budowski, P., I. Ascarelli, J. Gross and I. Nir, 1963. Provitamin A_2 from lutein. Science, 142: 969-971.

Chen, T.S. and S. Saad, 1981. Folic acid in Egyptian vegetables: The effect of drying method as storage on the folacin content of mulukhiyah (*Corchorus olitorius*). Ecol. Food Nutr., 10: 249-255.

El-Qudah, J.M., 1997. Biochemical researches on the nature, nutrition and utilization of carotenoids pigments from vegetables cultivated in Jordan. Ph.D. Thesis, Babes-Bolyai University, Cluj-Napoca, Romania.

El-Qudah, J.M., 2008. Dietary intake of selected common vegetable foods and their total carotenoids determination. Am. J. Agric. Biol. Sci., 3: 729-733.

El-Qudah, J.M., 2009. Identification and quantification of major carotenoids in some vegetables. Am. J. Applied Sci., 6: 492-497.

El-Sohemy, A., A. Baylin, E. Kabagambe, A. Ascherio, D. Spiegelman and H. Campos, 2002. Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake. Am. J. Clin. Nutr., 76: 172-179.

FAOUN, 1982. Food Composition Tables for the Near East. Food and Agriculture Organization of the United Nations, Rome, Italy Pages: 263.

Franceschi, S., E. Bidoli, C.L. Veccia, R. Talamini, D. Avanzo and E. Negri, 1994. Tomatoes and risk of digestive-tract cancers. Int. J. Cancer, 59: 181-184.

Jansen, M.C., A.L. van Kappel, M.C. Ocke, P. van't Veer, H.C. Boshuizen, E. Riboli and H.B. Bueno-de-Mesquita, 2004. Plasma carotenoid levels in Dutch men and women and the relation with vegetable and fruit consumption. Eur. J. Clin. Nutr., 58: 1386-1395.

Kalt, W., 2005. Effect of production and processing factors on major fruits and vegetable antioxidants. J. Food Sci., 70: R11-R19.

Krinsky, N.I. and E.J. Johnson, 2005. Carotenoid actions and their relation to health and disease. Mol. Aspects Med., 26: 459-516.

Li, L., J. Qin, S. Yin and G. Tang, 2007. Effects of mobile phase ratios on the separation of plant carotenoids by HPLC with a C30 column. Cromatographia, 65: 91-94.

Martini, M.C., D.R. Campbell, M.D. Gross, G.A. Grandits, J.D. Potter and J.L. Slavin, 1995. Plasma carotenoids as biomarkers of vegetable intake: The University of minnesota cancer prevention research unit feeding studies. Cancer Epidemiol. Biomarkers Prev., 4: 491-496.

Mayne, S.T., D.T. Janerich, P. Greenwald, S. Chorost and C. Tucci *et al*., 1994. Dietary β carotene and lung cancer risk in U.S. nonsmokers. J. Natl. Cancer Inst., 86: 33-38.

Moeller, M.S., P.F. Jacques and J.B. Blumberg, 2000. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. J. Am. Coll. Nutr., 19: 522S-527S.

Murakoshi, M., H. Nishino, Y. Satomi, J. Takayasu and T. Hasegawa *et al*., 1992. Potent preventive action of α-carotene against carcinogenesis: Spontaneous liver carcinogenesis and promoting stage of lung and skin carcinogenesis in mice are suppressed more effectively by α-carotene than β-carotene. Cancer Res., 52: 6583-6587.

Murkovic, M., K. Gams, S. Draxl and W. Pfannhauser, 2000. Development of an austrian carotenoid database. J. Food Compos. Anal., 13: 435-440.

Nasreddine, L., N. Hwalla, A. Sibai, M. Hamze and D. Parent-Massin, 2006. Food consumption patterns in an adult urban population in Beirut, Lebanon. Public Health Nutr., 9: 194-203.

National Rural Living Science Institute, 2001. Food Composition Table. 6th Edn., Rural development Administration, Suwon, Republic of Korea.

Okusanya, O.T., 1979. Quantitative analysis of the effects of photoperiod, temperature, salinity and soil types on the germination and growth of *Corchorus olitorius*. Oikos, 33: 444-450.

Pellett, P. and S. Shadarvian, 1970. Food Composition Tables for Use in the Middle East. 2nd Edn., American University of Beirut, Lebanon.

Rahman, A., 1978. Effects of gibberellic acid on the germination of *Corchorus capsularis* and *Corchorus olitorius*. Bangladesh J. Sci. Ind. Res., 13: 243-246.

Rao, A.V. and L.G. Rao, 2007. Carotenoids and human health. Pharmacol. Res., 55: 207-216.

Riso, P. and M. Porrini, 1997. Determination of carotenoids in vegetable foods and plasma. Int. J. Vitamin Nutr. Res., 67: 47-54.

Slattery, M., J. Benson, K. Curtin, D. Schaeffer and J. Potter, 2000. Carotenoids and colon cancer. Am. J. Clin. Nutr., 71: 575-582.

Stivala, L., M. Savio, O. Cazzalini, R. Pizzala and L. Rehak *et al*., 1996. Effect of β-carotene on cell cycle progression of human fibroblasts. Carcinogenesis, 17: 2395-2401.

Takrury, H., 1986. The effects of storage on carotene values in some plants. Dirasat, Uni. Jordan, 13: 87-93.

U.S. Department of Agriculture, Agricultural Research Service, 2006. USDA nutrient database for standard reference, rlease 18. Nutrient Data Laboratory, http://www.nal.usda.gov/fnic/foodcomp/search/

Verma, M.M. and N. Arora, 1978. Further studies on seed testing procedures for Jute (*C. capsularis* and *C. olitorius*). Seed Res., 6: 151-157.

Weinberger, K. and J. Msuya, 2004. Indogenous vegatables in Tanzania. Technical Bulletin no. 31, http://www.Avrdc.org