Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 8 (4): 162-167, 2014
ISSN 1819-3471 / DOI: 10.3923/rjphyto.2014.162.167

Quantification of Antioxidant Phytochemicals in Fresh Vegetables Using High Performance Liquid Chromatography

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ABSTRACT

The antioxidant phytochemicals viz., α-carotene, β-carotene, xanthophyll and lycopene were estimated in fresh samples of tomato, bitter gourd, brinjal, bottle gourd, pumpkin, cucumber, pointed gourd, chilli, okra and radish at the edible maturity stage using reverse phase HPLC system. The mean value of α-carotene, β-carotene and xanthophyll were 0.611, 4.637 and 0.995 mg/100 g, respectively. The mean lycopene content in tomato was found 7.231 mg/100 g. The range of α-carotene, β-carotene and xanthophyll were 0.049-1.919, 0.721-12.34 and 0.047-2.545 mg/100 g, respectively. Results indicate that vegetables are relatively abundant source of natural antioxidants.

Key words: α-carotene, β-carotene, xanthophyll, lycopene, antioxidants, vegetables

INTRODUCTION

Vegetables offer a cheap but rich source of a number of micronutrients and other phytochemicals having antioxidant properties. Vegetable are rich source of β-carotene (provitamin-A), thiamin, niacin, folic acid, lycopene and vitamin C. Tomato (*Lycopersicon esculentum* Mill) is known for its outstanding nutritive value and is popular as one of the most important protective food (Rawat and Kumar, 1996). The tomato fruit has attracted considerable attention, since the red pigment lycopene in tomato fruits, is a powerful antioxidant (Dwyer *et al.*, 2001). The antioxidant capacity of lycopene is at least twice that of β-carotene. Although lycopene provides no provitamin A activity, still lycopene attracts considerable attention because of its other potential health benefits (Giovannucci, 1999) by protecting against oxidative damage implicated in the pathogenesis of several human chronic diseases. In addition, the protective role of lycopene may also be related to its non antioxidant properties such as the reduction of cellular proliferation and modulation of intercellular gap-junction communication (Stahl *et al.*, 2002). It is known to reduce prostate cancer risk significantly (Giovannucci, 1999). Carotenoids are important nutritionally as antioxidants in the prevention of atherosclerosis and in the prevention of Age-related Macular Degeneration (AMD) (Palozza and Krinsky, 1992; Dwyer *et al.*, 2001; Moeller *et al.*, 2000). The hydrocarbon carotenoids have provitamin A activity and the oxygenated carotenoids like xanthophyll are linked to a lower risk of cancer (Beecher and Khachik, 1984). β-carotene has been shown to prevent peroxidation caused by singlet oxygen and also by scavenging free radicals. Some workers have reported the possibility that certain carotenes or their isomers have anticancer potential (Beems, 1987; Hennekens, 1986). Bitter gourd

(*Momordica charantia*), bottle gourd (*Lagenaria siceraria*), pointed gourd (*Tricosanthes dioica*) pumpkin (*Cucurbita pepo*) and cucumber (*Cucumis sativus*) all belong to the same botanical family Cucurbitaceae and are a valuable source of conventional antioxidant nutrients including vitamin C and β-carotene.

Interest in the role of antioxidants in human health has prompted research in the fields of food science and horticulture to assess vegetable antioxidants. There is a great need for quantitative data on the antioxidant content in vegetables. The objective of this study was to quantify the major antioxidant phytochemicals based on HPLC estimations in major vegetables.

MATERIALS AND METHODS

Ten different vegetables were purchased from the local markets. Each vegetable samples were taken separately and mixed together to get a composite sample of each vegetable. From composite sample, 100 g of lab sample was taken and from that, 5 g of sub sample of each vegetable was selected for extraction. The samples were washed with double distilled water, air dried and were stored at -4°C temperature.

Extraction and analysis of β-carotene, lycopine and lutein: The procedure described by Kurilich *et al.* (1999) was followed for the analysis of β-carotene as well as lutein. The 300-500 mg of sample was taken in a test tube and added to it 10 mL of ethanol containing 0.1 g of BHT. The test tube along with the sample was placed in a water bath at 70°C for 15 min. After removing the tubes from the water bath, added 180 μL of 80% KOH to each tube. The sample was vortexed and then saponified at 70°C for 30 min. Saponification was essential for maximum extraction of carotene and their esters. The samples were placed directly on ice bath and 2.5 mL of de-ionized water and 2.5 mL Hexane/toluene mixture (10:8). Then the tubes were vortexed and then centrifuged at 2100 rpm for 5 min. The upper layer hexane/toluene fraction was then transferred to a separate test tube. The hexane/toluene extraction was repeated for two more times. The combined hexane/toluene fractions were dried using a Speed-vac concentrator. The residue was reconstituted in 200-400 μL THF. The solution was filtered on a 0.2 μL filter and 20 μL of the filtered solution was injected in the Shimadzu High performance liquid chromatograph. The mobile phase consisted of Acetonitrile: Methanol: THF (52:40:8) (v/v/v) at a flow rate of 2.0 mL min^{-1}. The absorbance was recorded at 450 nm for β-carotene and lutein. The retention time for the standard β-carotene was recorded as 6.19 min, for lutein 2.35 min and for vitamin A at 4.4 min (Fig. 1-3).

The identification of α/β-carotene as well as xanthophyll and lycopene was made using retention time and peak area comparison of the chromatograms of commercially available standards of the above antioxidants (Sigma Chemical Co., St. Louis, MO).

RESULTS AND DISCUSSION

The aim of this study was to characterize the antioxidant phytochemicals of the vegetables being consumed by the Indian population with particular attention to β-carotene, α-carotene, lycopene and xanthophyll. In this study the results indicate that among the ten vegetables tested, the range of β-carotene ranged from 0.721-12.345 mg/100 g, α-carotene varied from 0.049-1.919 mg/100 g and xanthophyll from 0.047-2.545 mg/100 g on fresh weight basis,

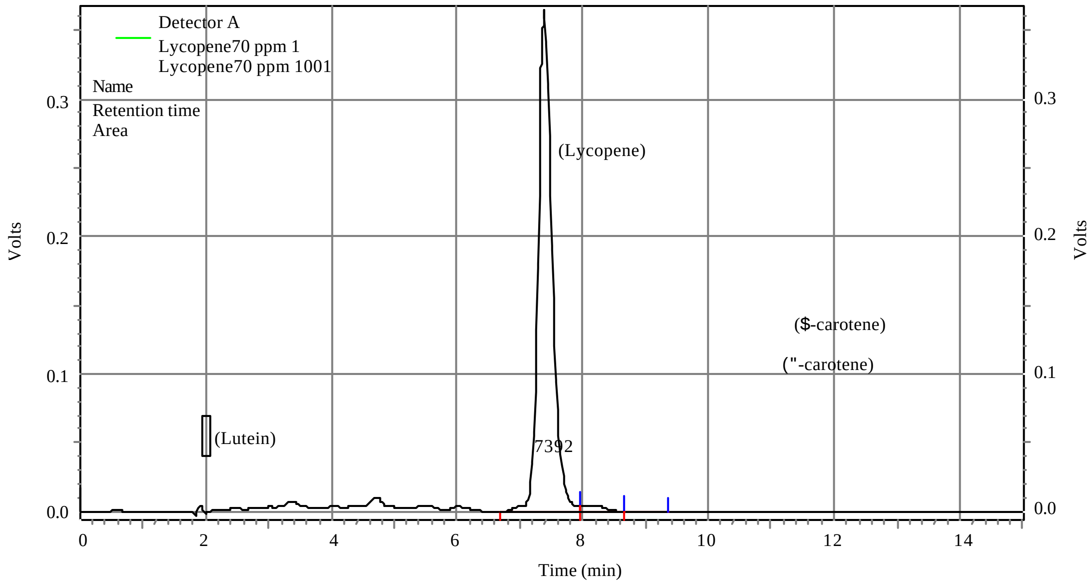

Fig. 1: Chromatogram of standard peak of lycopene

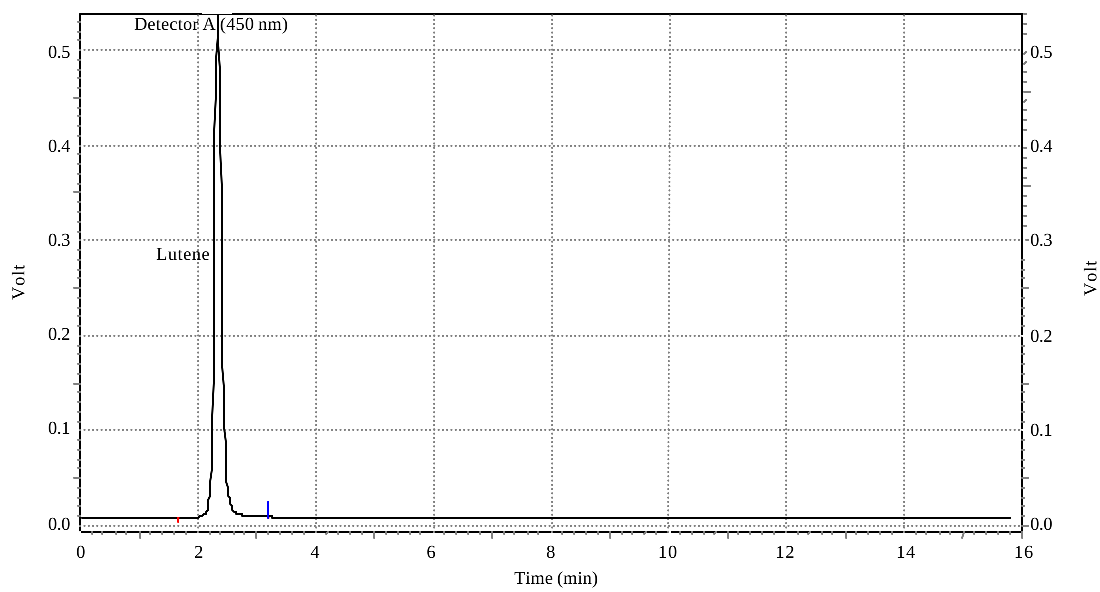

Fig. 2: Chromatogram of standard peak of xanthophyll

respectively (Table 1). The mean value of α-carotene, **β**-carotene and xanthophyll were 0.611, 4.637 and 0.995 mg/100 g, respectively in the selected vegetables. The maximum **β**-carotene content was found in tomato 12.345 mg/100 g and minimum in bottle gourd 0.721 mg/100 g. The maximum α-carotene content was also found in tomato (1.919 mg/100 g) and minimum in bottle gourd (0.049 mg/100 g). Previous studies reported **β**-carotene content in carrot (10.11 mg/100 g), spinach (8.23 mg/100 g), lettuce (3.94 mg/100 g) and tomato (1.93 mg/100 g). Williams and Caliendo

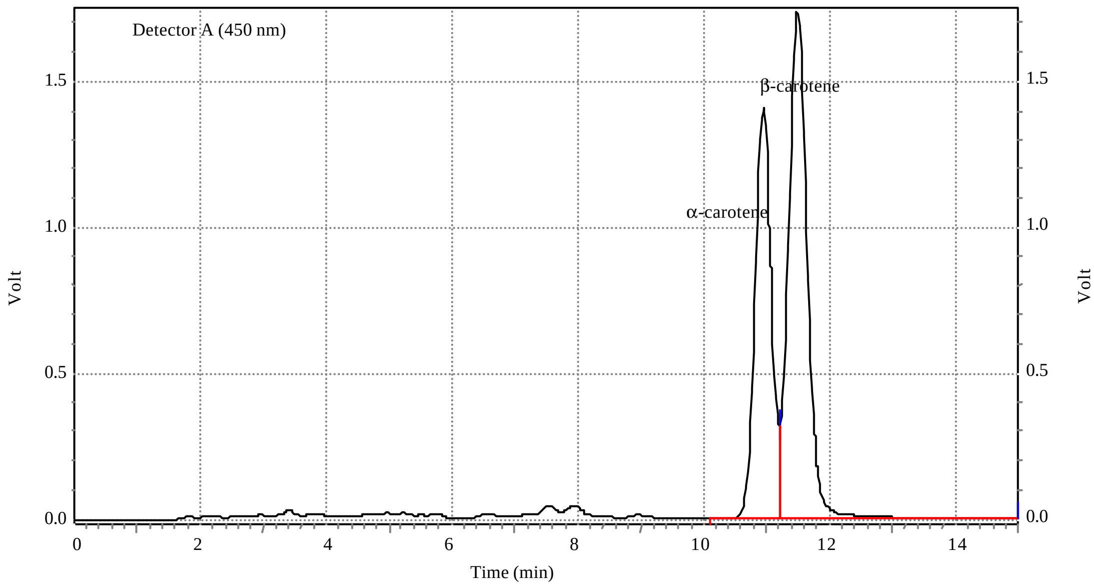

Fig. 3: Chromatogram of standard peak of α-carotene and β-carotene

Table 1: Carotenoids content (mg/100 g FW) in some raw vegetables

Vegetables	β-carotene	α-carotene	Xanthophyll	Lycopene
Tomato (*Lycopersicon esculentum* Mill)	12.345	1.919	0.310	7.231
Biter gourd (*Momordica charantica* L.)	2.511	0.669	1.024	-
Brinjal (*Solanum melongena* L.)	0.739	0.246	0.280	-
Bottle gourd (*Lagenaria siceraria* (Molina) Standl)	0.721	0.049	0.779	-
Pumpkin (*Cucurbita pepo*)	3.345	0.582	0.795	-
Cucumber (*Cucumis Sativus* Mill.)	5.976	0.235	1.535	-
Pointed gourd (*Trichosanthes dioica* Roxb)	6.570	1.104	2.545	-
Chilli (*Capsicum annuum* L.)	5.089	0.416	1.285	-
Okra (*Abelmoschus esculentus* (L.)	7.663	0.276	1.354	-
Radish (*Raphanus sativus* L.)	1.411	-	0.047	-
Mean	4.637	0.611	0.9954	-
Range	0.721-12.345	0.049-1.919	0.047-2.545	-

(1984), reported β-carotene content in lady finger (0.520 mg/100 g). Agte *et al*. (2000), analyzed 24 green vegetables for β-carotene and reported that β-carotene content in green vegetables ranges from 0.08-9.20 mg/100 g. The β-carotene content of these vegetables also corresponds to those reported by Robinson *et al*. (1986). However, Niizu and Rodriguez-Amaya (2005) reported much lower content of β-carotene in carrot (5.34 mg/100 g) and in contrast, they reported much higher content of β-carotene in tomato (3.50 mg/100 g).

Tharasena and Lawan (2012) reported the β-carotene content in brinjal (3.05±0.08 mg/100 g) and okra 31.3±1.09 mg/100 g). The mean β-carotene content (12.34 mg/100 g) for tomato reported in the present study was higher than the values reported earlier (Niizu and Rodriguez-Amaya, 2005), however, the value for β-carotene content in okra (7.663 mg/100 g) was lower than value reported earlier (Tharasena and Lawan, 2012). The large variation in β-carotene content of tomato may be due to the use of immature samples, because the content of β-carotene drops by 77% during the ripening process (Rigo *et al*., 1999). The amount of α-and β-carotene is also influenced by growing season and storage time prior to analysis (Goodwin, 1980). Azevedo-Meleiro and Rodriguez-Amaya (2007) studied the qualitative and quantitative differences in carotenoid composition among *Cucurbita moschata, Cucurbita maxima* and *Cucurbita pepo*. The principal carotenoids in *C. moschata* were β-carotene and α-carotene, whereas, lutein and β-carotene dominate in *C. maxima* and *C. pepo*.

Among the ten vegetables tested for xanthophyll content, the maximum xanthophyll content was recorded in pointed gourd (2.545 mg/100 g) and minimum in brinjal (0.280 mg/100 g). In an earlier study, Tharasena and Lawan (2012) reported value for xanthophyll content in brinjal (0.29±0.01 mg/100 g) which is very close to the values reported in the present study, however, in contrast to this very high values for xanthophyll content was reported by the same authors for Okra (9.56±0.15 mg/100 g) in comparison to the value reported in the present study for okra (1.35 mg/100 g). The variation in the data compared to others may be due to varietal differences as well difference in experimental condition, extraction procedures and different solvents used as mobile phase in HPLC.

The mean lycopene content in tomato (7.231 mg/100 g) recorded in the present study was in range to values reported earlier (Garcia and Barrett, 2006), where mean lycopene values ranged from 78.8-131.2 mg kg^{-1}. In another study (Hyman *et al*., 2004), it was reported that fruit lycopene content ranged from 0-246 μg g^{-1} Fresh Weight (FW), whereas, β-carotene content ranged from <1-12 μg g^{-1} FW across the 24 tomato genotypes. The lycopene content of tomatoes depends on the cultivar and maturity stage and is affected by growing conditions, temperature and humidity, among other factors (Sharma and Le Maguer, 1996; Giovannucci, 1999; Abushita *et al*., 2000; Dumas *et al*., 2003).

ACKNOWLEDGMENT

The authors are thankful to Prof. Ganga Bisht, Head Chemistry Department, Kumaun University, Nainital for encouragement and necessary laboratory facilities.

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