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Estimation of Carotenoid Contents of Selected Mediterranean Legumes by HPLC

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Abstract: Vegetables, fruits and legumes are important foods for humans and their consumption maintain health and may reduce the risk of diseases. Carotenoids and provitamins A are considered important for human health. Four legumes (faba bean, chickpea, lentil and dry beans) were analyzed for their carotenoid composition and content by using high performance liquid chromatography (HPLC). Neoxanthin and violaxanthin were found in all samples except dry beans. Lutein was present in all the legumes studied. With the exception of faba bean for Zeaxanthin, both Zeaxanthin and β-Carotene were found in all samples. Faba bean, chickpea, lentil and dry beans contained appreciable amounts of β-carotene (0.089, 0.077, 0.157 and 0.039 μg g^{-1}, respectively). Lentil had the highest concentration of lutein (7.348 μg g^{-1}). In conclusion, the results revealed from this study agree with those reported in the literature. Although, legumes are considered as a poor source of carotenoids, they can provide appreciable amounts of carotenoids that are considered essential to human health.

Key words: Carotenoids · Legumes · Mediterranean Foods · HPLC

INTRODUCTION

Carotenoids comprise a class of natural lipid-soluble pigments which are found in numerous vegetables and fruits [1]. They are a class of naturally occurring lipophilic pigments and about 50 of them occur in foods with plant origin. Epidemiologic studies showed that high carotenoid intake such as α-carotene, β-carotene and lycopene, is associated with a decrease in the incidence of many chronic diseases [2-4]. This is due to the facts that carotenoids are considered to be antioxidants in biological tissues as well as in food systems [5].

Legumes are considered as a nutrient dense foods, they are low in calories and are a good source of vitamins and minerals, dietary fiber and phytochemicals including carotenoids.

Legumes such as beans chickpea and lentils are grown and consumed in most countries of the world. They are considered a staple protein source in many Asian, African and Middle Eastern countries.The quality of protein found in legumes is considered to be high.For example Chickpea, which ranks third among the worlds food legumes [6], has high amounts of most essential amino acids. As staple foods, these legumes are widely consumed among populations in countries with low economic status and are viewed as a health-food in Western countries [7-10].The level of carotenoids concentrations of its seeds is above the engineered beta-carotene-containing "golden rice" level [11]. There is an increasing need for these legumes due to their high nutritional value [12-14]. Overall, they are considered as healthy foods and, could have beneficial effects in combination with other cereals and pulses on prevention of human illnesses [15].

In Jordan, like most Middle East countries, legumes consumption is relatively high. In our previous works, we identified some rich sources of carotenoids from different foods [16-18].In this work, carotenoid composition and content for four legumes (Faba bean, chickpea, lentil and dry beans) were studied by HPLC.

MATERIALS AND METHODS

The study was conducted at Carotenoids and Health Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA), Tufts University, Boston, USA, during summer 2007. Legumes were obtained from Boston, MA, USA.

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Reagents and chemicals:All-trans- β-carotene, lutein, zeaxanthin, ammonium acetate (analytical grade) and Methyl-Tert-Butyl Ether (MTBE) were obtained from Sigma Company (St Louis, MO, USA). Milli Q water was obtained from Water Purification Systems and used for all procedures using water. Methanol and Tetra Hydro Furan (THF) were purchased from JT Baker Chemical (Philipsburg,NJ), USA.

Chromatography, HPLC and analysis of carotenoids:Carotenoids were extracted as previously reported with minor modification [19] and described in full in our previous works [16-18]. Briefly, an HPLC system equipped with C30 column (3 ìm, 150×4.6 mm, YMC, Wilmington, NC, USA) was used to quantify carotenoids. 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. Determining peak areas under the curve in the high-performance liquid chromatograms calibrated against known amounts of standards were used to quantify carotenoids. Each peak was confirmed by the retention time and characteristic spectra of the standards.

RESULTS

Common Arabic, English and Scientific names of legume foods included in this study were found in table 1. Chromatographic profiles of legumes (dry beans, faba bean, chickpea and lentil) were shown in Fig. (1a,b,c and d, respectively). Major identified peaks were neoxanthin, violaxanthin, lutein, Zeaxanthin and β-carotene. A number of unidentified compounds (U1-U5) were present in chickpea and lentil.

Carotenoid contents of legumes were given in table 2. Lutein was found in all the legumes studied and was clearly the major carotenoid in the legumes. Neoxanthin and violaxanthin were contained in all samples except dry beans. With the exception of faba bean for Zeaxanthin, both Zeaxanthin and β-Carotene were found in all samples. Faba bean, chickpea, lentil and dry beans

Table 1: Common Arabic, English and Scientific names of foods included in the study

Common Arabic Name	English Name	Scientific name
Foul	Faba bean	*Vicia faba*
Hummus	Chickpea	*Cicer arietinum* L.
Adas	Lentil	*Lens esculenta*
Fasolia Jaffeh	Dry beans	*Phaseolus vulgaris*

Table 2: Carotenoid contents, edible portion, of studied legumes μg g^{-1}

Vegetables	Neoxanthin	Violaxanthin	Lutein	Zeaxanthin	β-Carotene
Faba bean [a]	0.131	0.196	2.683	-[b]	0.089
Chickpea	0.163	0.552	3.357	1.484	0.077
Lentil	0.252	1.139	7.348	2.773	0.157
Dry beans	-	-	0.094	0.017	0.039

[a] Values represent mean of three measurements

[b] Not determined

Table 3: Chromatographic properties and concentration of unidentified compounds in selected components of the Mediterranean legumes

Peak	U1	U2	U3	U4	U5
Chickpea					
Retention time (min)	15.51	17.03	17.61		
Absorption maxima (nm)	440.6 464.8 485.4	440.6 467.2	422.5 440.6 485.4		
Concentration (μg g^{-1})	1.171	0.779	0.101		
Lentil					
Retention time (min)				17.13	17.69
Absorption maxima (nm)				440.6	440.6 485.4
Concentration (μg g^{-1})				0.150	0.139

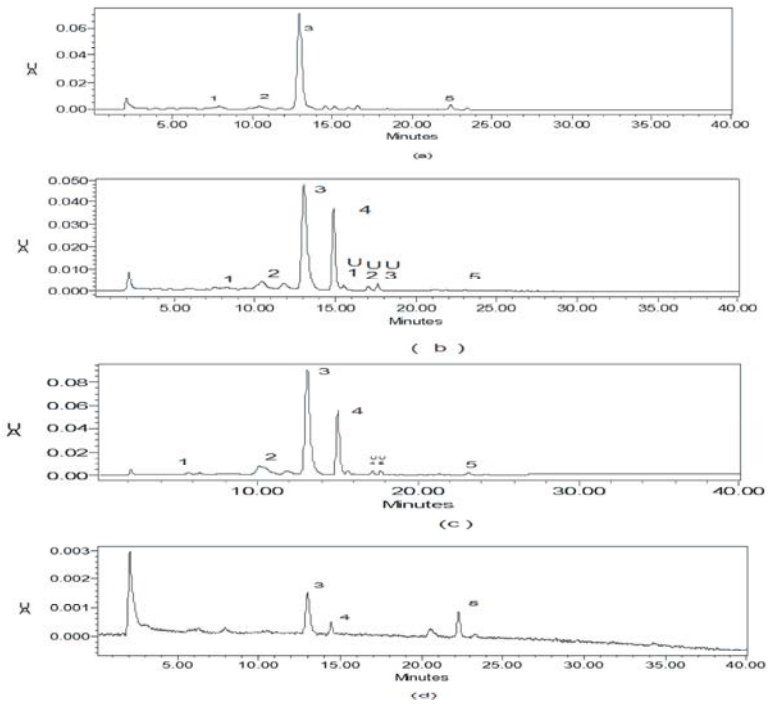

Fig. 1: Typical HPLC chromatograms of the carotenoids of (a) faba bean (b) chickpea (c) lentil and (d) dry beans. Peak identification: (1) neoxanthin, (2) violaxanthin, (3) lutein, (4) zeaxanthin, (5) β-carotene. Unidentified peaks: U1-U5.

contained appreciable amounts of β-carotene (0.089, 0.077, 0.157 and 0.039 μg g^{-1}, respectively). Lentil had the highest content of lutein (7.348 μg g^{+}).

The absorption maxima, Retention time and concentrations of unidentified compounds in chickpea (U1-U3) and lentil (U4 and U5) were shown in Table 3.

DISCUSSION

It is a common knowledge that legumes contain many nutrients. In the Middle East, traditional foods, including legumes, are important source of nutrients and vitamins for many persons in the community.

In this work,we identified appreciable amounts of carotenoid in legumes. In general, legumes are poor sources of β-carotene. β-carotene is a main precursor of retinol in human body [20]. Deficiency in vitamin A in diet has long been identified as a serious nutrition problem [21]. Wide variation in the content of β-carotene as well as other types of carotenoid was observed in the samples of dry beans, faba bean, chickpea and lentil (Table 2).

This study revealed that lentil contained the highest level of lutein, Zeaxanthin and β-carotene, at least two times greater than any other legume evaluated. In this study β-Carotene content ranged from 0.039 μg g^{-1} (dry beans) to 0.157 μg g^{-1} (lentil) and for the lutein content similar variation was observed too. Similar results were obtained by other studies [22-25].

USDA databases [26] showed that the content of vitamin A, RAE in faba beans, lentils and chickpeas are 3, 2 and 3 μg /100g, respectively. FAO, database [27] showed concentration of 62.5 μg / 100g of β-Carotene in lentil. Kandlakunta *et al.* [22] found concentration of 580 ± 13.8 μg / 100g of total carotenoids and 2 ± 1.14 μg / 100g of β-Carotene in lentil, while they were 1260 ± 34.6 and 393 ± 23.6 μg / 100g in dry beans, respectively.

Lako *et al.* [28], found that dry beans (*Phaseolus vulgaris*) has β-Carotene in concentration of 0.78 mg/100g. E-Siong *et al.* [23] reported for lutein and β-carotene in chickpea 683 and 40 (μg / 100g) respectively and in Egyptian bean 169 and 7 μg / 100g respectively. These results are generally in agreement with those in table (2).

Meals prepared with legumes are common in many Middle-Eastern countries [29] including Jordan [30]. Besides, Olive oil is widely used in food preparation as part of a Mediterranean diet, which enhances absorption of fat soluble compounds such as carotenoids [31].

It is well known that in comparison with the vegetables and fruits, the carotenoid concentration in legumes, are much lower. Most of green leafy vegetables reported by many researchers had carotenoid contents several times higher than in the legumes[32-34].

In this work, we identified appreciable amounts of carotenoid in the legumes analyzed.

Although all the legumes studied are considered to be a poor sources of carotenoids and β-Carotene, they are still of nutritional importance. If these foods are consumed in higher amounts, they can contribute significantly to total vitamin A intake.

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