International Journal of Horticultural Science 2011, 17 (4–5): 15–18.
Agroinform Publishing House, Budapest, Printed in Hungary
ISSN 1585-0404

Effect of organic and integrated farming on carotenoid and tocopherol content of apricot fruits

Hussein, G.D.[1], Bánáti, D.[1], Nyéki, J.[2] & Szabó, Z.[2]

[1]*Central Food research Institute, Herman Ottó u. 15, 1022 Budapest, Hungary (h.daood@cfri.hu)*
[2]*University of Debrecen, Centre of Agricultural Sciences Institute for Research and Development*

Summary: In modern and healthy diets antioxidants play an important role providing natural defence against serious diseases. Therefore it is recommended to include fruits and vegetables having high antioxidant capacity in daily diet in a due course. Apricot is one of the fruits receiving an increasing attention in this field. This study was conducted to investigate the composition and content of fat-soluble carotenoids and tocopherols in different varieties of apricot using recently developed liquid chromatographic methods. Also it was aimed to compare organic and integrated farming in their effect on carotenoid and tocopherol content of the fruits. The results showed that apricot fruit are rich in vital carotenoids and bioactive tocopherols with significant variation between different varieties. The organic farming had favourable effect on the level of the major carotenoids and depending on variety this technology either increases or does not have significant influence on vitamin E content.

Key words: apricot, carotenoid, tocopherol

Introduction

Apricot is among the fruits having special importance from nutritional point of view. The edible part of the fruit accumulate high amounts of biologically active carotenoids other macro- and micro-nutrients (*Mangels* et al., 1993; *Kita* et al., 2007). Carotenoids and tocopherols, as naturally occurring bioactive nutrients, have received an increasing interest during the last decades. The reactive oxygen species-scavenging activity is associated with reduced risk of serious diseases including cancer (*Keleman* et al., 2006; *Larsson* et al., 2010), cardiovascular disease (*Arab and Steck,* 2000) and age-related degeneration and disorder caused by deficiency of vital carotenoids and other bio-antioxidants (*Bone* et al., 2000; *Mozaffariech* et al., 2003; *Frankel and German,* 2003; *Schneidr,* 2005).

At post-harvest of plant crops, particularly during processing, carotenoids undergo some chemical alteration, such as isomerisation and oxidation on their molecules (*Niizu and Rodriguez-Amaya,* 2005). Such a chemical alteration can change, to a considerable extent, their chemical and biological properties (*Granado,* 1992). Under certain biological conditions all-trans to cis geometrical isomerisation occur not only in processed foods but also in human serum (*Molnár,* 2009)

The organic production of certain horticultural crops is increasing from year to year due to the increasing demand for fertilizer- and pesticide-free products. In conventional production fertilizer and pesticide residues threaten the health of the consumers since they increase the risk of serious diseases such as cancer. Due to the increasing level of environmental pollution and food contamination there is a great need to raise the level of endogenous antioxidants such as carotenoids, vitamin C and vitamin E in fruits and vegetables to neutralize the oxidizing effect of many dangerous pollutants. Stracke and co-workers (2009) found slight difference between organically and conventionally produced 'Golden Delicious' apple fruits in their poly-phenol contents and antioxidant capacities. The authors concluded that the impact of season climate is more effective than the cultivation technology. Carbonaro and Mattera (2001) found significantly higher polyphenol level in organic peaches, and more polyphenol oxidase activity in organic peaches and pears.

The objective of the present work was to study the carotenoids and tocopherol composition and content in apricot fruits cultivated under organic farming and integrated conditions, by newly developed HPLC methods.

Materials and methods

Freshly harvested fruits were obtained from the experimental fields of the University of Debrecen in Penedics and Boldogkőváralja. Standard all-trans β-carotene and lutein were purchased from Sigma-Aldricht (Budapest, Hungary). β-cryptoxanthin and lycopene were obtained from the Institute of Bio- and Medical Chemistry of the University of Péch (Hungary). HPLC- and analytical grade organic solvents were from Merck (Darmstat, Germany). A cross-linked Nucleodur ISIS, C-18 UPLC column was from Symetron Ltd (Budapest).

Extraction of carotenoids and tocopherols:

Five grams of fresh edible part ofthe fruit were crushed in a crucible mortar in presence of 1 g quartz sand and 0,5g ascorbic acid. The extraction procedure started with macerating with methanol to bind the water followed by addition of 1,2-dichloroethan and initiation of liquid-liquid phase extraction of carotenoids according to a previously described procedure (*Biacs and Daood* (1994)) After pooling, dehydration on anhydrous Na_2SO_4 of the pigment-containing phase, the solvent was evaporated under vacuum and the residues were re-dissolved in pure acetone. To saponify the extract it was re-dissolved in 20 ml petroleum ether and subjected to alkaline hydrolysis with 20 ml of 30%KOH in methanol for 1 h at dark place. Carotenoids were then extracted with ethyl acetate, which was pooled and washed with double distilled water. The organic layer was then dried on anhydrous Na_2SO_4 and evaporated to dryness under vacuum at 40 °C. The pigment was re-dissolved in HPLC acetone before injection on reversed-phase column. For tocopherol analysis the residues were re-dissolved in HPLC grade n-hexane before injection onto normal-phase silica column.

HPLC conditions and instrument:

A Waters Alliance liquid chromatographic instrument consisting of a Model 2695 Separation Module (Gradient pump, auto-sampler and column heater), a Model 2975 Photodiode-Array Detector and a Model 2475 fluorescence detector was used. Operation and data processing were performed by Empower software.

Separation of carotenoids was performed on Nucleodur ISIS, 3µm, 15 cm x 4,6mm, column (Macherey Nagel, Düren, Germany) with gradient elution of (A) water and (B) acetone. The elution started with 20% A in B, which changed to 10% A in B in 7 min then to 5% A in B in 5 min and stayed isocratic for 5 min and turned to 20% A in B in 3 min. The flow rate was 0.7 ml min^{-1}. Detection of carotenoids was between 200 and 700 nm. For quantification, each compound was detected at the maximum wavelength of its light absorbance. Identification of peaks from sample extract was based on comparison of their retention time and spectral characteristics with those of standard materials available in our laboratories and with data from literature.

As for tocopherol analysis, the separation was performed with isocratic elution on Nucleosil 100 Silica gel, 5 m, 250 x 4,6 mm column using mobile phase consisting of 99.6:0.4 n-hexane-absolute ethanol. The separated compounds were detected at EX: 295 nm and EM: 320 nm. Peak identification and quantification was based on use of external standard materials for γ-, β-, γ-, δ- tocopherol analogues.

Results and Discussion

HPLC profiles of carotenoids from un-hydrolysed extract of ripe apricot fruit is shown in *Figure1*. In such a crop, carotenoids occur un-esterified with fatty acids. According to the HPLC profile β-carotene and its cis-isomers were dominant carotenoids followed by all trans-lycopene, its cis isomer, neo-lycopene, rubixanthin and γ-carotene. As minor individuals, β-cryptoxanthin, α-carotene, and some unidentified carotenoids could be detected. This composition agrees, to a high extent, with that reported by *Baurenfiend* (1981) and *Mangels* et al., (1993) with more compounds could be separated and detected in the HPLC analysis developed recently by our group. Mono- and di-esters of zeaxanthin could also be detected. It is to be mention that esterification of bioactive carotenoids such as zeaxanthin and β-cryptoxanthin with fatty acid does not reduce their biological activity because mono-and di-esters can be hydrolysed by de-esterase enzyme in the human intestine, furthermore, antioxidant activity of carotenoid esters containing saturated fatty acids is almost the same of the correspondent free forms (*Grenado* et al., 1992)

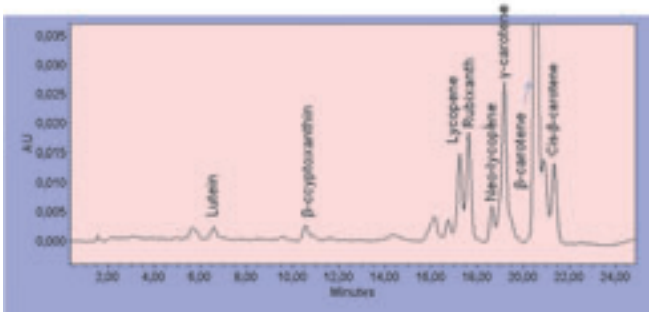

Figure 1. HPLC profile of apricot fruit carotenoids separated on cross-liked ISIS column with gradient elution of water in acetone and detected at 450 nm. For details see text.

Table1 shows the data obtained for different carotenoids in ripe fruits of different apricot varieties cultivated under integrated and organic farming conditions. The highest level of carotenoids was recorded in Tomcot fruits, which look pink in colour due to relatively high concentration of most carotenoids particularly all-trans lycopene and its isomers. The fruits of Tomcot variety contained also the highest level of β-carotene, the biologically most active and the major vitamin A precursor carotenoid in plant kingdom, revealing the high nutritive and biological values of such variety. Goldstrike and kajszi ranked 2nd, while ceglédi óriás and Bergarouge ranked 3rd when grown under integrated cultivation conditions.

Figure 3 shows the content of the major carotenoids in fruits harvested from trees of two apricot varieties grown under organic farming and integrated cultivation conditions. It is evident that organic/bio farming increases significantly the content of the dominant carotenoids such lycopene, rubixanthin an β-carotene in both examined varietie.

This change accompanied by inverse change in the content of γ-carotene and cis- β-carotene indicating that organic farming has an effect on the enzyme system (isomerise enzymes) responsible for the formation such carotenoid derivatives. From nutritional point of view this change is favourable since all-trans carotenoids are more biologically active than γ- and cis- isomers.

Table 1. Carotenoid composition and content of different apricot varieties grown under organic and integrated farming conditions

Varieties	Concentration (ug/g)								
	Lutein	**β-crypto xanthin**	**Lycopene**	**γ-carotene**	**Rubixanthin**	**α-carotene**	**β-carotene**	**cis-β -carotene**	**Total**
Goldstrike integr.	0,36 ± 0,04	0,26 ± 0,06	1,80 ±0,21	1,63±0,26	1,04±0,06	0,41±0,04	25,76±2,95	6,92±,37	40,36± 7,21
Tomcot integr.	0,27 ± 0,04	0,76 ± 0,12	8,82± 1,04	2,62±0,32	2,26±0,22	0,31±0,07	39,66±4,30	5,02±1,11	67,75± 6,36
Magyar Kajszi integr.	0,16 ± 0,04	0,26 ± 0,04	4,96± 0,84	3,84±0,58	2,10±0,18	0,12±0,02	19,34±1,95	3,29±0,56	42,08± 4,10
Ceglédi óriás integr.	0,15 ± 0,03	0,27 ± 0,03	2,52± 0,19	2,52±0,20	1,15± 0,13	not detected	21,88±4,02	3,48±0,48	33,92± 3,14
Magyarkajszi Bio	not detected	0,17± 0,04	8,33± 2,60	1,17±0,15	10,39±1,09	not detected	24,25±2,54	2,56±0,91	47,76± 5,29
Ceglédi óriás Bio	not detected	0,15± 0,05	9,17± 1,30	1,14±0,18	10,32±0,98	0,19±0,07	23,22±4,12	3,07±0,46	47,37± 5,72
Bergarouge Integr.	not detected	0,24 ± 0,01	7,24± 0,95	1,12±0,17	4,16±0,55	not detected	19,78±0,23	1,37±0,18	33,95± 2,45

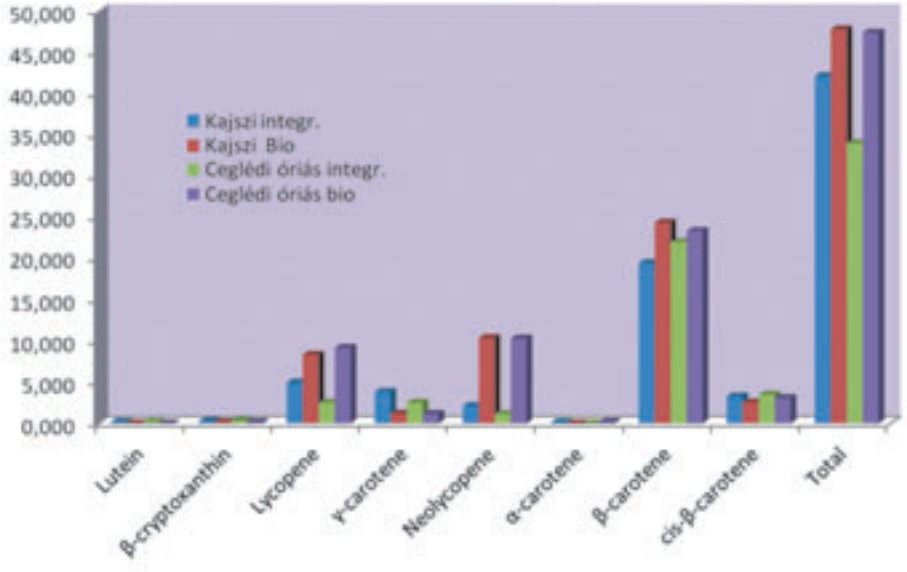

Figure 3. Carotenoid content of apricot fruits from Kajszi and Ceglédi óriás varieties grown under organic and integrated cultivation conditions.

The HPLC analysis of vitamin E components in the fat-soluble extract of apricot fruit after alkaline hydrolysis revealed that the α-tocopherol, the biologically active analogue of vitamin E, is dominant in the edible portion of apricot fruit *(Table 2*). The different varieties differed significantly in their content of vitamin E with that in Begarouge being the lowest followed by the content in Goldstrike among examined varieties. As for the effect of organic and integrated cultivation the results showed that the slight difference or change is variety-dependent since vitamin E content in Hungarian Kajszi is significantly higher than that found in fruits from harvested from trees of integrated cultivation, while a slight decrease was observed in the content of Ceglédi óriás apricot from bio farms. It is interesting and worthy to mention that β- and γ-tocopherol tended to significantly increase in organic/bio fruits. From chemical point of view the later analogue are more reactive, particularly as antioxidant, than α-tocophero.

Table2. Composition and content of vitamin E components extracted from apricot fruits of different varieties and analysed by HPLC

varieties	Concentration (ug/g)		
	α-tocopherol	**β-tocopherol**	**γ-tocopherol**
Goldstrike	7,25 ± 0,065	0,35 ± 0,026	0,52 ± 0,041
Begarouge Int	4,98 ± 0,290	0,23 ± 0,022	0,11 ± 0,011
Tomcot	9,67 ± 0,590	0,51 ± 0,091	0,67 ± 0,049
Kajszi integrated	10,09 ± 0,474	0,39 ± 0,071	0,83 ± 0,109
Kajszi Bio	11,58 ± 0,290	0,52 ± 0,120	1,10 ± 0,263
Ceglédi óriás integrated	9,29 ± 0,548	0,30 ± 0,030	0,65 ± 0,144
Ceglédi óriás Bio	8,55 ± 0,259	0,40 ± 0,032	0,97 ± 0,183

Conclusion

It can be concluded that apricot fruit is a remarkably good source of vital and biologically active carotenoids. It can substantially compensate the daily required intake of such nutrients. In addition it supplies the diet with considerable amount of vitamin E components, the highly reactive fat-soluble bio antioxidants. Production of apricot under organic farming conditions can increase its bio-activity and, thereby, improve functional properties of such a crop particularly, if the suitable variety is correctly selected.

Acknowledgement: This work was financially supported by the Hungarian Ministry of Agriculture and Rural Development and National Office for Research and Technology (NFÜ) under grant no. Biodeb07 and NFÜ TECH_08-A3/2-2008-0373 grant.

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