Carotenoids, Tocopherols and Antioxidant Activity of Lipophilic Extracts from Sea Buckthorn Berries (*Hippophae rhamnoides*), Apricot Pulp and Apricot Kernel (*Prunus Armeniaca*)

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**ABSTRACT**

A healthy human diet requires the daily consumption of fruits and vegetables rich in bioactive compounds. Sea buckthorn berries (*Hippophae rhamnoides* L.) and apricot fruits (*Prunus armeniaca* L.) are cultivated and appreciated in Romania both as fresh fruits and as derived products. Characterized by a complex chemical composition, sea buckthorn is rich in unsaturated lipids, carotenoids and tocopherols. Except for β-carotene content, less is known about other lipophilic compounds in apricot fruits.

The aim of this paper was to separate and quantify the individual carotenoids, tocopherols and tocotrienols in sea buckthorn, apricot pulp and kernels and also to determine the antioxidant activity of the lipophilic extracts using the TEAC method. Chemical characterization of lipophilic extract was performed by HPLC with PDA and fluorescence detection.

The total carotenoid content was 17.19±1.4 mg/100g F.W. in sea buckthorn; 3.51±0.25 mg/100g F.W. in apricot fruits and 0.58±0.04 mg/100g F.W. in apricot kernels. The major carotenoids in sea buckthorn were β-carotene, zeaxanthin and β-cryptoxanthin esters. Apricots fruits are rich in β-carotene and its geometric isomers while in kernels we could properly identified only lycopene. The α-tocopherol concentration was higher in sea buckthorn (46 mg/kg) than in apricot fruits (1.09 mg/kg) while apricot kernel contains large amounts of γ-tocopherol (111 mg/kg). Sea buckthorn fruits showed the highest antioxidant capacity, correlated with a high content of both tocopherols and carotenoids.

**Keywords**: apricot, antioxidant, carotenoids, tocopherols, sea buckthorn.

**INTRODUCTION**

Fruits and vegetables are highly recommended as components of a healthy diet due to their contribution to the intake of bioactive compounds, including antioxidants. Phenolic compounds, vitamin C, carotenoids and tocopherols are among the most powerful antioxidants and their presence in food is associated with a reduced risk of chronic and degenerative diseases and cancer (Sun, 2002; Rao and Rao, 2007).

Sea buckthorn (*Hippophae rhamnoides* L., fam. *Elaeagnaceae*) berries are exceptionally rich in bioactive compounds such as: unsaturated lipids, vitamins (C,E), carotenoids, flavonoids, aminoacids, oligoelements, etc. (Yang and Kallio, 2002; Kallio et al., 2002; Giuffrida et al., 2012; Pop et al., 2013). The oils extracted from flesh and kernels have regenerating, anti-inflammatory, anti-ulcerogenic, hepato-protective, cytoprotective properties...
which are often related to the antioxidant activity (Bal et al., 2011; Panossian and Wagner, 2013).

Apricot fruits (Prunus armeniaca L.) are appreciated and highly consumed fruits all over the world, both for their flavour and for nutritional qualities. The high content of sugars, proteins, minerals but also phytochemicals (phenolics and carotenoids) and vitamins, confer important biological properties such as antioxidant, antimicrobial, antimitagencic or anti-inflammatory (Erdogan-Orhan and Kartal, 2011). Apricot kernels have a high content of oil rich in oleic and linoleic acids, phytosterols and tocopherols (Turan et al., 2007).

The aim of this paper was to separate and quantify the individual carotenoids, tocopherols and tocotrienols in sea buckthorn (Hippophae rhamnoides L.) berries, apricot pulp and kernels and also to determine the antioxidant activity of the lipophilic fraction using the TEAC method.

MATERIALS AND METHODS

2.1 Plant material

Sea buckthorn berries (Hippophae rhamnoides L., var. Mara), were collected from Sibiu and was selected for uniform size and color and stored at -20°C until analysis. The apricot (Prunus armeniaca) were collected from Amman, Jordan and stored at -20°C until analysis.

2.2 Extraction of Lipophilic Fraction

The extraction was carried out starting from 20 g of freeze-dried fruit of sea-buckthorn, apricot and kernel apricot with a mixture of methanol/ethyl acetate/petroleum ether (1: 1: 1, v/v/v) (Breithaupt and Schwack, 2000). After filtering the extract, the residue was re-extracted twice using same solvent mixture. The combined extracts were partitioned in a separation funnel with the same solvent mixture. The combined extracts were partitioned in a separation funnel with the same solvent mixture.

2.3 Separation of carotenoids by RP-HPLC

Chromatographic separation of carotenoids was achieved by on Shimadzu LC20 AT high performance liquid chromatograph with a SPDM20A diode array detector and YMC C30 column (250x4.6 mm; 5 μm). The mobile phases consisted in: solvents A: methanol/tert-butyl methyl ether/water (81:15:4) and solvent B: tert-butyl methyl ether/methanol/water (90: 7:3). The gradient was: 0 min 0 % solvent B, 20 min 0 % B; 130 min – 82 % B; 132 min 0 % B, followed by equilibration of column for 10 min. The flow rate was fixed at 0.8 ml/min and the DAD detector was set at 450 nm. Standard compounds zeaxanthin, β-carotene and lycopene were provided by ChromaDex, USA; zeaxanthin dipalmitate and β-cryptoxanthin palmitate were obtained by semi synthesis and purified by HPLC in our laboratory (Pintea et al., 2013). The chromatographic data and UV-VIS spectra were compared using Shimadzu LC software.

2.4. Separation of tocopherols. Tocopherols and tocotrienols were separated using the same HPLC system but the detection was performed by a fluorescence detector RF20A operating at λ excitation = 290 nm and λ emission = 330 nm. A Lichrosorb Si60 column (250 x 4.6 mm; 5 μm) was used. The mobile phase consisted in hexane:2-propanol (99.5:0.5; v/v) operated in an isocratic mode with the flow 1 ml/min. Standard compounds α, γ and δ tocopherols and tocotrienols were provided from ChromaDex, USA. Calibration curves were performed with all standards in the concentration range 4-50 μg/ml.

2.4 Antioxidant activity of TEAC method

ABTS radical-scavenging activity for lipophilic fractions was determined according to Müller et al. (2011) with some modifications. In the present study, a distilled water solution of 5 mM aqueous solution ABTS (2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid) was filtered through manganese dioxide on a Whatman filter paper and then the excess of manganese dioxide was removed from the filtrate through PVDF syringe filter. Fresh ABTS⁺ was prepared fresh each day by dilution with PBS (75 mM) in order to obtain an absorbance of 0.700 at 734 nm. The diluted sample (100 μl) was mixed with 600 μl for 30s, centrifuged and then the absorbance was read using a Biotek Synergy HT microplate reader. A standard Trolox curve (r²=0.997) was obtained using solutions with concentration between 3.125 μM and 350 μM. Antioxidant activity of the extract was expressed as μM Trolox Equivalent.
RESULTS AND DISCUSSION

Carotenoids, tocopherols and tocotrienols in sea buckthorn berries, apricot fruits and apricot kernels.

Sea buckthorn berries are known as rich sources of carotenoids but the total amount and the profile of different type of compounds is highly variable with the genetic origin, conditions of cultivation and harvesting time. In the variety analyzed (Mara) in the present study a total amount of 17.19±1.4 mg/100 fresh weight was found. It was previously reported that the content of carotenoids in some Romanian varieties of Hippophae rhamnoides ranged between 53.1 – 96.7 mg/100 dry weight (Pop et al., 2014). Considering that the dry weight represents about 20 % of the berries, the total carotenoid content of this variety is high. As we used an unsaponified extract, the major compounds identified were zeaxanthin dipalmitate, other zeaxanthin esters, β-carotene, zeaxanthin, β-cryptoxanthin palmitate and lycopene (Fig. 1; Table 1). Other minor compounds were present, most of them esters of zeaxanthin, β-cryptoxanthin and lutein with various fatty acids (retention time 70-120 min) which could not be unequivocally identified by PDA detector. It is already known that xanthophylls esters represents a major fraction in the unsaponified sea buckthorn extract or oil, together with β-carotene (Weller and Breithaupt, 2005; Pintea et al., 2005; Giufridda et al., 2012; Pop et al. 2014). More than 62 % of the carotenoids in Mara variety are mono and diesters which is similar to data reporting an average of 71 % esterified carotenoids in other Romanian varieties (Pop et al., 2014) while in Swedish cultivars the average was 55 % (Andersson et al., 2009).

Apricot fruits contain significantly lower amounts of carotenoids 3.51±0.25 mg/100 fresh weight and have a completely different profile of pigments. The total amount of carotenoids is highly variable depending of the variety of apricots but was in the range of values reported by other authors (1.5-16 mg/100 g) (Ruiz et al., 2005; Sass-Kiss et al., 2005; Dragovic-Uzelac et al., 2007; Kurtz et al., 2008). The all trans isomer of β-carotene is the main carotenoid, followed by different other of its geometric isomers. The tentative identification of cis isomers was based on the presence of the „cis-peak“ which appears in the UV region of the absorption spectra of carotenoids (around 340 nm) (Britton et al., 1995b). Small amounts of β-cryptoxanthin and β-cryptoxanthin esters and unesterified lutein were also present. Similar profile of carotenoids was reported by Kurtz et al. (2008).

The HPLC-PDA analysis of apricot kernel extract showed a total carotenoid content 0.58±0.04 mg/100 fresh weight. Only one carotenoid could be identified - the lycopene, the others being in to low amounts to allow a proper identification based only on the characteristics

Fig. 1. HPLC-PDA C30 separation of carotenoids from apricot fruits, sea buckthorn berries and apricot kernel. Peaks identities presented in Table 1.
of UV-VIS spectrum. At our knowledge, there are no literature date regarding the presence or the content of carotenoids in apricot kernels.

Tocopherols were separated on a normal-phase column which has be proven to allow a good separation of both tocopherols and tocotrienols (Ruperez et al., 2001). Using a simple isocratic program we succeeded in the separation of standard compounds and tocopherols/tocotrienols in samples (Figure 2).

Sea buckthorn berries contains high levels of tocopherols in both soft part of the berries and in seeds. For the preparation of lipophilic extract we processed the whole berries, including seeds. α-tocopherol was found at 46.40 ± 2.1 mg/kg being the most important representative in sea

Tab. 1. Major carotenoids in sea buckthorn berries, apricot fruits and apricot kernel.

<table>
<thead>
<tr>
<th>Peak nr.</th>
<th>Compound</th>
<th>UV-VIS maxima</th>
<th>% of total carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sea buckthorn</td>
</tr>
<tr>
<td>1</td>
<td>Lutein</td>
<td>421,445,473</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>Zeaxanthin</td>
<td>426,450,475</td>
<td>4.38</td>
</tr>
<tr>
<td>3</td>
<td>β-cryptoxanthin</td>
<td>428,451,476</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>15-cis-β-carotene*</td>
<td>337,420,449,472</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>13-cis-β-carotene*</td>
<td>338,420,444,469</td>
<td>0.86</td>
</tr>
<tr>
<td>6</td>
<td>all trans β-carotene</td>
<td>421,452,478</td>
<td>24.96</td>
</tr>
<tr>
<td>7</td>
<td>α-carotene</td>
<td>422,444,473</td>
<td>2.54</td>
</tr>
<tr>
<td>8</td>
<td>9-cis-β-carotene*</td>
<td>345,421,447,473</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>β-cryptoxanthin ester</td>
<td>428,451,476</td>
<td>1.19</td>
</tr>
<tr>
<td>10</td>
<td>γ-carotene</td>
<td>434,461,488</td>
<td>2.59</td>
</tr>
<tr>
<td>11</td>
<td>β-cryptoxanthin palmitate</td>
<td>428,451,476</td>
<td>1.93</td>
</tr>
<tr>
<td>12</td>
<td>Zeaxanthin ester</td>
<td>427,450,476</td>
<td>12.27</td>
</tr>
<tr>
<td>13</td>
<td>Zeaxanthin dipalmitate</td>
<td>427,450,476</td>
<td>26.62</td>
</tr>
<tr>
<td>14</td>
<td>Lycopene</td>
<td>448,471,503</td>
<td>3.08</td>
</tr>
</tbody>
</table>

Total carotenoids (mg/100g f.w.) 17.19±1.4 3.51±0.25 0.58±0.04

*tentatively identified according to retention times and UV-VIS spectra

Fig. 2. NP-HPLC separation of tocopherols and tocotrienols
buckthorn berries. The amount is similar with that found by Kallio et al., (2002), who reported 40 ± 12 mg/kg α-tocopherol in the soft part of subspecies Hippophae rhamnoides. It was followed by γ-tocopherol and δ-tocopherol (Table 2). Smaller amounts of all α-, γ- and δ- tocotrienols were also detected. It has to be mentioned that due to the lack of standards for β-tocopherol and β-tocotrienol we were not able to identify them in the samples. However, according to the known order of elution of tocopherols on normal phase columns and hexane based mobile phases, it is highly probable that the compound with retention time 10.0 min and that with retention time 10.8 are β-tocopherols and, respectively β-tocotrienol. If we estimated their concentrations using the α-tocopherol calibration curve, they account for 8.5 and respectively 5.0 mg/kg. The proportion of α-tocopherol in our sample is 64.7 % of total tocopherols and tocotrienols, lower that that reported previously for soft part of the berries – 70-80 % (Kallio et al., 2002). This can be explained by the fact that we analyzed whole berries and it is known that the seeds have a higher proportion of γ-tocopherol (20-40 %) than the soft part of the berries.

Apricot fruits are characterized by low amounts of tocopherols – 1.43 mg/kg -compared to sea buckthorn berries. α-tocopherol was the major compound which accounts for almost 70 %, followed by α-tocotrienol. Small amounts of γ and δ tocopherols were also detected and, as in the case of sea buckthor berries, peaks corresponding to β-tocopherol have been observed but not properly identified. In contrast, apricot seeds had the highest amount of tocopherols and tocotrienols – 131.6 mg/kg kernel, which represents 516 mg/kg oil. The total tocopherols in apricot kernel oils from Turkey ranged between 373-600 mg/kg (Turan et al., 2007). The major compound in apricot kernel was γ-tocopherol which represented about 80 % of total tocopherols and tocotrienols. δ and α tocopherols represented 6.1 % and 5.7 % of tocopherols, while among tocotrienols, only the α-isomer could be detected.

Antioxidant capacity of lipophilic extracts from sea buckthorn berries, apricot fruits and apricot kernels.

The antioxidant capacity of the lipophilic extracts was determined using the TEAC (Trolox Equivalent Antioxidant Capacity) method (Miller et al., 1996), which has been adapted for lipophilic compounds (Müller et al., 2010). TEAC method is one of the mostly used antioxidant capacity assays based on single electron transfer (SET) reaction from antioxidant to ABTS•• radical which is reduced. The drop of absorption of the stable radical ABTS•• during the reaction with antioxidants is measured at 734 nm (Apak et al., 2013).

The antioxidant capacity of the lipophilic fraction from analyzed samples is presented in Table 3. Apricot fruits are rich in both in lipophilic antioxidants (mainly β-carotene) and hydrophilic antioxidants, such the polyphenols and ascorbic acid (Ruiz et al., 2005; Dragovic-Uzelac et al., 2007; Kurz et al., 2008; Sochor et al., 2010). A methanol extract of fresh Malatya apricots had 2.8-4.4 μM TE/g FW. (Güçlü et al., 2006), while Scalzo et al. (2005) reported 0.8-1.14 μM TE/g FW in some Italian varieties. Using the classical TEAC method Lecese et al. (2011) showed that the total antioxidant capacity of apricot fruits ranged between 1.49-11.72 μmols TE/g FW, depending on germplasm, and it was highly correlated with the phenol content (r=0.99) but not with carotenoids. About 85 % of the antioxidant capacity of the extract (sequential extraction with ethanol and tetrahydrofuran) was due to the presence of hydrophilic antioxidants (Lecese et al., 2011). The antioxidant capacity of apricot kernels is very low compared to that of sea buckthorn berries or fruits.

Similar to apricots, sea buckthorn berries have high nutrition value due to the high

Tab. 2. Tocopherols and tocotrienols in sea buckthorn berries, apricot fruits and apricot kernels

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sea buckthorn berries mg/kg</th>
<th>Apricot fruits mg/kg</th>
<th>Apricot kernels mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>46.40 ± 2.1</td>
<td>1.09 ± 0.08</td>
<td>8.01 ± 0.9</td>
</tr>
<tr>
<td>α-tocotrienol</td>
<td>0.97 ± 0.1</td>
<td>0.24 ± 0.04</td>
<td>2.77 ± 0.4</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>5.04 ± 0.4</td>
<td>0.06 ± 0.01</td>
<td>111.6 ± 6.2</td>
</tr>
<tr>
<td>γ-tocotrienol</td>
<td>1.49 ± 0.4</td>
<td>traces</td>
<td>traces</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>2.94 ± 0.3</td>
<td>0.04 ± 0.01</td>
<td>8.62 ± 0.5</td>
</tr>
<tr>
<td>δ-tocotrienol</td>
<td>1.33 ± 0.07</td>
<td>traces</td>
<td>traces</td>
</tr>
</tbody>
</table>
content of micronutrients, including lipophilic and hydrophilic antioxidants (Bal et al., 2011; Chen et al., 2013). Phenolic compounds and vitamin C are the most powerful hydrophilic antioxidants but their concentration decreases during the maturation of fruits as well as the hydrophilic antioxidant capacity. On the other side, carotenoids and tocopherols accumulate during maturation of fruits and it corresponds to an increase of antioxidant capacity of lipophilic extracts. It was shown that the lipophilic fractions of sea buckthorn were most effective when calculated as a ratio between antioxidant capacity and the amount of antioxidants in samples (Gao et al., 2000). More recently, sea buckthorn juice rich in carotenoids and tocopherols has been proved to be 20 times more active as antioxidant (80 μmol αTE/100 g; αTEAC assay) by all the tested methods than orange juice and more active than other fruits or vegetable juices (Müller et al., 2011).

Similar results were found when the antioxidant capacity of the same samples were determined by other methods: FRAP, DPPH and LPSC assay. The antioxidant capacity of tocopherols and related compounds seems to be influenced by the degree and pattern of methylation but also on the mechanism of action. When tested by αTEAC assay, no significant differences were found in the activity of tocopherols and tocotrienols (Müller et al., 2010). Carotenoids also exhibit different antioxidant capacity depending on their structural features (number of double bonds, type of ring, functional groups on the rings, etc.) but also on the type of assay. When the antioxidant capacity of pure compounds was determined by αTEAC assay, carotenones (β-carotene, lycopene) showed higher activities than xanthophylls (lutein, zeaxanthin, β-cryptoxanthin) and all of them had higher activity than α-tocopherol (Müller et al., 2011).

In sea buckthorn fruits, esterified zeaxanthin and β-cryptoxanthin are a major fraction. We already proved that esterification, especially with saturated fatty acids, do not significantly influence the antioxidant capacity of both xanthophylls (Pintea et al., 2013a, Pintea et al., 2013b), in consequence it can explain the high antioxidant capacity of sea buckthorn juice and extracts.

Previous studies have suggested that antioxidant activity is the result of the combination of each of the components of a complex mixture and that a synergetic or antagonist effect can be observed, depending on the environment (Sancho L.E. et al., 2013).

**CONCLUSION**

The total carotenoid content was 17.19±1.4 mg/100g F.W. in sea buckthorn; 3.51±0.25 mg/100g F.W. in apricot fruits and 0.58±0.04 mg/100 g F.W. in apricot kernels. The major carotenoids in sea buckthorn were β-carotene, zeaxanthin and β-cryptoxanthin esters. Apricots fruits are rich in β-carotene and its geometric isomers while in kernels we could properly identified only lycopene. The α-tocopherol concentration was higher in sea buckthorn (46 mg/kg) than in apricot fruits (1.09 mg/kg) while apricot kernels contain large amounts of γ-tocopherol (111 mg/kg). To the extent of our knowledge this is the first report of tocopherols and tocotrienols composition in apricot fruits and of carotenoids in apricot kernels. Lipophilic fraction rich in tocopherols and carotenoids has an important contribution to the antioxidant capacity of sea buckthorn and apricot fruits.

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**REFERENCES**


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**Tab. 3.** The antioxidant capacity of the lipophilic fraction from sea buckthorn berries, apricot fruits and seeds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total carotenoids mg/100g F.W*</th>
<th>Total tocopherols mg/100g F.W.</th>
<th>Antioxidant activity μM TE/g F.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea buckthorn berries</td>
<td>17.19</td>
<td>5.82</td>
<td>1.87</td>
</tr>
<tr>
<td>Apricot fruits</td>
<td>3.51</td>
<td>0.14</td>
<td>0.59</td>
</tr>
<tr>
<td>Apricot kernel</td>
<td>0.58</td>
<td>13.1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*F.W*—fresh weight


