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# Several Lipophilic Components of Five Elite Genotypes of Romanian Seabuckthorn (*Hippophae rhamnoides* subs. *carpatica*)

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**Abstract**

Seabuckthorn is a spinescent, deciduous and dioecious berry-producing shrub, with a high economical and ecological potential. It is frequently used as a pioneer species in anthropic and eroded soils due to its low pedoclimatic demands, strong rooting system and ability to fix atmospheric nitrogen. Seabuckthorn berries, leaves and bark have a high content of nutritive and active substances which promote this species for use in both food and medical industries. One of the most requested therapeutical products on the market is the seabuckthorn oil, extracted from both pulp and seeds. Two important parameters in analyzing seabuckthorn oil quality are fatty acids and tocopherols. In Transylvania region most of seabuckthorn orchards are established with local, low productive and less uniform planting material, randomly collected from wild flora. In order to assess the opportunity of introducing new seabuckthorn varieties in Transylvania, a selection process was initiated. In this context, five elites were selected from wild populations in the Danube Delta, using biometrical criteria. They were later compared to a representative individual from a local population and to a number of homologated cultivars, with respect to morphology and some lipophilic components (oil content, fatty acids and tocopherols). For both pulp and seeds, total lipids were extracted using a modified Folch method. Fatty acids were analyzed by gas chromatography using flame ionization detection. Tocopherols were analysed using a Shimadzu VP Series liquid chromatograph with a fluorescence detector FR-10 AXL.

***Keywords:*** seabuckthorn oil, fatty acids, tocopherols, selection

## Introduction

Seabuckthorn is a spinescent, deciduous, dioecious and anemophilous berry-producing shrub, of high economical value.

Because of its large spectrum of uses, from ecological and ornamental to nutritional and therapeutic, seabuckthorn is a multi-purpose species of great importance.

Raw materials and fruit-, leave-, and bark-derived products (*e.g.* seed and pulp extracted oil, juice, tea, cosmetics and pharmaceutics), constitute an entire industry based on this species (Dwivedi and Singh, 2003).

From an ecological point of view, due to its hardiness, strong rooting system with high sucker formation capacity and atmospheric nitrogen fixation capability, seabuckthorn can be successfully used as a pioneer species on very poor soils and highly eroded lands and it is also suitable for organic farming.

From a dietary point of view, seabuckthorn berries can be undoubtedly ranked among the richest soft fruits, due to their high content in several antioxidants (Antonelli *et al.*, 2005). Seabuckthorn fruits especially, but also leaves and bark, have a high content of nutritive and active substances such as: minerals (Ca, K, Na, Fe etc.), vitamins (provitamin-A, C, B, E etc.), fatty acids and proteins (Lu, 2005; Yang and Kallio, 2005a). Pulp oil is also rich in various carotenoids with high pharmaceutical value (Lu, 2005; Novruzov, 2005; Yang and Kallio, 2005a). Large quantities of vitamin E and carotenoids found in seabuckthorn oil, along with vitamin C from berries, have strong antioxidant properties with anti-aging effect, due to stabilization of cellular membrane (Gao, 2005).

Seabuckthorn oil has several pharmacological purposes and actions such as: diminishing inflammations; relieving pain; reducing the toxic effect of traditional drugs; improving the cardiovascular conditions; antibacterial properties; skin tissue regeneration after mechanical, chemical and burn injuries (especially the pulp oil); use in skin grafting; treatment of corneal wounds; antimutagen effects (Lu, 1992; Yang and Kallio, 2005b). Different studies reveal anticancer and anticarcinogenic effects of seabuckthorn oil, as well as positive effects on immune function, peroxidation, mucosa and skin (Singh, 2005). Seabuckthorn seed oil has significant antiatherogenic and cardioprotective activity (Gao, 2005). No toxic effect was noticed on regular consumption of seabuckthorn oil (Yang and Kallio, 2005b).

Seabuckthorn extracts proved also anti-radiation lesion properties (Goel and Bala, 2005)

Clinical tests performed in Romania proved efficiency of seabuckthorn oil used against skin physical and chemical burns, ocular damages and diabetes (Brad *et al.*, 2002).

Oils from both fruits and leaves are also used in cosmetic industry (Lu, 1992).

The market demand for seabuckthorn berries and their main product - the seed and pulp oil - encouraged us to

select new genotypes of high productivity and with large fruits. Once the elites were selected, several experiments were performed in order to determine the oil content and its composition in fatty acids and tocopherols. The results were compared, when available, to those obtained for other Romanian cultivars/seabuckthorn wild genotypes or to several literature references. The aim of this study also encompassed the implementation of lipophilic determination procedures for Romanian seabuckthorn (*H. rhamnoides* subsp. *carpatica*) in our laboratories.

**Materials and methods**

*Seabuckthorn berries*

Fresh seabuckthorn berries (*carpatica* subspecies) were collected from three different populations (referred to as CF, MF and SF) from Danube Delta area. Selection was made with regards to productivity, fruit weight, number of thorns, fruit color etc. After biometrical analyses, the elites of each population were selected. The control sample (referred to as CHF) was collected from a local seabuckthorn population considered as being phenotypically representative for Transylvanian Plateau. Main fruit characteristics are listed in Tab. 1.

*Extraction of lipids*

Total lipids were extracted employing a modified Folch method (Folch *et al.*, 1957). Fruit pedicles were removed and the seeds were separated manually. Soft parts (pulp plus peel, further referred to as pulp) and seeds were crushed and homogenized with an ultraturax apparatus, *i.e.* Heidolph Silent Crusher-M. Next, 1 g of fruit pulp/seeds was placed into a flask together with 60 mL of a chloroform/methanol solution (2:1, v/v). The flask was set on a magnetic agitator for two hours. Afterwards the solution containing the sample was filtered through a cellulose filter and moved into a separation funnel. 30 mL of distilled water were then added on top of the extract, in the separation funnel. After the separation of the two phases, the aqueous layer was discarded. The chloroformic fraction was further anhidrified using anhydrous sodium sulfate. The extract was dried using a vacuum rotary evaporator at 35°C.

The measurement of oil content was performed three times.

*Fatty acids*

Fatty acids (FA) were converted to methyl esters by reaction with boron trifluoride/methanol at 80°C for two

Tab. 1. Main characteristics of the seabuckthorn elites, control sample and cultivars

| Selections/Cultivars | Fruit color | Weight of 100 berries (g) | Dry weight (%) | Weight of 100 seeds (g) | Seed-berry ratio (w/w) | Oil content in fresh seed[m] | Oilcontent in fresh pulp[m] | Oil content in fresh berry | Oil content in dry berry |
|---|---|---|---|---|---|---|---|---|---|
| 'SF 7' | Orange | 45.15 | 15.61 | 2.20 | 4.87% | 16.43% | 3.63% | 4.25% | 27.23% |
| 'SF 8' | Orange | 63.25 | 18.14 | 2.99 | 4.73% | 7.20% | 6.30% | 6.34% | 34.96% |
| 'CF 2' | Light orange | 42.40 | 24.03 | 2.10 | 4.95% | 12.10% | 5.77% | 6.08% | 25.30% |
| 'CF 4' | Yellow | 73.08 | 19.87 | 2.78 | 3.80% | 8.60% | 6.63% | 6.70% | 33.75% |
| 'MF 3' | Light orange | 54.18 | 23.86 | 2.55 | 4.71% | 9.10% | 8.53% | 8.56% | 35.87% |
| Average | | 55.61 | 20.30 | 2.52 | 4.61% | 10.69% | 6.17% | 6.39% | 31.42% |
| CHF 1 (control) | Light orange | 25.20 | 19.66 | 0.96 | 3.81% | 13.61% | 5.62% | 5.92% | 30.13% |
| All 6 individuals average | | | | | 4.48% | 11.17% | 6.08% | 6.31% | 31.21% |
| Homologated cultivars* | | | | | | | | | |
| 'Auras' ('Sf. Gheorghe 5') | Light orange | 31.93 | 17.96 | | | | | 2.93% | 16.33% |
| 'Diana' (without spines) | Orange | 18.81 | 21.62 | | | | | 1.92% | 8.88% |
| 'Ovidiu' ('Sf. Gheorghe 9') | Light orange | 38.21 | 17.74 | | | | | 1.99% | 11.22% |
| 'Silvia' ('Serbanesti 4') | Orange | 17.74 | 20.29 | | | | | 2.21% | 10.90% |
| 'Tiberiu' ('Serbanesti 1') | Light orange | 37.71 | 12.74 | | | | | 1.41% | 11.10% |
| 'Victoria' ('Delta 60M') | Orange | 56.92 | 17.45 | | | | | 2.44% | 14.01% |
| Average | | 33.55 | 17.97 | | | | | 2.15% | 12.07% |

[m] Results are presented as mean value of three determinations. * Extracted from the official database of homologated cultivars in Romania for 2009; only Frutex SA homologated cultivars are presented; characteristics provided by Brad *et al.* (2002) and Rati and Rati (2003)

hours in a Pyrex glass tube. Esters were extracted twice using 1 mL n-hexane; the extracts were combined, neutralized and dried with anhydrous sodium sulfate and then filtered. The final step before starting the GC-FID analysis was to concentrate the filtrate under a nitrogen stream. FA were analyzed by gas chromatography (GC) with a flame ionization detection (FID). A 1 μL sample was injected into the Shimadzu GC-17 A series gas-chromatograph, equipped with a 30 m polyethylene glycol coated column (Alltech AT-WAX, 0.25 mm ID, 0.25 μm film thickness). Helium was used as the carrier gas at a pressure of 147 kPa. The injector and detector temperatures were set at 260°C. For the oven temperature the following programme was used: 70°C for 2 min then raised to 150°C at 10°C/min rate and held at 150°C for 3 min, then further raised up to 235°C at a 4°C/min.

*Tocopherols*

The tocopherols were analysed using a Shimadzu VP Series liquid chromatograph equipped with a degasser and a fluorescence detector FR-10 AXL with excitation wavelength of 290 nm and emission wavelength of 325 nm. Chromatographic separation was performed on an Alltima RP C-18 column (250 mm x 4.6 mm, 5 μm). The column was used at 40°C. The mobile phase was a mixture of acetonitrile and methanol (50:50, v/v) and eluted at a flow rate of 0.8 mL/min. Sample treatment included saponification of 90 mg oil in a Pyrex glass tube with 2 mL KOH 50% and 10 mL ethanol containing ascorbic acid (6%, m/v). The tube was transferred to a boiling water bath for 30 min. After sonication for 10 min, the digested sample was cooled on ice and 20 ml of 2% sodium chloride was added. Unsaponified lipids were extracted with two portions (each 10 mL) of n-hexane/diethyl ether (7:3, v/v). After separation of the phases, the organic layers were collected in a separatory funnel. Organic extracts were washed three times with water and then evaporated in a vacuum rotary evaporator at 35°C. The dried residue was extracted with 2 mL methanol and 2 mL acetonitrile by mixing on a vortex mixer for 2 min. The tocopherol peaks were identified by comparison of retention times with those of standard samples (Sigma). Tocopherol contents were calculated from the peak areas using data generated by the same standards mentioned before.

**Results and discussion**

*Seabuckthorn berries characteristics*

As shown in Tab. 1, both fruit weigh and dry matter averages of our selections are higher when compared to homologated cultivars and control sample. The elites have medium weight for 100 seeds, which is similar to other subspecies (Dwivedy *et al.*, 2005; Kallio *et al.*, 2002) and higher seed weight and seed/berry ratio compared to CHF1, which could translate into higher oil quantity for the same berry production/ha. CF4 had some of the largest seabuckthorn fruits ever reported (see Albrecht, 2003; Dwivedy *et al.*, 2005; Novruzov, 2005), except for some Russian cultivars in which case 100 fruits could weigh up to 120 g (Zubarev, 2008).

*Oil content*

Our results regarding oil content are summarized in Tab. 1. Differences can be seen between elites for both seed and pulp oil (7.2-16.4% in seed and 3.6-8.5% in fresh pulp). The average values for seed, pulp and especially for dry fruit oil content are similar to those of the control sample. The oil content in whole fresh and also dry fruit was calculated using seed/fruit ratio and dry weight. The average content we measured is three times higher then reported by Rati and Rati (2003) and Brad *et al.* (2002) for several Romanian cultivars; however, the two publications do not mention the methods used for oil extraction. Our results regarding pulp oil content are similar to those mentioned by Socaciu and Pintea (2005) and Shapiro *et al.* (1978) in Yang and Kallio (2005a) for *carpatica* ssp. (6.5% of fresh pulp and 4.2-6.6% of fresh fruits, respectively).

Oil percentage for different part of the fruits vary a lot because of different methods of determination and high diversity of seabuckthorn, even within the same subspecies (Tab. 4); however, the data we have selected can set the limits of oil content reported in the literature. Our results were compared exclusively to those obtained with similar extraction method (see Tab. 2). The oil content in seeds is closed to the average, especially to the *rhamnoides* ssp. content, while the pulp and whole fruit oil content of our elites seems to be higher than average but similar to previous results concerning *carpatica* ssp.

*Fatty acids*

Unsaturated FA are highly recommended for human health, especially linolenic and linoleic acids, which are active against cardiovascular diseases and high blood fat. These two types of acids can be found almost ten times more in seed oil compared to pulp oil (Lu, 1992). Alpha linolenic acid (18:3 omega-3) reduces blood pressure, blood cholesterol, lower risk of stroke and heart attack, prevent liver damage due to alcoholism, improve the condition of hair, nails and skin and kill cancer cells in tissue culture without harming normal cells (Singh, 2005).

FA composition of both pulp and seed oil are summarized in Tab. 3. One representative chromatogram for each type of oil is presented (Fig. 1 and 2). In seed oil the dominant FA are linoleic acid (40.1%), linolenic acid (26.3%) and oleic acid (17.9%). Seed oil has a high percentage of polyunsaturated FA (PUFA) (66.4%) and monounsaturated FA (MUFA) (21.5%) compared to saturated FA (SFA) (11.7%). When comparing the control sample to elites, a clear negative difference was noticed only for linoleic acid (31.2% in control sample) but higher content in linolenic acid (31.5%). In the pulp oil, the main FA are palmitic (36.55%), oleic (30.9%) and palmitoleic (20.45%) acids.

Tab. 2. Oil content in different parts of the seabuckthorn fruits, using chloroform/methanol (2:1) extraction method

| Species/subsp. | Average oil content (% of fresh weight) | | | |
|---|---|---|---|---|
| *Hippophae* genus | Seed oil | Pulp oil | Whole berry oil | References |
| *H. rhamnoides* ssp. *sinensis* | 7.3 | 1.7 | | Yang and Kallio (2001) |
| *H. rhamnoides* ssp. *sinensis* | 9.7 | | 4.1 | Kallio *et al.* (2002) |
| *H. rhamnoides* ssp. *sinensis* | 7.5 | 2.8 | 2.5 | Yang (2005a) |
| *H. rhamnoides* ssp. *rhamnoides* | 11.3 | 2.8 | | Yang and Kallio (2001) |
| *H. rhamnoides* ssp. *rhamnoides* | 10.6 | 3.2 | 3.6 | Yang (2005a) |
| *H. rhamnoides* ssp. *mongolica* | 12.6 | | 5.9 | Kallio *et al.* (2002) |
| *H. rhamnoides* ssp. *mongolica* | 13.3 | 5.4 | 7.2 | Yang (2005a) |
| *H. rhamnoides* L. (Poland- Belorussia) | | | 2.4 | Zadernowski *et al.* (2003) |
| *H. rhamnoides* L. (India) | | 3.3 | | |
| *H. salicifolia* | | 1.6 | | Ranjit *et al.* (2006) |
| *H. tibetana* | | 2.4 | | |
| *H. rhamnoides* ssp. *carpatica* | | 6.5 | | Socaciu and Pintea (2005) |
| Average | 10.3 | 3.0 | 4.3 | |
| Our results* | 11.2 | 6.08 | 6.31 | |

* Considered as average of all six individuals

MUFA (58.2%) and SFA (37.6%) are dominants in the pulp oil compared to PUFA (4.1%). The control sample has higher content in palmitoleic acid (32.3%) and smaller oleic and PUFA content compared to the average. Previous studies on FA of Romanian seabuckthorn (*e.g.* Brad *et al.,* 2002), reported that palmitic acid (34.2%), palmitoleic (31.5%) and oleic (25.7%) are the dominant FA in whole fruit oil; these results are very similar to FA composition in CHF1 pulp oil, while compared to the average FA content in pulp oil (the main component due to low mass percentage of seed within berry), we have similar results only for the palmitic acid.

Regardless of species and subspecies of seabuckthorn, the oil contains 20 types of FA with higher content in seed compared to pulp; in general, a higher percentage accounts for the unsaturated FA (Lu, 2005). Our results regarding unsaturated FA are very similar to those obtained by Lu (1992) for *sinensis* ssp., for both seed and pulp oil and by Korovina and Fefelov (2005) for oil extracted from whole berry of Russian origin.

Tab. 3. Major fatty acids composition

| *H. rhamnoides subsp. carpatica* Selections | Major fatty acid compositions in sea buckthorn seed and pulp oil, as mass percentage of total amount of fatty acids | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Berry part | 16:0 (Palmitic) | 16:1 (Palmitoleic) | 18:0 (Stearic) | 18:1n-9 (Oleic) | 18:1n-7 (*cis*-Vaccenic) | 18:2n-6 (Linoleic) | 18:3n-3 (Linolenic) | SFA (%) | MUFA (%) | PUFA (%) |
| 'CF 2' | Seed | 9.15 | 1.71 | 3.17 | 20.09 | 2.1 | 40.2 | 23.55 | 12.32 | 23.91 | 63.75 |
| | Pulp | 35.86 | 13.35 | 0.95 | 40.49 | 6.8 | 1.14 | 0.87 | 36.82 | 60.65 | 2.32 |
| 'CF 4' | Seed | 6.55 | 0.65 | 3.83 | 16.23 | 2.07 | 41.05 | 29.39 | 10.38 | 18.96 | 70.45 |
| | Pulp | 39.98 | 19.26 | 1.02 | 28.59 | 6.04 | 3.89 | 1.07 | 41.02 | 53.91 | 4.96 |
| 'SF 7' | Seed | 8.09 | 1.25 | 3.28 | 15.84 | 2.24 | 43.57 | 25.69 | 11.38 | 19.34 | 69.27 |
| | Pulp | 40.19 | 20.69 | 0.8 | 27.17 | 6.22 | 3.23 | 1.67 | 40.99 | 54.09 | 4.9 |
| 'SF 8' | Seed | 7.11 | 0.39 | 3.44 | 18.43 | 1.88 | 43.73 | 24.76 | 10.55 | 20.71 | 68.49 |
| | Pulp | 32.75 | 20 | 1.41 | 33.38 | 7.29 | 3.9 | 1.14 | 34.17 | 60.68 | 5.04 |
| 'MF 3' | Seed | 10.3 | 1.89 | 3.26 | 18.45 | 2.38 | 40.86 | 22.83 | 13.56 | 22.74 | 63.69 |
| | Pulp | 33.83 | 17.11 | 1.16 | 35.82 | 7.14 | 3.68 | 0.97 | 35 | 60.08 | 4.65 |
| Elites average | Seed | 8.24 | 1.178 | 3.396 | 17.808 | 2.134 | 41.882 | 25.244 | 11.64 | 21.13 | 67.13 |
| | Pulp | 36.52 | 18.08 | 1.07 | 33.09 | 6.70 | 3.17 | 1.14 | 37.60 | 57.88 | 4.37 |
| 'CHF 1' | Seed | 9.25 | 2.38 | 2.67 | 18.14 | 2.67 | 31.16 | 31.51 | 11.92 | 23.2 | 62.67 |
| | Pulp | 36.68 | 32.29 | 0.6 | 19.96 | 7.25 | 2.01 | 0.88 | 37.28 | 59.51 | 2.89 |
| *H.r. ssp. carpatica** | Seed | 8.41 | 1.38 | 3.28 | 17.86 | 2.22 | 40.10 | 26.29 | 11.69 | 21.48 | 66.39 |
| | Pulp | 36.55 | 20.45 | 0.99 | 30.90 | 6.79 | 2.98 | 1.10 | 37.55 | 58.15 | 4.13 |

* Considered as average of all six individuals; however, further investigations with higher number of investigated samples/different populations are required

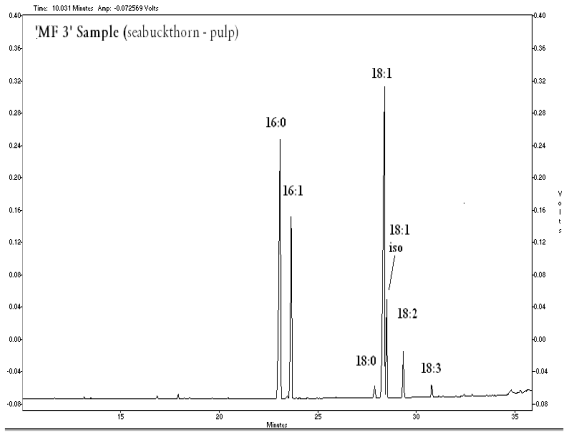


Fig. 1. Chromatograms of fatty acids composition in seabuckthorn pulp oil

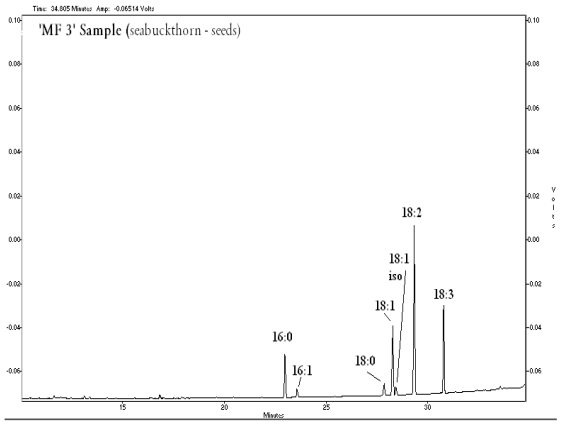


Fig. 2. Chromatograms of fatty acids composition in seabuckthorn seed oil

Regarding *H. rhamnoides* ssp. *turkestanica*, *mongolica* and German cultivars Hergo and Leikora, where palmitic and palmitoleic acids dominate in the pulp oil and linoleic and linolenic acids have the highest percentage in the seed oil (Jamyansan and Badgaa, 2005; Mörsel *et al.*, 2005), we had similar results for CHF1; however, the general average show that palmitic and oleic acids are dominants in the pulp oil of *carpatica* ssp. (most of the samples originating from the Danube Delta).

When comparing our results to the general average of several species and subspecies, a high similarity regarding the ratio of FA in seed oil can be observed. In this context, the most important are the linoleic, linolenic and oleic acids. The main acids within pulp oil were the palmitic, oleic and palmitoleic acids, along with a low percentage of linoleic acids; these results are similar to those obtained for *fluviatilis* ssp., but small differences can be noticed when compared to general average regarding palmitoleic, palmitic, oleic and linolenic acids. The ratio between saturated and unsaturated FA of Romanian seabuckthorn is very similar to the general average already mentioned.

*Tocopherols*

Between different types of tocopherols, the most bioactive are the α-tocopherol (accounts for 30% of total bioactivity), β-tocopherol (25-50%) and γ-tocopherol (10-35%) (Singh, 2005). Most of the investigations regarding seabuckthorn oil have been concentrated on the levels of α-tocopherol, total tocopherols or ratios between the tocopherols/tocotrienols components.

Tocopherols, and especially the α-tocopherol, are also known as vitamin E, an important dietary antioxidant. Vitamin E is also considered to have anti-sterility effect (Brad *et al.*, 2002). Vitamin E deficiency in humans causes defects in development of nervous system in children and may result into certain types of cancer and atherosclerosis (Singh, 2005). Yang and Kallio (2005a) found higher content of vitamin E in seabuckthorn pulp oil (up to 248.5 mg% of oil in ssp. *sinensis*) compared to seed oil (up to 159.4 mg% oil in ssp. *turkestanica*), while Lu (2005) found the highest content in the seed oil of *H. salicifolia* (248.2 mg% of oil) (see Tab. 4).

The results regarding major tocopherol components in Romanian seabuckthorn are summarized in Tab. 5 and two significant chromatograms for seed and pulp oil tocopherol composition are also presented in Fig. 3 and 4. A good separation was obtained between α-tocopherol and β+γ tocopherols, while δ-tocopherol registered only small amounts in both pulp and seed oil. As shown in Tab. 5, there was a high variation between individuals regarding tocopherol amounts (78.9-178.3 mg% of oil for α-tocopherol and 82.7-181.7 mg% for β+γ tocopherols in the seed oil; 83.1-194.4 mg% of oil for α-tocopherol and 11.4-26.6 mg% for β+γ tocopherols in the pulp oil). A very similar ratio between α-tocopherol and β+γ tocopherols in seed oil was also noticed; on the other hand, the pulp oil had a much higher content of α-tocopherol (87.7% of total tocopherols analyzed) compared to β+γ tocopherols. SF elites had the highest content in tocopherols, while CHF1 content is similar to the elites average. Even if α-tocopherol content is higher in pulp oil compared to seed oil (up to 194.4 mg% of oil), the total tocopherols have highest concentration in seed oil (up to 360 mg% of oil).

The average values we obtained for total tocopherols, in both seed and pulp, have typical values according to Yang and Kallio (2005a), while the SF8 elite had higher values (360 mg% in the seed oil) compared to other works (Tab. 6).

While FA composition proved not to be influenced by the oil extraction method, the tocopherol content in oil is highly influenced by it (Cenkowski *et al.*, 2006; Vlase *et al.*, 2006). The literature data regarding tocopherols composition in the seabuckthorn oil are insufficient, especially for oils extracted from different parts of the berries; at the same time, the extraction protocols are not always mentioned, and this is why comparisons are difficult to be made.

Tab. 4. Fatty acid composition in seabuckthorn oil of different origin

| Species/subsp. *Hippophae* genus | Berry part | Oil content (% of fresh weight) | Vit. E (mg% oil) | Major fatty acid compositions in sea buckthorn seed and pulp oil, as mass percentage of total amount of fatty acids | | | | | | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 16:0 (Palmitic) | 16:1 (Palmitoleic) | 18:0 (Stearic) | 18: 1n-9 (Oleic) | 18:2n-6 (Linoleic) | 18:3n-3 (Linolenic) | |
| *H. rhamnoides* ssp. *sinensis* | Seed | 9.5 | 154.7 | 10.3 | 0.7 | 2.7 | 17.4 | 28.2 | 36.1 | Lu (2005) |
| | Pulp | 9.34 | 133 | 34.2 | 30.3 | 1.3 | 15.8 | 6.8 | 1.7 | |
| *H. rhamnoides* ssp. *sinensis* | Seed | 7.3 | - | 8.7 | - | 2.5 | 19.4 | 40.9 | 26.6 | Yang and Kallio (2001) |
| | Pulp | 1.7 | - | 26.7 | 27.2 | 1.3 | 17.1 | 12.7 | 7.1 | |
| *H. r.* ssp. *turkestanica* | Seed | 12.23 | 193.7 | 9.9 | 3.9 | 2.3 | 13.1 | 43.3 | 23 | Lu (2005) |
| | Pulp | 5.44 | 116.2 | 40 | 40.8 | 0.9 | 2.8 | 7 | 0.6 | |
| *H. r.*ssp. *turkestanica* | Seed | - | - | 11.8 | 3.7 | 3.7 | 19.8 | 31.8 | 17.1 | Singh (2005b) |
| | Pulp | - | - | 18.4 | 42.4 | 0.8 | 5.5 | 7.9 | 0.7 | |
| *H. r.* ssp. *mongolica* | Seed | 11.87 | 205.3 | 9.1 | 4.4 | 2.8 | 16.2 | 36.7 | 26.4 | Lu (2005) |
| | Pulp | 3.93 | 136.2 | 31.7 | 35.1 | 1.3 | 7.9 | 12.1 | 0.6 | |
| *H. r.* ssp. *mongolica/ turkestanica* | Seed | - | - | 7.2 | 1.8 | 2.8 | 18.4 | 39 | 30.3 | Jamyansan and Badgaa (2005) |
| | Pulp | - | - | 30.1 | 37.6 | 1.1 | 11.2 | 14.9 | 1.8 | |
| *H. r.* ssp. *rhamnoides* | Seed | 12.36 | 173.1 | 8.9 | 2.1 | 2.7 | 18.5 | 34.6 | 27.8 | Lu (2005) |
| | Pulp | 2.83 | 135.6 | 31.2 | 26.9 | 1.2 | 26.3 | 7.2 | 1.2 | |
| *H. r.* ssp. *rhamnoides* | Seed | - | - | 7.5 | - | 2.8 | 13.4 | 36.3 | 35.9 | Cenkowsky *et al.* (2006) |
| | Pulp | - | - | 34.8 | 34.8 | 1.2 | 3.4 | 13.5 | 2.0 | |
| *H. r.* ssp. *rhamnoides* | Seed | 11.3 | - | 7.4 | - | 3 | 17.1 | 39.1 | 30.6 | Yang and Kallio (2001) |
| | Pulp | 2.8 | - | 27.8 | 32.8 | 0.8 | 17.3 | 9 | 3.2 | |
| *H. r.* ssp. *fluviatilis* | Seed | 9.85 | - | 10.3 | 3.2 | 2.5 | 21.7 | 32.8 | 28.7 | Lu (2005) |
| | Pulp | 2.62 | - | 40.7 | 26.8 | 0.6 | 26.4 | 3.6 | 1.2 | |
| *H. r.* L. (India) | Pulp | 3.3 | - | 32.1 | 48.7 | 0.1 | 13.3 | 6.6 | 0.9 | |
| *H. tibetana* | Pulp | 2.4 | - | 27.1 | 39.1 | 39.1 | 24 | 88 | 4.6 | Ranjith *et al.* (2006) |
| *H. salicifolia* | Pulp | 1.6 | - | 28.5 | 37 | 0.4 | 15.2 | 15.2 | 0.8 | |
| *H. salicifolia* | Seed | 9.6 | 248.2 | 13.9 | 0.7 | 2.7 | 18.9 | 37.6 | 21.1 | Lu (2005) |
| | Pulp | 2.64 | 134.3 | 20.8 | 36 | 1 | 22 | 3.9 | 1.8 | |
| **H. r.* ssp. *sinensis*[M] | Seed | 9.7 | - | 9 | traces | 2.2 | 22.4 | 35.4 | 29 | Kallio *et al.* (2002) |
| | Berry | 4.1 | - | 27.4 | 21.9 | 1.5 | 20.2 | 13.2 | 9.7 | |
| **H. r.* ssp. *mongolica*[M] | Seed | 12.6 | - | 8.6 | Traces | 3.3 | 17.9 | 38.6 | 29.1 | Kallio *et al.* (2002) |
| | Berry | 5.9 | - | 33.9 | 32.8 | 1.2 | 4.6 | 15.5 | 5.6 | |
| **H. r.* L. (German cv.) | Berry | 3.4-4.9 | - | 33 | 34.2 | 0.3 | 28.4 | 3.3 | 1 | Mörsel *et al.* (2005); Albrecht (2003) |
| **H. r.* subsp. *carpatica* | Berry | 2.5 | - | 34.2 | 25.7 | 0.9 | 31.5 | 5.4 | 1.9 | Brad *et al.* (2002) |
| ** H. r.* ssp. *sinensis, rhamnoides* and *mongolica* | Seed | 6-14 | - | - | - | - | - | - | - | Yang (2001) |
| | Berry | 1.5-10.5 | - | - | - | - | - | - | - | |
| ** H. r.* ssp. *sinensis, turkestanica* and *mongolica* | Seed | 4.2-16.5 | - | - | - | - | - | - | - | Yang and Kallio (2005a) |
| | Berry | 1.5-13 | - | - | - | - | - | - | - | |
| **H. r.* L. (India) | Seed | 9-16.9 | - | - | - | - | - | - | - | Dwivedy *et al.* (2005) |
| **H. r.* L. (Pakistan) | Seed | 7.8-12.4 | - | - | - | - | - | - | - | Shah *et al.* (2007) |
| **H. r.* L. (Azerbaijan) | Seed | 8.3-13.6 | - | - | - | - | - | - | - | Novruzov (2005) |
| | Berry | 2.8-6.4 | - | - | - | - | - | - | - | |
| **H. r.* L. (Russian cv.) | Berry | 1.6-17.8 (3.9-8) | - | - | - | - | - | - | - | Korovina and Fefelov (2005); Solonenko and Privalov (2005); Zubarev (2008) |
| Average percentage | Seed | 10.50 | 195.00 | 9.55 | 2.56 | 2.77 | 17.63 | 36.39 | 27.60 | |
| | Pulp | 3.51 | 131.06 | 30.29 | 35.39 | 3.65 | 14.87 | 14.89 | 2.01 | |
| *H. rhamnoides* ssp. *carpatica* | Seed | 11.17 | - | 8.41 | 1.38 | 3.28 | 17.86 | 40.10 | 26.29 | This study** |
| | Pulp | 6.08 | - | 36.55 | 20.45 | 0.99 | 30.90 | 2.98 | 1.10 | |
| | Berry | 6.31 | - | - | - | - | - | - | - | |

[M] fatty acids are presented as molar percentage from triacylglycerols; * Results not included in the average; ** Considered as average of all six individuals

Tab. 5. Major tocopherol components and concentrations in Romanian seabuckthorn

| *H. rhamnoides* subsp. *carpatica* Selections | Berry part | Major tocopherol components (mg% of oil) | | | | |
|---|---|---|---|---|---|---|
| | | α-tocopherol | % from total tocopherols | β+γ-tocopherols | %from total tocopherols | α+ β+γ-tocopherols |
| 'CF 2' | Seed | 78.89 | 48.84% | 82.65 | 51.16% | 161.54 |
| | Pulp | 83.11 | 87.96% | 11.38 | 12.04% | 94.49 |
| 'CF 4' | Seed | 95.31 | 52.11% | 87.59 | 47.89% | 182.90 |
| | Pulp | 121.76 | 87.18% | 17.91 | 12.82% | 139.67 |
| 'SF 7' | Seed | 147.12 | 48.90% | 153.76 | 51.10% | 300.88 |
| | Pulp | 156.44 | 87.87% | 21.60 | 12.13% | 178.04 |
| 'SF 8' | Seed | 178.25 | 49.52% | 181.68 | 50.48% | 359.93 |
| | Pulp | 194.44 | 87.98% | 26.56 | 12.02% | 221.00 |
| 'MF 3' | Seed | 88.22 | 48.37% | 94.16 | 51.63% | 182.38 |
| | Pulp | 96.11 | 87.68% | 13.51 | 12.32% | 109.62 |
| Average | Seed | 117.56 | 49.49% | 119.97 | 50.51% | 237.53 |
| | Pulp | 130.37 | 87.76% | 18.19 | 12.24% | 148.56 |
| 'CHF 1' | Seed | 129.56 | 53.75% | 111.47 | 46.25% | 241.03 |
| | Pulp | 145.57 | 87.41% | 20.97 | 12.59% | 166.54 |
| *H. rhamnoides* subsp. *carpatica** | Seed | 119.56 | 50.21% | 118.55 | 49.79% | 238.11 |
| | Pulp | 132.91 | 87.69% | 18.66 | 12.31% | 151.57 |

* Considered as average of all six individuals

Tab. 6. Major tocopherol components and their concentrations in seabuckthorn oil

| Species/subsp. *Hippophae* genus | Berry part | Tocopherol component (mg% of oil or mass percentage) | | | | | | | | Total tocopherols (mg% of oil) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | α (mg%) | α (%) | β (mg%) | β (%) | γ (mg%) | γ (%) | δ (mg%) | δ (%) | | |
| *H. r.* L. ssp. *rhamnoides* | Pulp | 220.8 | 81.9% | 21.1 | 7.83% | 11.1 | 4.1% | 6.5 | 2.4% | 269.5 | Cenkowsky *et al.* (2006) |
| | Seed | 121 | 45.3% | 9.5 | 3.56% | 130 | 48.7% | 6.4 | 2.4% | 266.9 | |
| *H. r.* L. ssp. *mongolica/ turkestanica* | Pulp | 189 | 57.2% | 67.7 | 20.49% | 57.2 | 17.3% | - | | 330.4 | Jamyansan and Badgaa (2005) |
| | Seed | 144 | 55.4% | 57.7 | | 41.6 | 16.0% | - | | 260 | |
| *H. r.* L. (Poland- Belorussia) | Berry | 73.4 | 64.1% | - | | traces-0.3 | | 39 | | 114.5 | Zadernowski *et al.* (2003) |
| *H. r.* L. (Uzbekistan) | Pulp | | 65% | | | traces | | | 35% | 300 | Bekker *et al.* (2005) |
| | Seed | | 65% | | | traces | | | 35% | 200 | |
| *H. r.* L. (Germany cv.) | Pulp | | 10.90% | | 0.30% | | 2.50% | | 86.3% | | Mörsel *et al.* (2005) |
| | Seed | | 26% | | 1.50% | | 6.60% | | 65.9% | | |
| *H. r.* L. (Azerbaijan) | Berry | | | | | | | | | 68.8-174 | Novruzov (2005) |
| *H. r.* L. (India) | Pulp | 67.9-104.6 | | 0.9-2.7 | | 8.2-17 | | 11.7-36.2[a] | | 130.1-178.8[b] | |
| *H. salicifolia* | Pulp | 35.4-51.7 | | 3.3-9.4 | | 3.3-9.4 | | 12.8-29.1[a] | | 66.6-90.2[b] | Ranjit *et al.* (2006) |
| *H. tibetana* | Pulp | 86.4-89.5 | | 1.5-1.9 | | 6.2-8.6 | | 33.6-43.1[a] | | 136.8-154.6[b] | |
| *H. r.* L. ssp. *mongolica* [c] | Berry | 136 | 75.7-89.2% | | 2.4-12.2% | | 4-10.8% | | 0.3-2.4% | 164[b] | Kallio *et al.* (2002) |
| | Seed | 129.4 | 17.2-66.1% | | 5-13.8% | | 25.3-55.8% | | 1.7-10.7% | 231[b] | |
| *H. r.* L. ssp. *sinensis* [c] | Berry | 194 | 75.7-89.2% | | 2.4-12.2% | | 4-10.8% | | 0.3-2.4% | 233[b] | Kallio *et al.* (2002) |
| | Seed | 44.4 | 17.2-66.1% | | 5-13.8% | | 25.3-55.8% | | 1.7-10.7% | 130[b] | |
| Not specified | Berry | 220 | 88.7% | 12.1 | 4.9% | 13.1 | 5.3% | 2.9 | 1.2% | 248.1 | Vlase *et al.* (2006) |

[a] δ-tocopherol + γ-tocotrienol; [b] Total tocopherols and tocotrienols, [c] Mass percentage related to whole beries and seeds weight

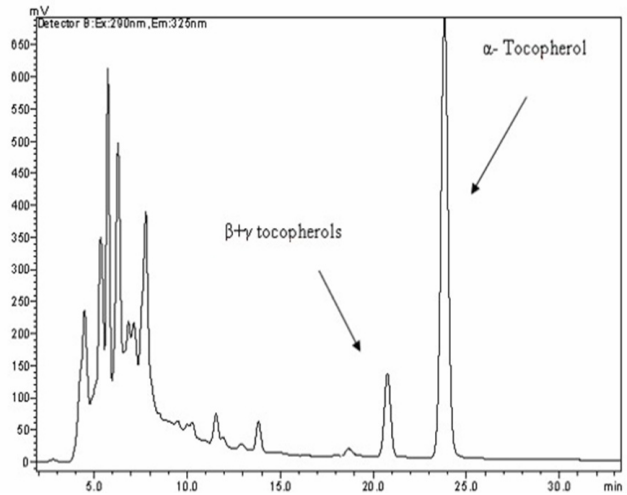


Fig. 3. Chromatogram of tocopherols in seabuckthorn pulp oil

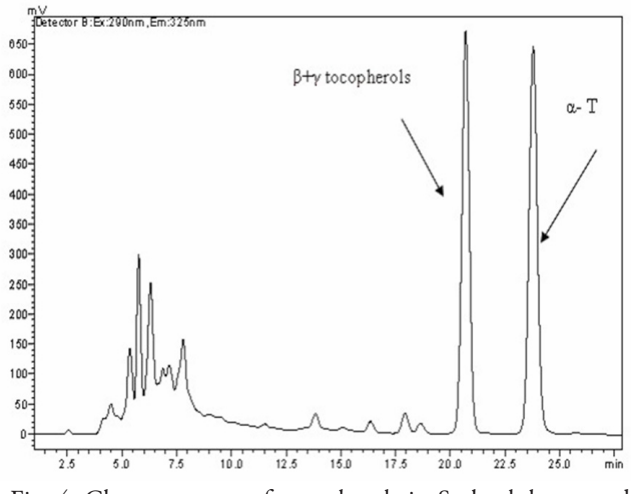


Fig. 4. Chromatogram of tocopherols in Seabuckthorn seed oil

As it can be seen in Tab. 6, there are large inconsistencies regarding results for both composition and concentration of tocopherols in the seabuckthorn oil. Our results are comparable to those obtained by Cenkowsky *et al.* (2006) for both seed and pulp oil of *H. rhamnoides* ssp. *rhamnoides*. However, some other results have similarities regarding only composition or content in tocopherols. Compared to Vlase *et al.* (2006), our samples had similar tocopherol composition in pulp, so the analyses may refer to same *carpatica* subspecies, even if not specified.

## Conclusions

High phenotypical and biochemical variations within seabuckthorn species, subspecies and even individuals of the same subspecies were observed.

Even if previous studies regarding oil extraction protocols, fatty acids composition and tocopherol composition in seabuckthorn oil have been published in Romania (Albulescu *et al*., 2006; Gruia *et al.,* 2007; Vlase *et al*., 2006, Brad *et al.*, 2002), no clear details are provided with regards to seabuckthorn berries or oil origin. No detailed references could be found on tocopherol composition of the oil extracted from fruits of *carpatica* subspecies and on pulp and seed oil tocopherol and fatty acids composition treated individually.

Compared to other results, our selections have larger fruits, medium seed weight, and similar oil content in seeds and in most of the cases higher in pulp. We have also obtained similar fatty acids composition in seed oil and some differences regarding pulp oil; however, the ratio between saturated and unsaturated fatty acids is very close to general average. Even if large inconsistencies were observed between different results regarding tocopherol content and composition in seed and pulp oil, our results are similar to those presented in other works (*e.g.* Cenkowsky *et al.*, 2006).

There are some clear differences between the control sample and the other selections, which stress the need for increasing the number of analyzed individuals and populations before extrapolating the results to *carpatica* subspecies; however, these results are very useful as the initial steps for further more complex analyses regarding Romanian seabuckthorn.

Based on phenotypical and biochemical characteristics of selected elites from *H. rhamnoides* ssp. *carpatica*, we can conclude that they have a high economical potential if introduced in culture.

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