Differences in SBT between Russian and German varieties

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Introduction

Seabuckthorn is one of the promising crops in many countries of the Asia, Europe and North America. It has outstanding resistance to dry conditions during summer time as well as cold tolerance during winter. Seabuckthorn has not specific demands to soil fertility and humidity. But the most valuable feature of seabuckthorn is unique biochemical composition including variety of vitamins and microelements.

State of the art technologies provide waste free utilization not only berries, but all plant as well. That is very important both for farmers and processing enterprises. Seabuckthorn become an important factor in economy of poor rural areas, in ecology for erosion control and natural reforestation of degraded areas.

Because of rapidly developing market of seabuckthorn products the quality control becomes is quite important issue to prevent possible adulterations and deceptions

This research work was primary initiated by the observance of adulterated SBT pulp oil in European Community market. Because of missing detailed information on composition and comparability of it between European and Asian cultivars it was necessary to have a closer look on this question. Taking into consideration that about 80% of total seabuckthorn market of Russia and quite a few of Chinese represented by varieties bred at the Lisavenko Institute (Barnaul, Russia), it was reasonable to put them in comparative estimation with most promising German varieties. Based on long time cooperation between UBF GmbH Altlandsberg and Lisavenko Institute (Barnaul) the investigations were carried out with authentic cultivars of German and Russian origin.

Seabuckthorn growing and processing is a multinational and diversified business. Raw materials, semi-products and final products are merchandised.

Buyer often do not know the origin of raw materials and have to trust their suppliers. Actually there is only a limited number of standards available on national basis, mostly focusing on berries as raw material or Seabuckthorn oil. Often quality control on delivery is only coarsely done. Own experimental surveys shows that not really seldom advertised qualities does not really exist. So e.g. pesticides are frequently found in berries, juice and oil labeled as to be 'organic'.

Price of Seabuckthorn oil on world market is high, especially when of organic origin. As in some countries price is calculated based on carotenoids content, adulteration of oils with cheap synthetic carotenoids is a profitable but dishonest procedure. One important question we dealt with was to answer if there is naturally occurring capsanthin in Seabuckthorn oil. This was especially a real task because of more then 100 registered varieties worldwide in an area from far east Asia to western Europe.

Material and Methods

Samples were collected during the years 2010, 2011 and 2012. The materials were taken from experimental fields of Lisavenko institute in Barnaul and from orchards around Berlin, mainly in Werder region southwest from Berlin. All samples were from authentic plantations where origin and

identity of planting materials where well known. So classification to varieties and cultivars were certain and unchecked cross-breeding was excluded. Samples were frozen directly after picking and stored until analysis at -18°C in refrigerator. For analysis frozen samples were taken without thawing the whole sample. Analyses were repeated for all three crop years. During analysis samples were protected against oxygen and light. Table 1 shows the used varieties and their origin.

Extraction of lipids was done by method of Folch et al. With chloroform and methanol 60:40. the method was slightly modified. Samples where first poured in semi boiling methanol for 5 minutes and then chloroform was added. The berries were grind before in frozen state. After water addition the chloroform layer was separated, dried with sodium sulphate and concentrated in rotary evaporator at temperatures below 40°C.

Fatty acid composition was determined after transesterification with TMSH in methanol. Method is according to DGF Method C III 2 (66). TMSH reagent was bought at Macherey Nagel Dueren. Determination of fatty acid esters was done by capillary GC in accordance to AOCS standard method Ce 1-62.

Carotenoids were determined by HPLC on Lichrosphere 250 mm x 4 mm. Solvent was iso-octane / ethyl acetate 96:4. Detection was visible at 450 nm. Alternatively the separation and determination was done using a CAMAG HPLC system. Separation was done on silicagel plates 200x200, solvent was methanol acetone 1:1. detection was done by scanning at 450 nm. Quantification in both cases was with external standards. Standards were obtained from Fluka, Germany.

Table 1: Sample materials and its origin

Variety	Origin	Variety	Origin
Inja	Barnaul, Siberia	Zlata	Barnaul, Siberia
Essel		Chuyskaya	
Sudarushka		Avgustina	
Elisaveta		Rosinka	
Azhurnaja		Klavdia	
Altaiskaya		Leikora	Berin area, Germany
721-95-2		Hergo	
Dzhemovaj		Ascola*	
Zhemchuzhnitsa		Frugana*	

* not determined in all years

Tocopherols and sterol were determined after saponification. Saponification was done by DGF method G-III 6b (57). The obtained extracts were used for determination. Samples were resolved in iso-octane and kept under argon layer to prevent from oxidation. Saponification was also done with argon as shielding gas.

Tocopherols were analyzed on normal phase HPLC, 300 mm x 4,6 mm, mob. phase i-octane / ethyl acetate 96/4 according to DGF method F II 4a. Detection was in UV at 295 nm in UV region. Quantification was done by external calibration.

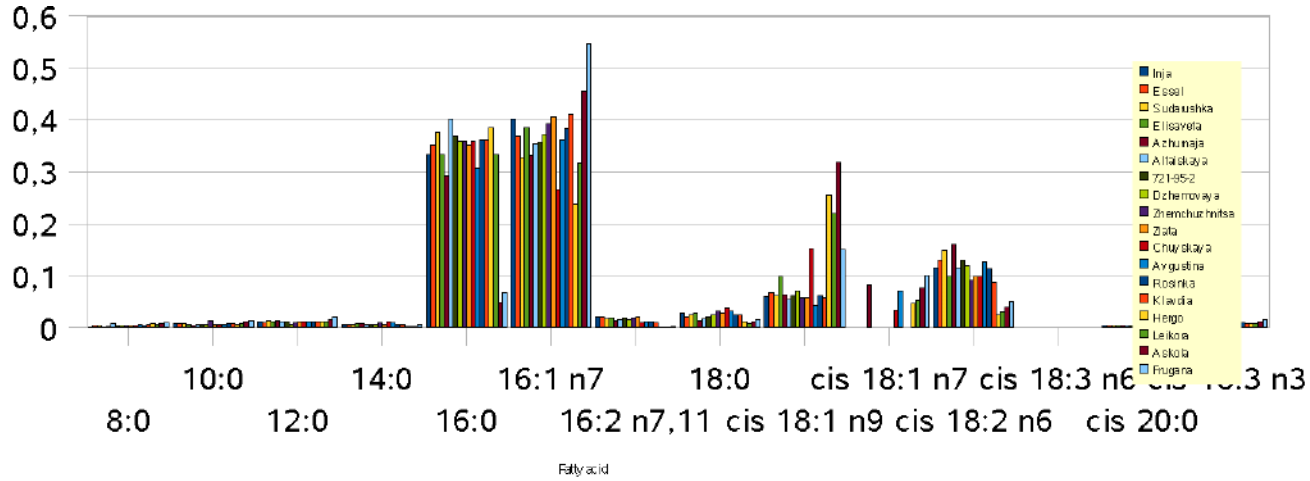
Sterols determination was carried out after derivatisation with BSTFA. Sample are transferred to a 1.6 ml vial, solvent completely evaporated with a flow of argon. The sample is resolved in 500 µl of acetonitril and then 100 µl of BSTFA reagent (methanolic solution, Macherey-Nagel, Germany) is added.

The vials are capped with Teflon covered silicon septum caps and mixed in ultrasound bath for 5 minutes following reaction at 50°C for 15 minutes in an oven. 10 µl are used for GC analysis. GC parameters are as described in DGF method F II 4a.

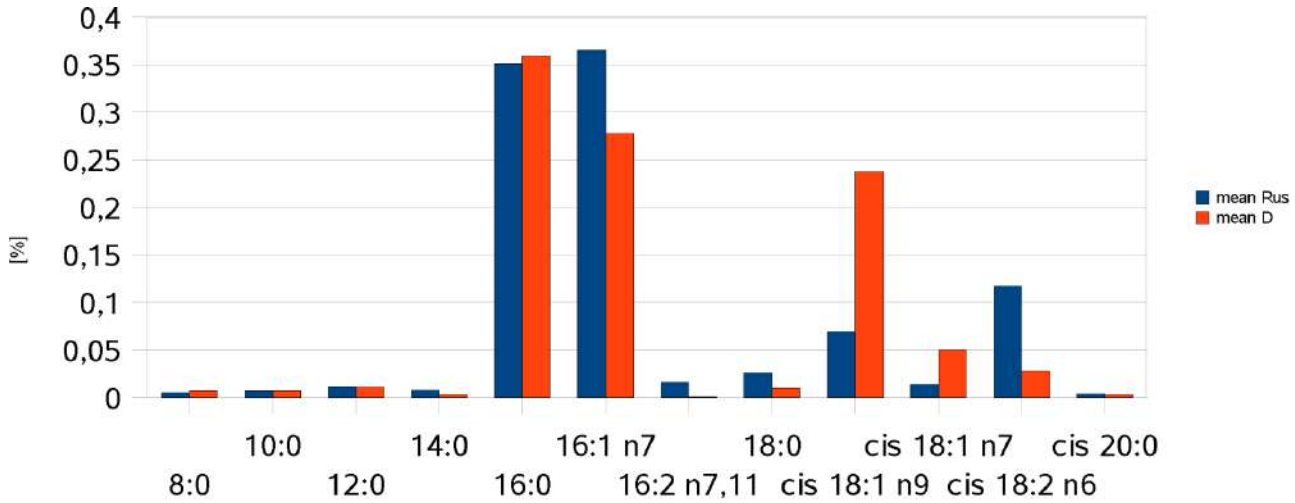
Results and discussion

Picture 1 shows the fatty acid composition of the investigated varieties ad cultivars. The important differences are figured out in 1a. It can be seen from the picture that important differences are found in 18:1 n-9, 18:1 n-7, 18:2. This may be a result of differences in climate. German cultivars have higher concentration in 18:1 species where Russian are higher in linoleic acid and palmitolenic acid.

Picture 2 show tocopherol pattern observed in the sample materials. Main tocopherol isomer is alpha-tocopherol followed by gamma-tocopherol. This composition is also reported in literature and agree with the common knowledge. A special observation is the generally higher concentration of delta-tocopherol in German varieties. This might be a dependence on the different genetical resources of German and Russian varieties and cultivars.

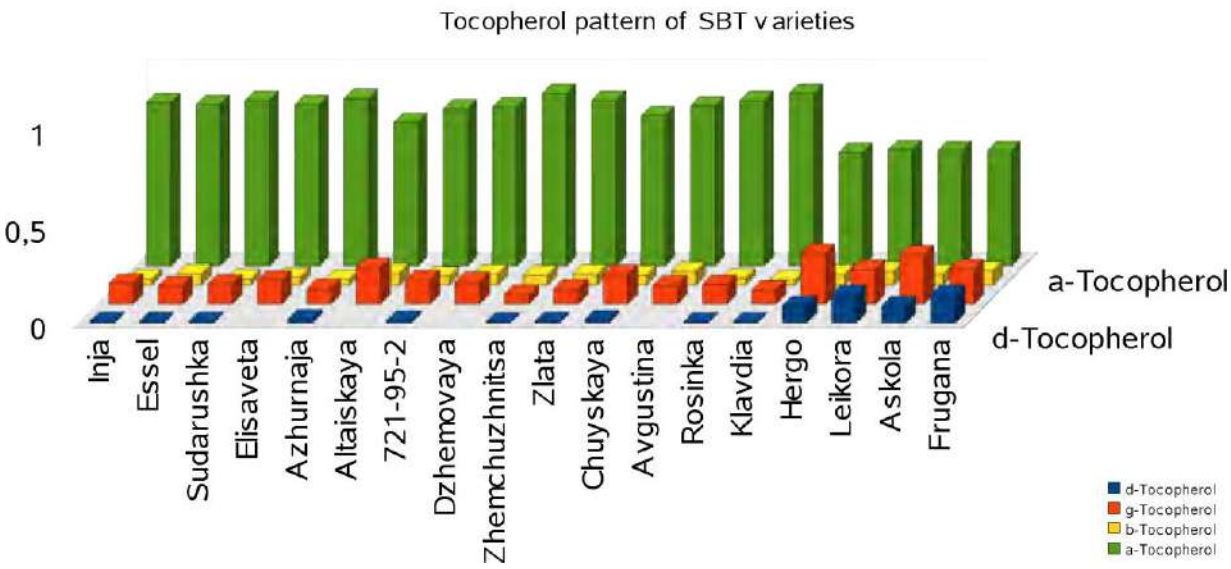


Picture 1a. Fatty acid composition of investigated varieties

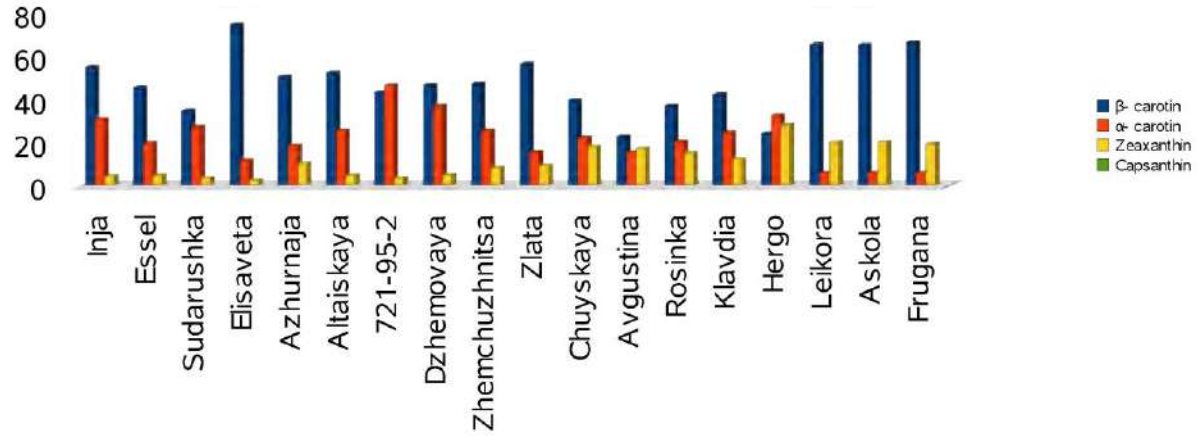


Picture 1b. Fatty acid composition – key parameters comparison between Russian and German varieties

Carotenoids are a group of terpene based chromophores found in mostly all plant lipids. The group contains over 200 individual compounds. One of the most important is β-carotene. Picture 3 list the most important carotenoids and the expected possible tampering compound, capsanthin.

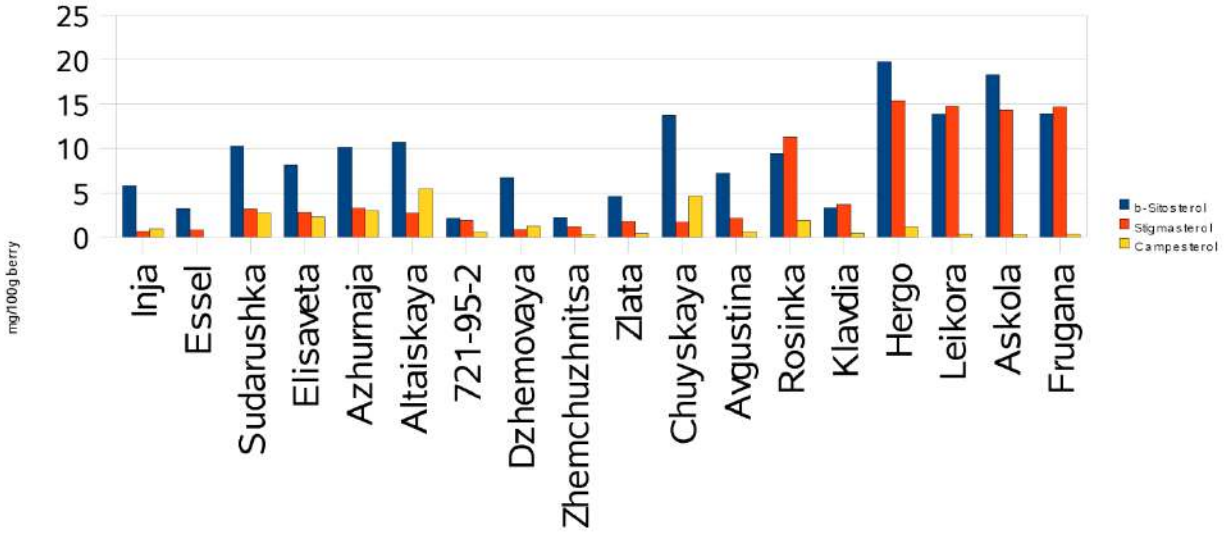


Picture 2: tocopherol composition



Picture 3. Carotenoids in Seabuckthorn pulp oil

There are two visible results from this investigation. Neither in Russian nor German varieties capsanthin was found. The second interesting fact is the low concentration of α-carotene in German brands. The β-carotene is also remarkable high.



Picture 4 Sterol pattern in Seabuckthorn oil

Sterol pattern show a significant higher sterol content in German cultivars and a remarkable high level of stigmasterol. Table 2 compares the quotients of stigmasterol and β-sitosterol.

Table 2: Stigmasterol and β-Sitosterol quotient

Variety	***Inja***	***Essel***	***Sudarushka***	***Elisaveta***	***Azhurnaja***	***Altaiskaya***	***721-95-2***	***Dzhemovaya***	***Zhemchuzhnitsa***	***Zlata***	***Chuyaskaya***	***Avgustina***	***Rosinka***	***Klavdia***	***Hergo***	***Leikora***	***Askola***
Quotient	0,11	0,25	0,31	0,34	0,32	0,25	0,9	0,13	0,57	0,39	0,12	0,29	1,19	1,13	0,78	1,07	0,78
Average Russia	0,33																
Average Germany	0,92																

Conclusions

Typical biochemical substances of Seabuckthom oil like fatty acids, tocopherols, sterols can characterize not only nature of Seabuckthom oil in general, but difference between varieties it was obtained from. Russian and German varieties belong to different subspecies (*H. rhamnoides sbsp. mongolica, H. rhamnoides sbsp. rhamnoides*). These differences combined with regional and climatic factors can be seen in chemical composition. Tocopherols and sterols are reliable indicators for both subspecies. Fatty acid combination also shows some differences but not so significant.

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