Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2016) 5 (2) 55-61
Food in Health and Disease, scientific-professional journal of nutrition and dietetics (2016) 5 (2) 55-61

SUPERCRITICAL CO_2 EXTRACTION OF SEA BUCKTHORN

Nika Pavlović[1*], Kristina Valek Lendić[1], Maja Miškulin[2], Tihomir Moslavac[3], Stela Jokić[3]

[1]Institute of Public Health for the Osijek-Baranja County, Franje Krezme 1, HR-31000 Osijek, Croatia
[2]Josip Juraj Strossmayer University of Osijek, Faculty of Medicine, Cara Hadrijana 10E, HR-31000 Osijek, Croatia
[3]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhaca 20, HR-31000 Osijek, Croatia

Original scientific paper

Summary

Sea buckthorn is one of the most prominent plants containing important nutrients essential for our health. Bioactive compounds are found in the pulp and in the seeds of the fruit. Sea buckthorn oil can be used in food supplementation, pharmaceutical and cosmetic industry.
In this study supercritical CO_2 extraction of oil from sea buckthorn berries was performed at pressure 300 bar, temperature 40 °C and CO_2 flow rate 2 kg/h. Fatty acid composition of oil was determined by gas chromatography, and amount of tocopherols using HPLC. In defatted cake after supercritical extraction the amount of remained oil, fibre and protein content were also determined.
The initial oil content in sea buckthorn berries was 11.60%. The major fatty acids in oil were palmitic (35%), palmitoleic (20%) and oleic acids (32-35%). The amount of α-tocopherol was 35.99 mg/100g oil, and total tocopherol amount was 71.62 mg/100g oil. In defatted cake cellulose content was determined to be 11.56%, proteins 14.78%, moisture 5.68% and ash 3.16%.
Because of high content of bioactive compounds and unique oil composition, this oil is connected with benefits on certain diseases. Defatted cake, which is also rich in many components, can be used as by-product in food industry.

Keywords: sea buckthorn oil, supercritical CO_2 extraction, bioactive compounds, defatted cake

Introduction

Sea buckthorn (SBT) (*Hippophae rhamnoides* L. Elaeagnaceae), one of the most valuable plants, receives increasing attention worldwide because of it's nutritional, medical and pharmaceutical potential. It possess large quantities of bioactive substances like tocopherols, carotenoids, flavonoids, essential fatty acids and large proportion of polyunsaturated fatty acids (PUFA), and also some essential amino acids. Flavonoids are contained in all parts of the plant and have the best activity in protecting our cells from oxidative damage and genetic mutation (Chauhan & Varshneya, 2012). Sea buckthorn also contains important organic acids like malic, quinic, oxalic, citric and tartaric acids. The leaves of the plant are good source of some nutrients like phenolic components, tannins and vitamin C. Plant fruits of SBT are oval, orange to red berries, consisting from the pulp and the seeds (Bal et al., 2011). The carotenoids are fully responsible for the plant color (St. George & Cenkowski, 2007). The chemical composition, content and quality of the berries depend on climate conditions, the size of the fruit, ripeness, processing and upon the maturity level at harvest (Bal et al., 2011). Low temperatures and freezing can cause crystal formation and physical changes which can increase the possibility of oxidation, a common cause of oil degradation. Various factors influence on oxidation phenomena such as light, air, water activity, pro-oxidants and enzymes (St George & Cenkowski, 2007). There are two types of oil that can be extracted from SBT, the oil from the pulp and oil from seeds of SBT. Some studies show that fresh pulp oil recovery is from 4-17%, dry pulp oil 20-25%, and seed oil 8-20%. This mostly depends on specific cultivars and harvest time. It is scientifically proven that SBT pulp has the highest concentration of palmitoleic fatty acid 16:1, n-7, (omega 7 fatty acid) up to 43%. SBT seed oil has the linoleic acid to linolenic acid ratio 1:1, which is characteristic only for SBT oil (Bal et al., 2011; Yang & Kallio, 2002; Zadernowski et al., 1997). Seed of SBT mostly contains fatty acids (FA) with 18 carbon atoms per molecule (linoleic and linolenic acid), and the oil from the pulp of the plant consists mainly from FA with 16 carbon atoms per molecule (palmitic and palmitoleic acid) (Cossuta et al., 2007). In addition, FA composition differs from different parts of the plant. Seed of the SBT plant is rich with PUFA while the skin and pulp of the plant abounds with saturated (SA) and monounsaturated fatty acids (MUFA) (Zadernowski et al., 1997). When expressing the influence on health, it can be noted

*Corresponding author: nika.felicita@gmail.com

that SBT has positive effect on some mental functions such as memory loss in elders, and skin disease treatments, especially creams containing its extracts (Beveridge et al., 1999; Krejacarova et al., 2015). Many countries around the world use different parts of this plant in traditional medicine. It was shown to have great effects on cardiovascular system, atherosclerosis, diabetes, anti-inflammatory effect and antitumor effect because of its benefits (Beveridge et al., 1999; Wani et al., 2016). The seeds and the pulp of SBT are also an excellent source of tocopherols. Oil obtained from these berries slows down the oxidation process, promote the healing of wounds and reduces skin dermatitis. Introducing this SBT oil into the daily diet such as bread, juice and yoghurt leads to some new trends in application of this plant and also in food industry. The high concentration of PUFA and some other lipid nutrients such as carotenoids and tocopherols, make it sensitive to oxidation and consequently that, the usage of this oil is limited. Solution to the problem may be a microencapsulation, which converts the oil into a powder, protecting it from oxidation, especially when antioxidants are added what increases the stability of oil and improves its shelf life (Yang & Kallio, 2002). It is even desirable to mix SBT oil with other oils to improve their efficiency. Some oils, like olive oil, can act synergistically with SBT oil enhancing its properties and activity (Edraki et al., 2014).

SBT, as one of the healthies berries in the world, is rich with nutrients and phytonutrients that are necessary for normal functioning of our body, and therefore, because of it's effectiveness has impressive properties. Drained juices of these berries are beneficial for colds, fever, exhaustion and cramps while SBT oil is applicable for liver diseases, inflammation, gastrointestinal disorders and ulcers, wounds, eczema, burns, rosacea, conjunctivitis and vaginal problems (Bal et al., 2011). There are no evidence of any allergy occurrence or toxic reactions after consuming SBT oil (Gupta & Upadhyay, 2011). SBT oil, as treasure of energy in a bottle, can easily fit in "Food to Health" theory.

To extract oil from SBT berries, different extraction methods have been used, and priority is increasingly given to supercritical fluid extraction (SFE), especially with carbon dioxide (CO_2) as a solvent. SFE, compared to other conventional extraction techniques with organic solvents, gains increasing popularity because of its advantages, such as lower viscosity, better diffusion and surface tension, causing the supercritical solvent to penetrate better in the material from which the desired substance is extracted. The solvent power and selectivity can be controlled by changing its temperature and pressure and is also very easy to remove it from the extract. CO_2 is an environmental friendly solvent and generally recognized as safe (GRAS) (Jokić et al., 2011).

The aim of this study was to perform SFE of SBT berries to obtain the oil. The amount of oil, fibre and protein content in defatted cake which remain after SFE, as well as moisture content and ash were determined. The fatty acid composition between oils obtained by Soxhlet extraction and SFE was also studied and compared. In the oil obtained by SFE the tocopherol content was determined.

Material and methods

Material

Dried SBT berries (Fig. 1a) were purchased from „Planet Health", the supplier, on-line store for food supplements and Eco products. Country of origin these dried berries was Germany. The purity of CO_2 used for extraction was 99.97% (w/w) (Messer, Osijek, Croatia). Industry FAME mix 37 standard for fatty acids was purchased from Restek (USA). α-tocopherol (Dr. Ehrenstorfer Cat No. 17924300), β-tocopherol (Supelco Cat No. 46401-U), γ-tocopherol (Supelco Cat No. 4-7785) and δ-tocopherol (Supelco Cat No. 4-7784) were used. All other solvents were of analytical grade and purchased from J.T. Baker (PA, USA).

Fig. 1. Sea buckthorn (from left to right): a) purchased dried berries b) grinded dried berries before SFE extraction c) defatted cake after SFE extraction

Determination of initial oil content

The initial oil content in dried SBT berries was measured by automatic extraction systems Soxterm by Gerdhart with *n*-hexane (Aladić et al., 2014).

Determination of particle size distribution of SBT berries with sieving

The grounded dried SBT berries (Fig. 1b) were sieved for 20 minutes using a vertical vibratory sieve shaker (Labortechnik Gmbh, Ilmenau, Germany). About 200 g were used at each sieving. The raw material size distribution was determined using a nest of 9 sieves of aperture sizes 1.4, 0.8, 0.63, 0.5, 0.4, 0.315, 0.2, 0.1 and 0.05 mm. The mass of fragments remaining on each sieve was used to calculate the distribution of fragments, which was then normalized in respect of the total mass. For evaluation of sieve analysis results, the Rosin-Rammler-Bennet (RRB) distribution (Allen, 1981) was chosen. The percentage by mass of particles (R) greater than screen size (d) is given as (Eq. 1):

$$R = 100\exp\left[-\left(\frac{d}{d_0}\right)^n\right] \qquad (1)$$

where d_0 represents the particle size corresponding to the 36.8th percentile of the cumulative probability distribution (size constant), and n controls the shape of the distribution (uniformity coefficient). The function of the sum of sieve residue (R) was fitted to the experimental data by changing the representative particle size d_0 and the uniformity coefficient n, minimizing the sum of the mean square error using *STATISTICA 12.0* software (Stat Soft Inc., USA).

Supercritical CO_2 extraction of SBT oil

The experiment was performed in SFE system explained in detail elsewhere (Jokić et al., 2014; Jokić et al., 2015). The process scheme for SFE system is given in Fig. 2. The grounded dried SBT berries of 100 g were placed into extractor vessel. The extracts were collected in previously weighed glass tubes. Extraction process took 90 minutes until the all amount of oil were extracted (each 15 minutes the amount of obtained extracts were weight). The amount of extract obtained after defined time was established by weight using a balance with a precision of ±0.0001 g. Separator conditions were 15 bar and 25 °C. The SFE was performed at extraction of pressure 300 bar and temperature of 40 °C at mass flow rate of 2 kg/h.

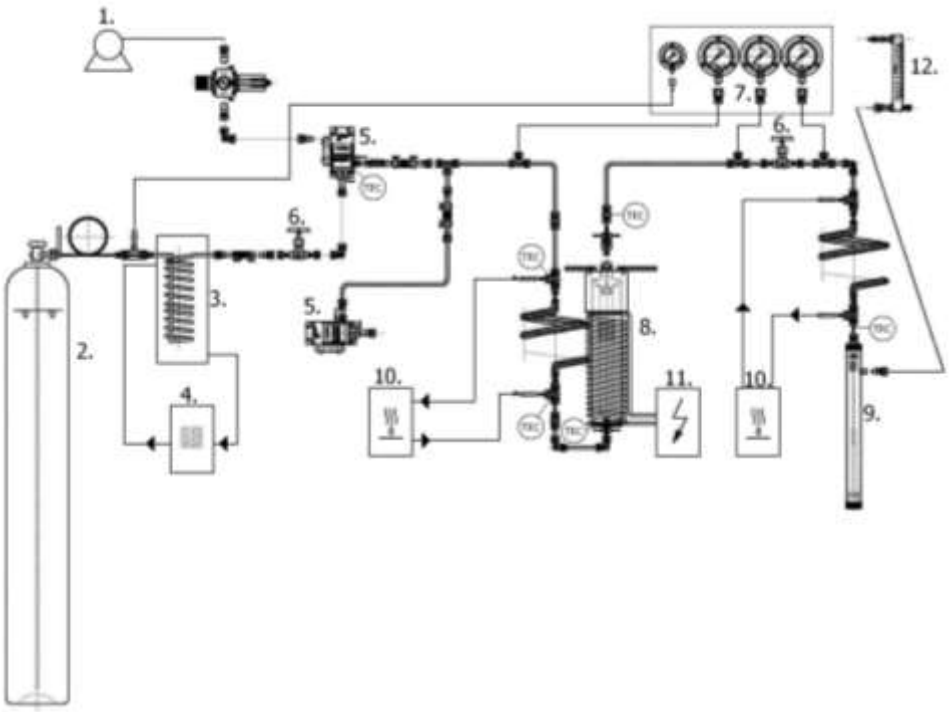

Fig. 2. Process scheme for SFE system
1. Compressor; 2. CO_2 Tank; 3. Stainless steel coil; 4. Cooling bath; 5. Air driven fluid pump Haskel MS-71; 6. Valves (B-HV); 7. Manometers; 8. Extraction vessel; 9. Separator vessel; 10. Water bath; 11. Centralized system glass fiber heater; 12. Flow meter

Determination of fatty acids composition of oil

Preparation of fatty acid methyl esters was carried out according to HRN EN ISO 12966-2:2011 standard by saponification of glycerides with NaOH in methanol after which the soaps were converted into FA methyl esters by reaction with BF3 methanol complex. Prepared fatty acid methyl esters were analyzed by gas chromatography according to HRN EN ISO 12966-1:2015. Gas chromatograph 7890B (Agilent Technologies, Lake Forest, USA) with a capillary column: Rtx®-2560 (biscyanopropyl polysiloxane) 100 m long with a diameter of 0.25 mm and the thickness of the stationary phase 0.20 microns (Restek, USA), a splitless injector (temperature 225 °C) and a flame-ionization detector (temperature 250 °C) was used with a sample volume of 1 μL. Start column temperature was 100 °C with holding time for 4 minutes. The oven temperature was increased with a rate of 3 °C/min to 240 °C/min, holding for 11 minutes. Carrier gas was nitrogen (99.9999%) at constant flow rate of 1.2 ml/min. The hydrogen flow was 30 ml/min, air flow was 250 ml/min, and the makeup gas flow (nitrogen) was 45 ml/min.
FA methyl esters in samples were identified by comparison with retention times of 37 FA methyl ester standard at the same conditions. Prior to standard and sample analysis, certified reference material (CRM) was prepared and analysed at the same conditions. The results were expressed as percentage (%) of individual fatty acids to total fatty acids. The detection limit was 0.01%. The analysis were conducted in two replications.

Determination of tocopherol content in oil

Determination of tocopherol content in SBT oil obtained by SFE (α, β+γ, δ) was done according to modified HRN EN 12822:2014 standard (Bele et al., 2013). Analysis was done on reversed-phase High Performance Liquid Chromatography (HPLC) Infinity 1290 Agilent Technologies (USA) instrument using fluorescence detection (FLD). The excitation and emission wavelengths were set at 290 and 325 nm, respectively. The instrument configuration had an Autosampler G4226A and 1260 FLD G1321C with quaternary pump G4204A. A Zorbax Eclipse XDB 5μm- C18 column that was used, was 250 mm long and the mobile phase was acetonitrile : methanol (50:50) with gradient run time of 16 minutes. In the beginning flow was 2ml/min holding for 7 minutes, decreasing to 1.5ml/min. Injection sample volume was 20μl and column temperature was set to 25 ºC. Sample preparation of oil was done by weighing a certain amount of oil and dissolving it in given volume of isopropanol which gave a good and accurate response (88-99% recovery). The prepared solution was filtered through 0.2 μm filter and was placed in apparatus for measurement.
The identification of tocopherols was done after method calibration for each component, by comparing retention times with standards. Amounts of tocopherols were expressed in mg/100g of oil. The analysis were conducted in two replications.

Determination of fibre and protein content, moisture and ash in defatted cake

Determination of crude fibre in the defatted cake after SFE (Fig. 1c) was done according to Scharrer-Kurschner, by method developed in the laboratory following the modified standards HRN ISO 5498:1999 and HRN ISO 6541:2001. Determination of proteins in dried berries of SBT was done using modified standards HRN ISO 1871:1999 and HRN ISO 5983-2:2010 by block digestion and steam distillation method. Also, ash was determined at 550 ºC according to modified standard HRN ISO 5984:2004, and moisture by drying on 105 ºC to a constant mass according to modified standard HRN ISO 6496:2001 (Trajković et al., 1983). Dry matter was determined according to identical standard by calculation. The analysis were conducted in two replications.

Results and discussion

Characteristic of SBT berries, oil and defatted cake

Some chemical characteristics (average values of two repetitions) of SBT dried berries were given in Table 1. The initial oil content in SBT berries was determined to be 11.60 ± 0.13% which is consistent with earlier reported values of some previous studies. For example, Damian et al. (2013) have reported 6-15% of oil for seed parts of SBT and 3-5% for soft parts of SBT. Yang & Kallio (2001, 2002) have published that oil content in SBT seeds was 10% up to 15-16% in berries depending on different cultivars. Bal et al. (2011) published that oil content range from 5.3 to 19.5% in SBT. Maturity is also one of main factors which affects oil content in SBT fruit which makes it to vary from 4.2 to 17% what is related to our research (Zadernowski et al., 1997). The moisture content in dried berries in our study was found to be 6.86 ± 0.11% which is similar to some previous studies where moisture content varied from 5.43 to 21.9% (Bal et al., 2011; Beveridge et al., 1999).

Table 1. Some chemical characteristics of SBT dried berries

Compound	%
Oil	11.60
Moisture	6.86
Saturated fatty acids	37.20
Unsaturated fatty acids	62.80
MUFA	54.42
PUFA	8.40

Before further extraction experiments, dried berries were milled and the average particle size were determined to be 0.365 ± 0.31 mm. Supercritical CO_2 extraction was performed at pressure 300 bar, temperature 40 °C and CO_2 flow rate 2 kg/h during 2 hours until the whole amount of oil was extracted. The extracted oil by *n*-hexane and by supercritical CO_2 were analyses by gas chromatography to determine the fatty acid composition (Table 2).

Table 2. Fatty acid composition (weight % of total fatty acids) of SBT oils obtained by different extraction methods

Fatty acid	Soxhlet extraction W(%)	SFE W(%)
myristic acid	0.17	0.19
palmitic acid	35.57	34.40
palmitoleic acid (cis-9)	20.39	20.26
stearic acid	1.22	1.33
oleic acid (cis-9)	35.09	31.91
linoleic acid (cis-9,12)	4.02	7.09
linolenic acid (cis-9,12,15)	2.44	3.18

The major fatty acids were palmitic (35%), palmitoleic (20%) and oleic acids (32-35%) for both types of extraction (Soxhlet and SFE). Obtained values for saturated and unsaturated fatty acids were similar for both extraction methods and ranged between 36-37% for saturated and 62-63% for unsaturated fatty acids. The results obtained from this study show similarity to other studies (Andrei et al., 2014; Dulf, 2012; Kallio et al., 2002; Pintea et al., 2001; Zadernowski et al., 1997) for the obtained contents of major fatty acids like palmitic, palmitoleic, oleic acids which together represent approximately cca 90% of total fatty acids. Dulf (2012) confirmed presence mainly for palmitic (16:0) (23-40%), oleic (18:1n-9) (20-53%) and palmitoleic (16:1n-7) (11-27%) which corresponds to this research. He also confirms similarity with his studies for MUFA and PUFA (53-70%, 3-7%). From this study and some related research, it is evident that SBT oil contains larger amounts of palmitoleic acid than in other fruit oils. Fatty acid composition also greatly depends on time of fruit collection (Zadernowski et al., 1997) and also on different varieties or origin (Yang & Kallio, 2001). When comparing two extraction techniques (Soxhlet and SFE) it can be concluded that they both provide comparable results for fatty acid composition in SBT oil. It is necessary to emphasize the development of SFE technique due to its many advantages over other conventional methods (Jokić et al., 2011).

Tocopherol content in SBT oil obtain by SFE were given in Table 3. α-tocopherol content was determined to be 35.99 mg/100g oil, and total tocopherol amount was 71.62 mg/100g oil, what again demonstrates the prevalence of α-tocopherol over 50%, what matches with other studies. In study by Beveridge et al. (1999) total tocopherol amount in oil of SBT berries ranged from 40.1 to 103 mg/100g, and in the research by Bal et al. (2011) ranged from 40.1 to 113 mg/100g of oil.

Table 3. Tocopherol concentrations in SBT oil obtained by SFE

Tocopherols	mg/100g oil
α-tocopherol	35.99
$\beta+\gamma$-tocopherol	24.17
δ-tocopherol	1.46
Total tocopherol amount	71.62

Beside SBT oil, which is the main product of extraction process, defatted cake after SFE was also investigated and the result of it's chemical composition are given in Table 4. In defatted cake after supercritical CO_2 extraction, cellulose content was 11.56%, protein content 14.78%, moisture 5.68% and ash content 3.16%.

Table 4. Chemical composition of SBT defatted cake after SFE

Compound	%
Moisture content	5.68
Cellulose	11.56
Proteins	14.78
Ash	3.16
Dry matter	94.32
Oil	0.58

This study shows similarity for moisture content in defatted cake with other studies (7.0%) (St George & Cenkowski, 2009). The remaining oil in defatted cake after SFE was determined to be 0.58 ± 0.09%, which means that using supercritical CO_2 it is possible to completely recover the oil from initial material and such defatted cake can be used further in other processes. For example, this potentially valuable by-product could be mixed with other products or just used further in food or pharmaceutical industry.

The results of protein content in SBT defatted cake after SFE were very close to results of some other researches. Nuernberg et al. (2015) obtained almost exactly the same amount of 14.6% for protein content while Ben-Mahmoud et al. (2014) reported value of 27.7 to 33.2% for protein content. He also published results for crude fiber in defatted cake which were 15.0-21.9% what is less than in this research, and total ash content 2.7-3.6% similar to this research. Kaushal & Sharma (2011) pointed out also very similar total ash content of 2.7-3.59%.

Conclusions

SBT fruit that was discovered long time ago but neglected in some parts of the world, grows on poor soils and unpresentable location, often imperceptible and unsightly. This plant with an interesting fatty acid composition and large number of bioactive components that are found in all part of the plant could in our days be fully utilized due to newer extraction techniques like supercritical CO_2 extraction. This more ecological way of extraction enables complete oil extraction in the safest way, with non-solvent residues, while beneficial components remain unharmed. Oil from berries of SBT contains high proportions of unsaturated fatty acids, such as palmitoleic fatty acid or so called omega 7 fatty acid, which has also some potentially therapeutic properties. Defatted cake, remaining after the supercritical CO_2 extraction, can also be utilized as a by-product to produce nutritive-rich formulations due to its content.

References

Aladić, K., Jokić, S., Moslavac, T., Tomas, S., Vidović, S., Vladić, J., Šubarić, D. (2014): Cold pressing and supercritical CO_2 extraction of hemp (*Cannabis sativa*) seed oil. *Chem. Biochem. Eng.* 28 (4), 481-490.

Allen, T. (1981): Particle Size Measurement, London UK: Chapman and Hall, pp. 139-140.

Andrei, S., Bunea. A., Pop, E. A., Dulf, F., Pintea, A. (2014): The protective effect of *Hippophae Rhamnoides* carotenoid extract against lipid peroxidation in crude vegetable oils. *Bulletin UASVM Food Sci. Tech.* 71 (2), 101-106.

Bal, L. M., Meda, V., Naik, S. N., Satya, S. (2011): Sea Buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmeceuticals. *Food Res. Int.* 44 (7), 1718-1727.

Bele, C., Matea, C., T., Raducu, C., Miresan, V., Negrea, O. (2013): Tocopherol content in vegetable oils using a rapid HPLC fluorescence detection method. *Not. Bot. Horti. Agrobo.* 41 (1), 93-96.

Ben-Mahmoud, Z., Mohamed, M. S., Bláha, J., Lukešová, D., Kunc, P. (2014): The effect of sea buckthorn (*Hippophae Rhamnoides* L.) residues in compound feeds on the performance and skin color of broilers. *Indian J. Anim. Res.* 48 (6), 548-555.

Beveridge, T., Li, T. S., Oomah, B. D., Smith, A. (1999): Sea Buckthorn products: Manufacture and composition. *J. Agric. Food Chem.* 47 (9), 3480-3488.

Chauhan, S., Varshneya, C. (2012): The profile of bioactive compounds in seabuckthorn: Berries and seed oil. *Int. J. Theor. Appl. Sci.* 4 (2), 216-220.

Cossuta, D., Simandi, B., Hohmann, J., Doleschall, F., Keve, T. (2007): Supercritical carbon dioxide extraction of sea buckthorn (*Hippophae rhamnoides* L.) pomace. *J. Sci. Food Agric.* 87 (13), 2472-2481.

Damian, C., Leahu, A., Oroian, M., Avramiuc, M., Carpiuc, N. (2013): Antioxidant activity in extracts of sea buckthorn. *Lucrări Ştiinţifice-Seria Zootehnie.* 60, 151-154.

Dulf, F. V. (2012): Fatty acids in berry lipids of six sea buckthorn (*Hippophae rhamnoides* L., subspecies carpatica) cultivars grown in Romania. *Chem. Cent. J.* 6 (106), 1-12.

Edraki, M., Akbarzadeh, A., Hosseinzadeh, M., Tanideh, N., Salehi, A., Koohi-Hosseinabadi, O. (2014): Healing effect of sea buckthorn, olive oil, and their mixture on full-thickness burn wounds. *World J. Plast. Surg.* 27 (7), 317-23.

Gupta, A., Upadhyay, N. K. (2011): Sea Buckthorn (*Hippophae rhamnoides* L.) Seed Oil: Usage in Burns, Ulcers, and Mucosal Injuries. In: Nuts and Seeds in Health and Disease Prevention, Preedy, V.R., Watson, R.R., Patel, V.B (eds.), London, Academic Press, pp. 1011-1018.

HRN EN 12822:2014: Foodstuffs – Determination of vitamin E by high performance liquid chromatography – Measurement of α-, β-, γ-, δ-tochopherol.

HRN EN ISO 12966-1:2015 Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters.

HRN EN ISO 12966-2:2011 Animal and vegetable fats and oils – gas chromatography of fatty acid methyl esters – Part.2: Preparation of methyl esters of fatty acids.

HRN EN ISO 5983-2:2010 Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content – part 2: Block digestion and steam destillation method.

HRN ISO 1871:1999 Agricultural food products – General directions for the determination of nitrogen by Kjeldahl method.

HRN ISO 5498:1999 Agricultutal food products – Determination of crude fiber – General method.

HRN ISO 5984:2004 Animal feeding stuffs – Determination of crude ash.

HRN ISO 6496:2001 Animal feeding stuffs – Determination of moisture and other volatile matter content.

HRN ISO 6541:2001 Agricultural food products – determination of crude fibre content – Modified Scharrer method.

Jokić, S., Horvat, G., Aladić, K. (2015): Design of SFE system using a holistic approach - problems and challenges. In: Supercritical Fluid Extraction: Technology, Applications and Limitations, Lindy, J. (ed.), New York, USA: Nova Science Publishers, Inc., pp. 95-122.

Jokić, S., Moslavac, T., Bošnjak, A., Aladić, K., Rajić, M., Bilić, M. (2014): Optimization of walnut oil production. *Croat. J. Food Sci. Technol.* 6 (1), 27-35.

Jokić, S., Nagy, B., Velić, D., Bucić-Kojić, A., Bilić, M. (2011): Kinetic models for supercritical CO_2 extraction of oilseeds – a review. *Croat. J. Food Sci. Technol.* 3 (2), 39-54.

Kallio, H., Yang, B., Peippo, P., Tahvonen, R., Pan, R. (2002): Triaylglycerols, glycerophospholipids, tocopherols, and tocotrienols in berries and seeds of two subspecies (ssp. Sinensis and mongolica) of Sea Buckthorn (*Hippophaë rhamnoides*). *J. Afgric. Food Chem.* 50 (10), 3004-3009.

Kaushal, M., Sharma, P. C. (2011): Nutritional and antimicrobial property of sea buckthorn (*Hippophae* sp.) seed oil. *J. Sci. Ind. Res.* 70 (12), 1033-1036.

Krejcarová, J., Straková, E., Suchý, P., Herzig, I., Karásková, K. (2015): Sea buckthorn (*Hippophae rhamnoides* L.) as a potential source of nutraceutics and its therapeutic possibilities – a review. *Acta Vet. Brno.* 84 (3), 257-268.

Nuernberg, K., Nuernberg, G., Priepke, A., Dannenberger, D. (2015): Sea buckthorn pomace supplementation in the finishing diets of pigs – are there effects on meat quality and muscle fatty acids? *Arch. Anim. Breed.* 58 (1), 107-113.

Pintea, A., Marpeau, A., Faye, M., Soccaciu, C., Gleizes, M. (2001): Polar lipid and fatty acid distribution in carotenolipoprotein complexes extracted from sea buckthorn fruits. *Phyt. Anal.* 12 (5), 293-298.

St George, S. D., Cenkowski, S. J. (2007): Influence of harvest time on the quality of oil-based compounds in sea buckthorn (*Hippophae rhamnoides* L. ssp. sinensis) seed and fruit. *J. Agric. Food Chem.* 55 (20), 8054-61.

St George, S. D., Cenkowski, S. J. (2009): The Influence of drying on levels of bioactive compounds in pulp/peel oil of sea buckthorn (*Hippophae Rhamnoides* L. ssp. sinensis) berries. *J. Food Process. Preserv.* 33 (s1), 91-109.

Trajković, J., Mirić, M., Baras, J., Šiler, S. (1983): Analysis of foodstuffs, Faculty of Technology and Metallurgy, Belgrade, pp. 13-14, 29-30, 478-479.

Wani, T. A., Wani, S. M., Mukhtar, A., Mudasir, A., Gani, A., Masoodi, F. A. (2016): Bioactive profile, health benefits and safety evaluation of sea buckthorn (*Hippophae rhamnoides* L.): A review. *Cogent Food Agric.* 2 (1), 1-9.

Yang, B., Kallio, H. (2002): Composition and physiological effects of sea buckthorn (*Hippophaë*) lipids. *Trends Food Sci. Tech.* 13 (5), 160-167.

Yang, B., Kallio, H. P. (2001): Fatty Acid Composition of Lipid sin Sea Buckthorn (*Hippophaë rhamnoides* L.) Berries of Different Origins. *J. Agric. Food Chem.* 49 (4), 1939-1947.

Zadernowski, R., Nowak-Polakowska, H., Lossow, B., Nesterowitz, J. (1997): Sea-Buckthorn lipids. *J. Food Lipids.* 4 (3), 165-172.