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Fatty acid composition of sea buckthorn *(Hippophae rhamnoides* L.) pulp oil of Lithuanian origin stored at different temperatures

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The purpose of the present research was to analyze samples of sea buckthorn pulp (*Hippophae rhamnoides* L.) oil produced in Lithuania, in order to determine the quantitative fatty acid (FA) composition. Samples were stored for 360 days at 0 °C and –20 °C and FA methyl esters were analyzed by gas chromatography. Analysis of sea buckthorn berry oil samples and the comparison of its results with data from other sources showed differences in the quantity of separate FA. The storage temperature (0 °C and –20 °C) did not seem to significantly affect the total percentage of saturated, monounsaturated and polyunsaturated FA. We, however, determined differences of individual FA: the oil stored at –20 °C contained more oleic acid ($p < 0.02$), the content of linoleic acid was by 10% lower ($p < 0.0001$) and of α-linolenic acid by 25.4% higher ($p < 0.01$) than in the oil stored at 0 °C.

Key words: sea buckthorn pulp oil, fatty acid

INTRODUCTION

Besides FA, sea buckthorn oil contains also organic acids, flavonoids, vitamins P, E, carotenoids, microelements (kalium, calcium, etc.), and sterols [1–4].

The qualitative and quantitative composition of sea buckthorn berries depends on the geographic environment of their habitat, development throughout a specific period, genetic characteristics of the plant, soil structure, climatic conditions and some other factors difficult to predict and rate [5]. The characteristics of sea buckthorn oil depend both on the berry components (seeds, peel, pulp) and technology methods (plain and extractive).

Sea buckthorn oil is effective for treating skin and mucous injuries [1, 2]. A special study conducted earlier confirmed the curative effect of this oil on inflammatory atopic dermatitis (AD). AD patients have a defective Δ6-desaturation enzyme which transforms linoleic (C18:2) and linolenic (C18:3) acids into γ-linolenic (C18:3) and octadecanotetraenoic (C18:4) acids. This results in the reduction of long chain desaturated products which are needed for the synthesis of eicosanoids in plasma phospholipids [1].

It has been found that oil from sea buckthorn seeds and pulp helps to stabilize cell membrane structure [2]. Polyunsaturated fatty acids (PUFA), in particular monounsaturated fatty acids (MUFA), a fair amount of which can be found in sea buckthorn oil, is a component of membrane sphingolipids and glycerophospholipids, which perform the epidermal barrier system function [1]. Being essential components of the cell membrane, MUFA and PUFA are important for maintaining the required fluidity of the membrane [6]. They are also important for the functioning of receptors, enzymes, ion channels, and other substance transportation systems [1]. Skin glycerophospholipids form a source of linoleic acid needed for the synthesis of acylceramides and 13-hydroxyoctadecadienoic acid (13-HODE).

Acylceramides are the key components determining the epidermal barrier function. 13-HODE helps to lower the epidermal hyperproliferation and very likely reduce inflammation [1].

Furthermore, in patients taking pulp oil, the concentration of high density cholesterol (HDL-Ch) increased significantly (from 1.38 to1.53 mmol/l). This is an important indicator reducing the risks of heart and vascular diseases and preventing the development of atherosclerosis [1, 2].

Experiment-based analysis has confirmed that sea buckthorn oil has antioxidant characteristics that protect against the negative action of free radicals [2]. These

antioxidant characteristics come from vitamin E, ascorbic acid, carotenoids (β-carotene), and flavonoids [2].

The data that can be found in scientific research papers point to the antimicrobial, anti-inflammatory, regenerating and biostimulating effects of sea buckthorn oil. Some of these qualities are linked with fatty acids. We therefore analyzed the composition of FA in sea buckthorn pulp oil stored at different temperature, and compared it to data from other sources.

MATERIALS AND METHODS

To produce high quality oil, only ripe berries were used. First they were cleared and washed. The pulp was separated mechanically from the seed and seed coat. Physical methods are used to destroy the emulsion that is naturally found in the pulp of the berries, and later, favorable conditions are created for water and oil phases to be separated. The oil phases are collected and analyzed.

In our investigation, samples of oil phase before determining FA content in them were stored in a sealed and light-protected place at different temperatures: six samples were stored at 0 ± 0.5 °C and the other six at –20 ± 0.5 °C with a 65% relative humidity in the surrounding environment. The samples were stored for 360 days before the analysis. Under such conditions, the results of the analysis of FA quantitative and qualitative indicators would allow determining the proper storage conditions of sea buckthorn pulp oil determined.

We used 20 ml of a chloroform: methanol mixture (2:1, v/v) (Folch method) [7] for lipid extraction. Fatty acids were transesterified with 10 ml of methanol and 1 ml of HCl [8] for 2 hours in a boiling water bath. FA were separated using hexane (2 ml/3 times). By using nitrogen, the content of the tube was concentrated up to the required volume, which was approximately 150–300 μl. FA methyl ethers were chromatographed on a 50 m × 0.25 mm glass capillary column coated with a 0.2 mm film of CP-SIL 88 *(Chrompack)*. Analysis was performed on a GC–2010 gas chromatograph (Shimadzu) containing a flame ionization detector. The injection type was no flow separation. The injector and detector temperatures were 250 °C and 260 °C, respectively. Nitrogen was used as a carrier gas. The total flow was 13.7 ml/min. The oven temperature was programmed: initially at 140 °C (kept for 3 min); increased at a rate of 18 °C/min up to 170 °C and isothermally for 8 min; raised 2 °C/min up to 205 °C. The separated FA were identified using a standard mixture of known fatty acids (Sigma Chemical Co, USA).

The quantities of various FA are presented in percentage (%) of their total quantity. Estimations were done based on the SPSS program.

RESULTS AND DISCUSSION

The data we received (Table 1) were compared with data on the oil of sea buckthorn pulp and seed compiled by other researchers. We can see that the researchers from Turku University failed to find myristic and myristoleic acids [6]. This had effect on the percentage of other FA. By comparing the FA spectrum results in the Lithuanian sea buckthorn pulp oil with that data, we found 12% more saturated FA in the oil we analyzed. This resulted from the quantity of myristic and in particular palmitic (10.2%) acids identified. We however found a lower quantity (6%) of monounsaturated. The oil samples we analyzed contained 17.5% less oleic acid, one of the dominating MUFA. The gross quantity of PUFA also was 13.5% lower in our samples. Linoleic acids were 24.4% lower, whereas α-linolenic acid was 6.2% above the quantity identified by our Finnish colleagues.

Table 1. **Comparison of FA composition (%) of Lithuanian sea buckthorn pulp oil with pulp oil and seed oil of another origin**

Fatty acids	Quantity, %			
	Pulp oil*	Pulp oil [6]	Pulp oil [2]	Seed oil [6]
Myristic C14:0	0.4	**	0.6	**
Palmitic C16:0	37.8	33.4	37.4	11.3
Stearic C18:0	0.9	1.0	2.1	2.6
Total	39.2	34.4	40.1	13.9
Myristoleic C14:1(n-5)	0.2	**	**	**
Palmitoleic C16:1(n-7)	24.9	24.9	26.1	4.4
Oleic C18:1(n-9)	22.3	26.2	22.5	18.9
Vakccenic C18:1(n-7)	7.5	7.3	**	3.2
Total	55.1	58.4	48.6	26.5
α-Linoleic C18:2(n-6)	4.1	5.1	5.5	34.1
Linolenic C18:3(n-3)	1.7	1.6	6.8	24.9
Total	5.9	6.7	12.3	59.0

*Average quantities of FA in sea buckthorn pulp oil samples stored at 0 °C and –20 °C.
** No data.

Table 2. **Effect of Lithuanian sea buckthorn pulp oil storage temperature on FA composition (%). All values are presented as means ± SD**

Fatty acids	Quantity, %	
	Pulp oil stored at 0 °C	Pulp oil stored at –20 °C
Myristic C14:0	0.425 ± 0.4	0.379 ± 0.02#
Palmitic C16:0	38.132 ± 0.79	37.533 ± 1.22
Stearic C18:0	0.992 ± 0.05	0.951 ± 0.10
Total	39.549 ± 0.79	38.863 ± 1.33
Myristoleic C14:1(n-5)	0.215 ± 0.02	0.151 ± 0.07
Palmitoleic C16:1(n-7)	24.812 ± 0.93	24.995 ± 1.24
Oleic C18:1(n-9)	22.060 ± 0.32	22.620 ± 0.56#
Vakccenic C18:1(n-7)	7.563 ± 0.09	7.472 ± 0.26
Total	54.709 ± 0.56	55.457 ± 1.56
Linoleic C18:2(n-6)	4.377 ± 0.19	3.932 ± 0.16#
α-Linolenic C18:3(n-3)	1.458 ± 0.11	1.955 ± 0.39#
Total	5.836 ± 0.31	5.888 ± 0.52

#$P < 0.05$.

To compare the data of the analysis of FA composition in sea buckthorn pulp oil with the data from the source [2], we can see that the oil samples analyzed by us had 2.3% less saturated FA, of which palmitic acid was 1% higher and myristic acid and stearic acid were 33%, 42.8% lower. We however discovered that the level of MUFA was 12% higher, although palmitoleic acid and oleic acid were respectively 4.6% and 0.8% lower. The failure by source [2] to unveil quantities of myristoleic and vakccenic effected the comparison of results. The quantity of PUFA discovered by us was as much as 2.1 times lower. We found 25.4% less of linoleic acid and 4 times less α-linolenic acid.

According to scientific data [6], sea buckthorn seed oil is much more beneficial than pulp oil, because it contains significant amounts of essential PUFA. Data about the level of FA in sea buckthorn seed oil is presented in Table 1.

The effect of storage temperature (from 0 °C to –20 °C) on the fatty composition of the sea buckthorn pulp oil is shown in table 2. It is obvious from the table that storage temperature had no effect on the total quantity of saturated FA, MUFA or PUFA in the sea buckthorn pulp oil. We however determined the most notable differences of individual FA: the oil stored at –20 °C contained more oleic acid ($p < 0.02$), linoleic acid was 10% lower ($p < 0.0001$), and α-linolenic acid was 25.4% higher ($p < 0.01$) than in the oil stored at 0 °C.

We discovered that the main portion of FA in sea buckthorn pulp oil of Lithuanian origin is composed of palmitic, palmitoleic, and oleic acid. This finding matches the data of the other researches. The storage conditions at 0 °C and –20 °C, as expected, have some effect on the composition of FA in sea buckthorn pulp oil. This is in particular true of individual PUFA, which are very sensitive to oxidation. Our data suggest that in order to protect highly unsaturated FA from oxidation sea buckthorn pulp oil should be rather stored at temperature of –20 °C.

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LIETUVOJE PAGAMINTO IR SKIRTINGOJE TEMPERATŪROJE LAIKYTO ŠALTALANKIŲ UOGŲ *(HIPPOPHAE RHAMNOIDES* L.) MINKŠTIMO ALIEJAUS RIEBALŲ RŪGŠČIŲ SUDĖTIES TYRIMAS

Santrauka

Tyrėme Lietuvoje pagaminto šaltalankių uogų (*Hippophae rhamnoides* L.) minkštimo aliejaus bandinių, laikytų 0 °C ir –20 °C temperatūroje, RR kiekybinę sudėtį,. Šis aliejus buvo gautas atskyrus uogų minkštime esančią aliejaus fazę. RR metilo esteriai buvo nustatyti dujinės chromatografijos metodu. Nustatėme, kad šaltalankių minkštimo aliejaus didžiąją RR dalį sudaro palmitino, palmitoleino ir oleino rūgštys. Šaltalankių uogų aliejaus bandinių RR sudėties palyginimas su kitų autorių duomenimis atskleidė nedidelius atskirų RR kiekių skirtumus. Šaltalankio aliejaus laikymo temperatūra (0 °C ir –20 °C) sočiųjų, mononesočiųjų ir polinesočiųjų RR bendrai procentinei sudėčiai didelės įtakos neturėjo. Nustatėme, kad žemesnėje temperatūroje laikytas aliejus turėjo daugiau oleino, α-linoleno rūgščių ir mažiau linolo rūgšties.