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IDENTIFICATION OF THE ORIGIN OF SEA BUCKTHORN OIL OF THE ALTAI KRAI BY DIFFERENTIAL SCANNING CALORIMETRY

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Abstract: The composition of lipids derived by extraction with Freon 22 and enzymatic hydrolysis from berries, berry shells, and seeds of the Chuy sea buckthorn cultivar has been studied. The fatty acid composition and acid and peroxide values of the samples have been analyzed; the differential scanning calorimetry (DSC) melting curves have been examined. The DSC method has been found to be appropriate for determining the origin of raw materials and the production method for sea buckthorn oil.

Keywords: sea buckthorn oil, the Altai Territory, production method, differential scanning calorimetry

1. INTRODUCTION

Sea buckthorn berries are rich in vitamins, carotenoids, flavonoids, proteins, antioxidants, amino acids, fatty acids, and phytosterols [1]. The most valuable component of sea buckthorn berries is their oil. The oil from the sea buckthorn pulp and seeds is characterized by a high content of lipids, including tocopherols, tocotrienols, carotenoids, and ω-3 and ω-6 polyunsaturated fatty acids [2, 3]. The composition of the sea buckthorn seeds and pulp varies in accordance with the subspecies, cultivar, soil and climate conditions, origin, cultivation activities, harvesting time, and extraction method [3]. The aim of this study is to explore the possibility of identifying samples of sea buckthorn oil derived from different parts of sea buckthorn berries by differential scanning calorimetry (DSC).

2. MATERIALS AND METHODS

2.1 *Berries*

Berries of the Chuy sea buckthorn cultivar harvested on commercial plantations of the Lisavenko Research Institute of Horticulture for Siberia of the Russian Academy of Agricultural Sciences in 2012 were used.

Samples of sea buckthorn oil extracted with difluorochloromethane (Freon 22) from the crushed pulp (prepared by juicing the berries), the kernel (seed), and the berry shells and oil samples prepared by the enzymatic method were studied.

2.2. *Sample Preparation*

The extraction of sea buckthorn oil was conducted in an extractor for 8 h with the subsequent removal of Freon 22.

The Protosubtilin and CelloLux-A enzymes in a ratio of 1 : 1 were used to derive oil by enzymatic hydrolysis.

2.3. *Study of Melting Process*

The melting of the samples was studied by DSC using a DSC-60 instrument (Shimadzu, Japan). The weighed portion was 10.0 ± 0.5 mg. The measuring cell was cooled with liquid nitrogen to a temperature of −100°C. The experiments were conducted in a temperature range of −100°C to 50°C at a heating rate of 10°C/min. The experiments were conducted in a nitrogen environment at a gas flow rate of 40 cm^3/min. The α-quartz was used to bring the system into the state of equilibrium. The instrument was calibrated against indium (T_{melt} = 156.6°C, H_f = 28.71 J/g). The calculated data were obtained using the DSC-60 software.

2.4. *Determination of Fatty Acid Composition*

The fatty acid composition of the oil samples was determined by gas chromatography (GC). The oil samples were converted to their methyl esters and analyzed on a Kristallyuks 4000 gas chromatograph using a flame ionization detector, a 50 m x 0.25 mm FFAP capillary column, and helium as a carrier gas (Hewlett-Packard, Palo Alto, CA). The thermostat temperature was programmed as follows: from 60°C (an isothermal mode for 1 min) to 190°C at a rate of 20°C/min and an isothermal period of 30 min at 190°C. The temperature of the injector and the detector was 250°C.

2.5. *Determination of Peroxide and Acid Values*

The peroxide and acid values of the samples were determined by standard methods [5, 6].

2.6. *Statistical Analysis*

All the studies were conducted at least twice. The measurement results were processed by the analysis of variance.

3. RESULTS

3.1. *Fatty Acid Composition*

The fatty acid composition of the prepared oil samples is shown in Table 1.

Table 1. Fatty acid composition of the oil samples (a measurement error of 2–6%)

Species, cultivar	Fatty acids								*Reference*
	14:0	**16:0**	**16:1**	**18:0**	**18:1 oleic**	**18:1 vaccenic**	**18:2**	**18:3**	
	from berry pulp and shells								
Hippophae rhamnoides	0.5	28.4	50.3	0.6	11.3	not detected	1.3	1.3	7
Hippophae rhamnoides	0.6	35.8	45.6	0.5	0.8	not detected	0.8	0.5	7
Hippophae rhamnoides	0.4	33.8	46.4	1.0	13.4	not detected	5.4	1.3	7
Hippophae salicifolia	0.3	29	32.9	2.9	17.6	not detected	16.1	0.6	7
Hippophae tibetana	1.1	25.7	32.1	0.5	26.0	not detected	9.3	5.2	7
Chuy	0.5	35.1	35.3	1.2	4.3	5.8	11.3	0.9	***
Quebec	-	35.3	40.1	0.8	3.2	6.5	10.6	0.9	4
carpatica	0.46	39.1	26.7	0.8	20.8	6.4	4.6	0.90	8
from seeds									
Chuy	0.3	11.0	5.5	2.2	15.1	not detected	35.1	25.6	***
quebec	- н.о.	8.0	2.8	3.1	13.1	2.2	32.4	37.2	4
carpatica	0.24	12.4	0.36	2.9	16.7	1.5	33.7	31.8	8
from berry shells									
Chuy	0.87	36.6	34.5	1.27	5.8	5.1	12.1	0.9	***
prepared by enzymatic hydrolysis									
Chuy	0.52	34.3	33.7	1.8	5.1	5.7	14.2	1.4	***
Jutland, Germany	0.3	33.0	34.2	0.3	28.4	not detected	3.3	1.0	9
Canada, Quebec	0.4	36.1	39.4	0.8	2.9	6.2	10.8	0.9	10
from whole berries									
carpatica	0.59	36.2	24.6	0.9	22.3	6.2	6.2	2.7	8

[a]Trace, ≤0.1%.
*Total amount of oleic and vaccenic acids.
**Arachidonic and behenic acids are also detected.
***The data obtained by the authors.

These data suggest the following.

–The composition of the oil samples prepared from the pulp, shells, and seeds of Chuy sea buckthorn berries is most similar to the composition of the samples of oil produced in the province of Quebec (Canada).

–The composition of the oil derived from berry shells is close to the composition of the pulp oil.

–The composition of the oil sample prepared by enzymatic hydrolysis is similar to the composition of the analogous Canadian sample and significantly differs from the German sample.

3.2. *Differential Scanning Calorimetry*

The melting curve of the seed oil is shown in Fig. 1.

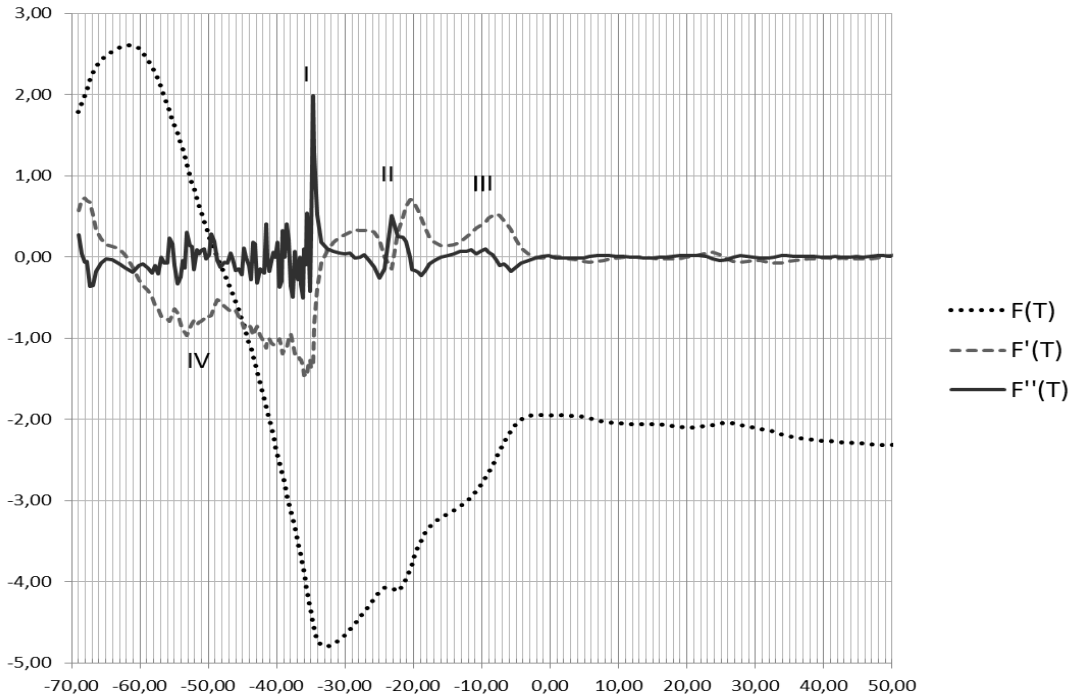

Fig. 1. Melting curve of the Chuy sea buckthorn seed oil.

These data suggest that the melting curve of the seed oil is a superposition of four overlapping peaks.

The characteristics of their total peak are listed below.

Peak position, °C	–33.5 ± 0.4
Endoeffect onset temperature, °C	–39.9 ± 0.4
Finishing melting temperature, °C	–12.0 ± 0.3
Melting heat, J/g	57.0 ± 1.5

The first-derivative analysis makes it possible to determine the peak positions of four endoeffects of melting of triglycerides at –54°C, –32°C, –19°C, and –8°C.

The melting curve of the oil from the berry shells is shown in Fig. 2.

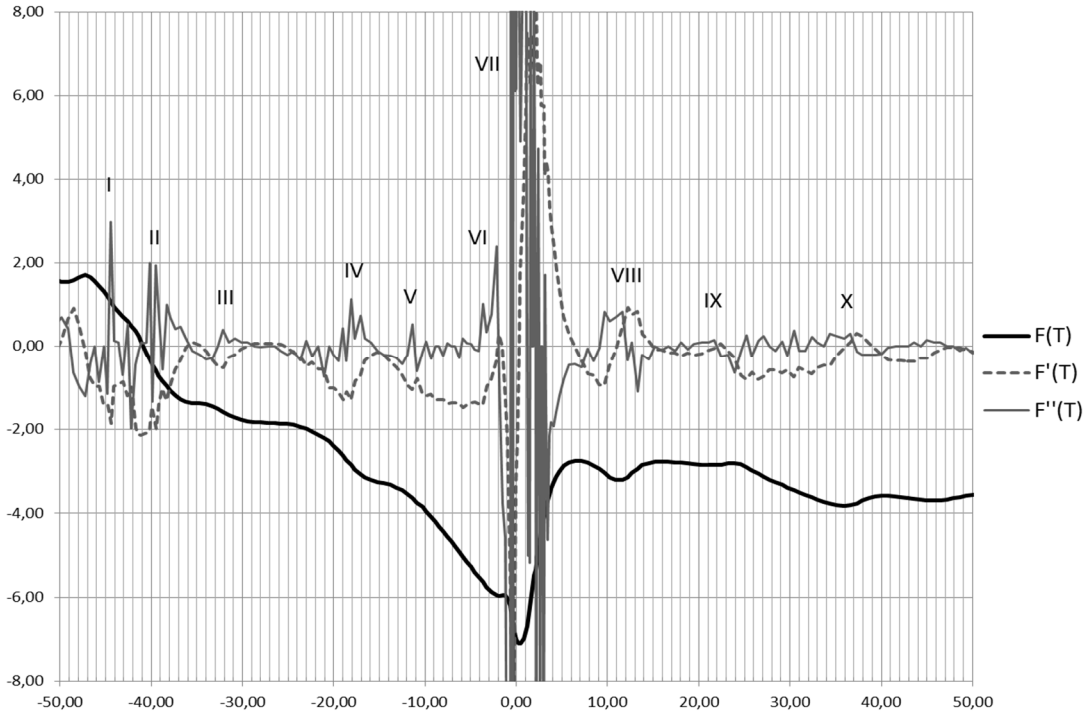

Fig. 2. Melting curve of the oil derived from Chuy sea buckthorn berry shells.

Unlike the previous sample, the melting curve of the oil derived from sea buckthorn berry shells is a superposition of at least ten peaks.

The data on five of them that could be identified with the data processing program are shown in Table 2.

However, the first-derivative analysis of the melting curve reveals two additional peaks in a range of –30 to –45°C, which should be apparently attributed to the melting of triglycerides of unsaturated acids. The peak position exhibits a divergence of 2–4 deg, which requires a unified approach to the method of analysis of these complex melting curves.

The melting curve of the oil sample prepared by enzymatic hydrolysis is shown in Fig. 3.

The melting curve of this sample is a superposition of four overlapping peaks. Their parameters are listed in Table 3.

The melting curve of the sea buckthorn oil extracted with Freon is also a superposition of four overlapping peaks (Fig. 4).

Table 2. Parameters of the melting curve of the oil derived from sea buckthorn berry shells

Peak	Parameter	Values
I	Peak position, °C	–24.9 ± 0.4
	Endoeffect onset temperature, °C	–37.9 ± 0.4
	Finishing melting temperature, °C	–21.3 ± 0.4
	Melting heat, J/g	2.8 ± 0.2
II	Peak position, °C	–22.2 ± 0.4
	Endoeffect onset temperature, °C	–21.9 ± 0.4
	Finishing melting temperature, °C	–15.1 ± 0.4
	Melting heat, J/g	0.8 ± 0.1
III	Peak position, °C	0.7± 0.4
	Endoeffect onset temperature, °C	–2.5 ± 0.4
	Finishing melting temperature, °C	4.0 ± 0.4
	Melting heat, J/g	21.9 ± 1.4
IV	Peak position, °C	11.5 ± 0.4
	Endoeffect onset temperature, °C	8.3 ± 0.4
	Finishing melting temperature, °C	14.0± 0.4
	Melting heat, J/g	0.9 ± 0.1
V	Peak position, °C	39.9 ± 0.4
	Endoeffect onset temperature, °C	19.6 ± 0.4
	Finishing melting temperature, °C	42.5 ± 0.4
	Melting heat, J/g	2.6 ± 0.2

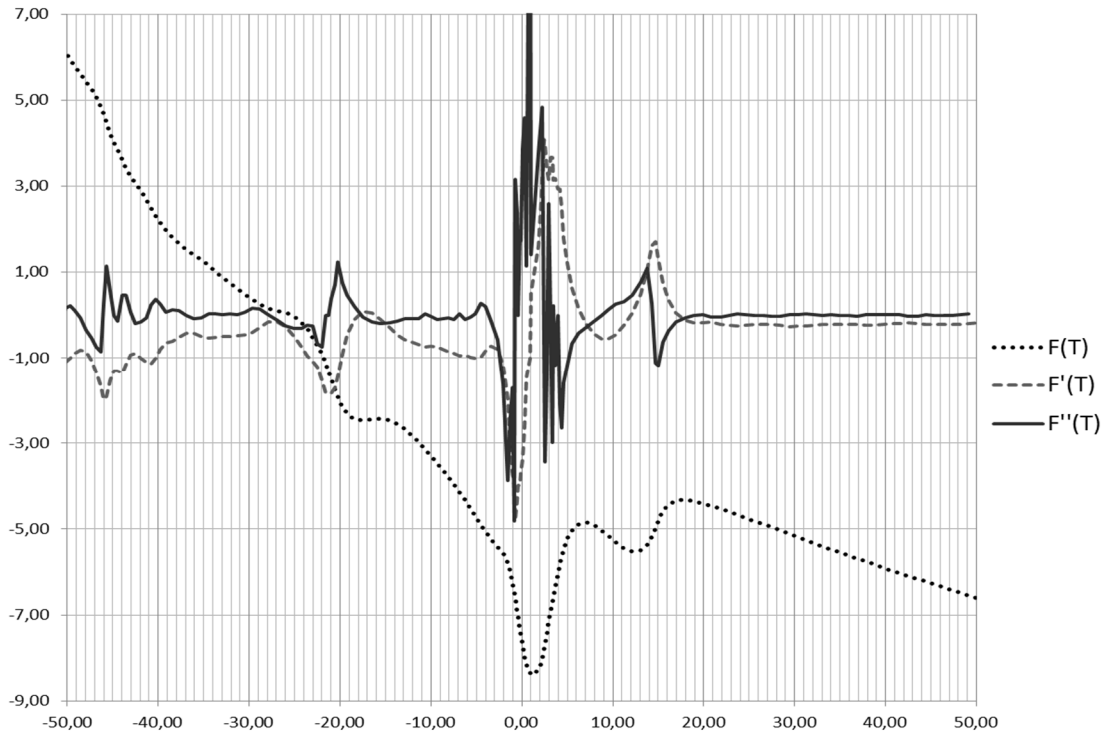

Fig. 3. Melting curve of the oil derived by enzymatic hydrolysis.

Table 3. Parameters of the melting curve of the sea buckthorn oil derived by enzymatic hydrolysis

Peak	Parameter	Value
I	Peak position, °C	–19.8 ± 0.4
	Endoeffect onset temperature, °C	–23.3 ± 0.4
	Finishing melting temperature, °C	–15.0 ± 0.4
	Melting heat, J/g	2.6 ± 0.4
II+III	Peak position, °C	1.2 ± 0.4
	Endoeffect onset temperature, °C	–1.5 ± 0.4
	Finishing melting temperature, °C	5.0 ± 0.4
	Melting heat, J/g	11.7 ± 0.8
IV	Peak position, °C	12.8 ± 0.4
	Endoeffect onset temperature, °C	7.5 ± 0.4
	Finishing melting temperature, °C	16.0 ± 0.4
	Melting heat, J/g	2.9 ± 0.2

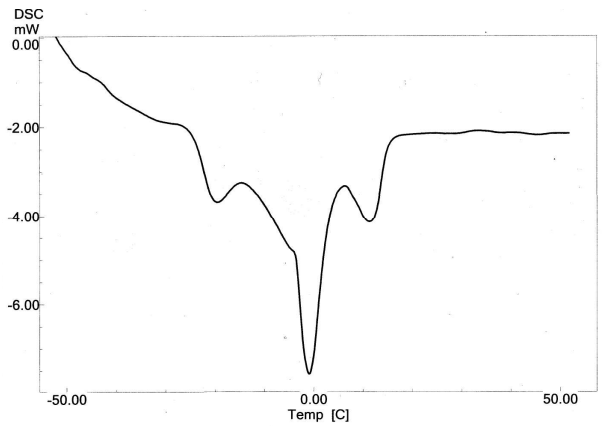

Fig. 4. Melting curve of the oil extracted with Freon.

These data suggest that the melting curve is a superposition of four overlapping peaks. Their parameters are listed in Table 4.

Table 4. Parameters of the melting curve of the sea buckthorn pulp oil extracted with Freon

Peak	Parameter	Value
I	Peak position, °C	–19.6± 0.4
	Endoeffect onset temperature, °C	–24.0± 0.4
	Finishing melting temperature, °C	–15.3± 0.4
	Melting heat, J/g	3.6± 0.4
II+III	Peak position, °C	–1.0± 0.4
	Endoeffect onset temperature, °C	–4.4± 0.4
	Finishing melting temperature, °C	3.7± 0.4
	Melting heat, J/g	16.7 ± 0.8
IV	Peak position, °C	11.3 ± 0.4
	Endoeffect onset temperature, °C	6.9 ± 0.4
	Finishing melting temperature, °C	15.1 ± 0.4
	Melting heat, J/g	3.5 ± 0.4

It should also be noted that the total heat of melting of the oil samples is 17 to 57 J/g, which is comparable to the data for vegetable oils [10].

3.3. *Acid and Peroxide Values*

The acid and peroxide values of the oil samples are listed in Table 5.

Table 5. Acid and peroxide values of the sea buckthorn oil samples

Oil sample	Acid value, mg KOH/1 g of oil	Peroxide value, ? O_2 mmol/kg
extracted with Freon 22	12.05	0.7
from berry shells	6.85	4.8
from seeds	7.2	4.4
enzymatic hydrolysis	2.6	0.5
from dried berries [3]	-	1.8–4.0; 5.4 ± 0.3; 3.0 ± 1.9

According to the set of parameters, the oil sample prepared by enzymatic hydrolysis is the most promising for use in cosmetic formulations.

4. DISCUSSION

The above data suggests that the oil derived from Chuy sea buckthorn berries has a similar qualitative composition and peroxide value as the oil from other subspecies of sea buckthorn.

Differences in the fatty acid composition lead to differences in the melting curves: four to ten peaks were recorded for the studied samples. An assumption of the nature of these endothermic effects was made only by the Canadian authors in [4]. Table 6 shows a comparison of the DSC data on the melting peak temperatures of our and Canadian samples.

The data suggest that the samples mostly exhibit three minima corresponding to the melting of three triglycerides or their complexes. With allowance for the melting point of even-numbered fatty acids and the triglycerides formed by them, these acids can be arranged in the following series with respect to increasing melting point: linolenic > linoleic > palmitoleic > oleic > myristic > palmitic > stearic.

Taking into account that the mass fraction of a number of acids is negligible and they cannot be shown as a separate peak in the melting curve and given the fact that they are isostructural with homologs, the following three groups of triglycerides can be distinguished: a high-temperature group composed of C18:0, C16:0, and C14:0 acids; a medium-temperature group consisting of C16:1 and C18:1; and a low-temperature comprising C18:2 and C18:3. However, if we take into account that the melting point of pure triglycerides of the saturated acid series is significantly higher (e.g., stearic acid triglyceride is melted at 75°C), then we can conclude that the DSC curves describe the melting process of multicomponent eutectic mixtures, as described for milk fat [12, 13], rather than individual triglycerides.

Table 6. Parameters of the melting curves of the oil samples

Sample	Endoeffect peak temperature, °C for the peak				
	I	II	III	IV	V
seeds	–33.5				
berry shells	–24.9	–22.2	0.7	11.5	39.9
enzymatic	–25.8	1.2		12.8	
extracted with Freon 22		–19.6	–1.0	11.3	
[3]	–22.5		–4.0	10.0	
[11]	–24.4		–4.1	10.7	

Note also that none of the samples contain water; therefore, the extensive endothermic effect in the region of 0°C is not attributed to the melting of ice.

Taking into account that the fatty acid composition of sea buckthorn oil depends not only on the cultivar, geographical location, climatic conditions, and cultivation activities, the derived melting curves are individual and can be used to determine the composition of the feedstock for the production of sea buckthorn oil in the Altai Territory and in the used extraction technology.

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