

UDC 582.284+58.088+58.04+577.13+577.115+577.115

EXTRACTIVE SUBSTANCES OF SAPROTROPHIC MUSHROOMS: *FLAMMULINA VELUTIPES*, *HYPHOLOMA CAPNOIDES*, *ARMILLARIA* *BOREALIS*, *ARMILLARIA CEPISTIPES*. HEAVY METAL CONTENT

© V.V. Bakanov¹, D.N. Vedernikov^{1*}, I.V. Kruchina-Bogdanov², L.S. Eltsova³

¹ St. Petersburg State Forest Technical University named after S.M. Kirov, Institutskiy per., 5, St-Petersburg, 194021 (Russia), e-mail: dimitriy-4@yandex.ru

² AMT, Ltd., ul. Novorossiyskaya, 50, St-Petersburg, 194021 (Russia)

³ Cherepovets State University, pr. Lunacharskogo, 5, Cherepovets, 162600 (Russia)

The article is concerned to the study and comparison of the extractive substances chemical composition of typical saprotrophic mushrooms that grow in the forests of St. Petersburg and the Leningrad region: *Flammulina velutipes*, *Hypholoma capnoides*, *Armillaria borealis*, *Armillaria cepistipes*. The article analyzed and compared the chemical composition of low-molecular compounds of caps and legs separately. The composition of heavy metals was compared: cadmium, mercury, iron. The content of heavy metals in the studied fungi was compared with those studied earlier (*Lentinula edodes* and *Pholiota squarrosa*). Cadmium and mercury are found mainly in the caps of autumn mushrooms.

The composition of fatty acids, sterols, carbohydrates and sugar alcohols in various parts of mushrooms (stems and caps) was determined and compared. It was revealed that glycerin is presented in all types of mushrooms and is contained in large quantities in the stems and caps of *F. velutipes*. The qualitative composition of sugar alcohols is approximately the same in all mushrooms, but the quantitative composition has specific differences. Sugar alcohol threitol is contained in *Armillaria*.

Keywords: saprotrophic mushrooms, *Flammulina velutipes*, *Hypholoma capnoides*, *Armillaria borealis*, *Armillaria cepistipes*, extractives substance, heavy metals.

The study was funded by a grant Russian Science Foundation No 22-26-00345.

Introduction

The studied mushrooms of the *Armillaria* genus belong to the group of edible mushrooms. Fungi of the *Hypholoma* genus are inedible or slightly poisonous [1]. Mushrooms of the *Flammulina* genus are edible, but it is recommended to boil them before use [2]. *F. velutipes* belongs to the class *Agaricomycetes*, the family *Physalacrylic*, and the genus *Flammulina* [3]. Some compounds, including carbohydrates, proteins, lipids, glycoproteins, phenols and sesquiterpenes have been extracted from various parts of this mushroom [4].

The genus *Hypholoma* P. Kumm. belongs to the family *Strophariaceae* Singer & A. H. Sm. (*Agaricales*, *Basidiomycota*), the subfamily *Stropharioideae* (Singer) Singer.

The mushrooms of the genus *Armillaria*, namely *A. borealis* and *A. cepistipes* have been researched also. *A. borealis* belongs to the class – *Agaricomycetes*, the family – *Physalacrylic*, the genus – *Armillaria* [5]. The *A. cepistipes* belongs to the class – *Agaricomycetes*, the family – *Physalacrylic*, the genus – *Armillaria* [3]. The mushrooms of these two species have been poorly studied and official information, their chemical composition and extractive substances hasn't been found.

At the moment, only *F. velutipes* is industrially grown, because it is in great demand, both for consumption, because of its taste, and for use in scientific research [6]. It is known that *F. velutipes* have anti-inflammatory, antitumor and antioxidant activities [6–8].

Bakanov Vyacheslav Vadimovich – graduate student,
e-mail: bakanovvyachuslav@mail.ru

Vedernikov Dmitriy Nikolaevich – Professor, Doctor of
Chemistry, e-mail: dimitriy-4@yandex.ru

Kruchina-Bogdanov Igor Vadimovich – General Director,
e-mail: igogo011@gmail.com

Eltsova Liubov Sergeevna – researcher of the Ecological and
Analytical Laboratory, e-mail: khabarovals@yandex.ru

In this regard, the aim of our work was to obtain preliminary data on the extractive substances of the saprotrophic fungus, as well as to clarify the differences in the composition of the parts of the fruiting bodies of these fungi, since the lower and upper parts are often used in different ways. Since it is known that fungi often accumulate heavy metals, the contents of mercury, cadmium and iron in different parts of the fungi were determined.

Fungi growing in our climate (temperate) are very attractive regarding their research. Chemical composition and biological activity are of great interest.

Experimental

Mushrooms grown in natural conditions were used for the research: *F. velutipes*, 2 types of *Armillaria*, and *H. capnoides*. *F. velutipes* were collected 25.11.2020 in the village Sablino along the river Tosnenki on the rotten, vertically standing osiers. *A. borealis*, *A. cepistipes* and *H. capnoides* were collected 25.09.2020 in the forest on the border of the Novgorod and Leningrad regions. Belonging to a certain type of fungi was determined by Ph.D. Volobuev S.V. Laboratory of Systematics and Geography of fungi of the V.L. Komarov Botanical Institute.

Before the research the experimental samples were washed from the substrate residues, and then dried for 12 hours at a temperature 40–45 °C with the use of an electric dryer to the humidity of 10–15%. The initial humidity was 80–90%.

Mushrooms were extracted with isopropyl alcohol in a Soxhlet extractor for 12 hours. The alcohol extract was evaporated on a rotary evaporator under reduced pressure. The evaporation residue was extracted several times with methyl tert-butyl ether (MTBE) at the boil until the fatty acids were removed. The control was carried out by TLC. Silica gel Aldrich–Sigma TLC plate was used. Eluent – hexane – MTBE ($V_1 : V_2 = 20 : 5$). Spots were visualized with a solution of 10% sulfuric acid in ethanol followed by heating.

The methyl tert-butyl (MTBE) extract was separated into acids and neutrals by washing with an aqueous solution of 1% NaOH. The substances were analyzed by gas chromatography-mass spectrometry (GC-MS). Hot water extraction was carried out at a temperature of 90 °C for 3 hours and the ratio of mushrooms and water 1 to 30.

Qualitative and quantitative analyzes of «volatile» components was carried out by chromatography-mass spectrometry according to the method given in [9]. Acids, sugar alcohol, carbohydrates analyzes were performed after preliminary derivatization [9]. The sample was prepared for the determination of triglycerides according to the method [10]. Neutral glycerides were separated by acetone at -20°C during 16 hours to remove phospholipids. A solvent was removed from an aliquot of 100 µl of acetone under vacuum and the absolute content of triglyceride content was determined according to [11]. Test conditions: Agilent DB-HT SimDis column (5 m × 0.54 mm × 0.15 µm); carrier gas N₂, 20 cm³/s. The thermostat temperature was programmed to increase from 60 to 400 °C at a rate of 5 °C per min and 10 min isotherm at 400 °C. Input temperature samples 350 °C, flow divider 1 : 30, sample volume 5 µl; flame ionization detector, temperature 410 °C, hydrogen supply rate – 40 ml/min., nitrogen – 25 ml/min., oxygen – 350 ml/min.

The metal content was determined in the mushrooms after extraction with isopropanol. Methods for the analysis of cadmium, iron and mercury are described in [9].

Results and discussion

Extractive substances. Substances soluble in MTBE (methyl tert-butyl ether) are more in the caps than in the stems in both types of *Armillaria*. Substances extracted by isopropanol alcohol predominate in the stems of *A. borealis* and *A. cepistipes*. There are more water-soluble substances in the stems of *A. borealis* than in the caps, and vice versa in *A. cepistipes*. Substances released by ether are more in the caps of *F. velutipes* than in the stems (6.5 and 4.2%, respectively). The substances released by ether from the caps of *F. velutipes* consist mainly of neutral substances (94%). The yield of isopropanol extract from the caps of *F. velutipes* is slightly higher than in the stems. (Table 1).

Fatty acids, neutral substances and compounds of isopropanol extract were identified in the researched mushrooms. In all mushrooms there is a classic series of fatty acids that are inherent for mushrooms, namely palmitic acid, linoleic acid, oleic acid and stearic acid. These acids are present in all mushrooms, but in different quantitative proportions. The qualitative composition of neutral substances is not uniform and there are some similarities and differences (tables 2–4).

Table 1. Experimental values of mushroom extraction

Groups extracted with	<i>F. velutipes</i>		<i>H. capnoides</i>		<i>A. borealis</i>		<i>A. cepistipes</i>	
	Stem	Cap	Stem	Cap	Stem	Cap	Stem	Cap
	Extract yield, % of dry matter							
Petroleum ether	1.0	1.7	0.4	0.8	0.3	1.1	0.2	1.3
MTBE	4.2	6.5	3.1	3.1	1.6	4.2	0.7	6.3
Extract containing neutral substances	94	49	35	36	63	68	24	68
acids	6	51	65	64	37	32	76	32
IPS	26.5	33.5	7.3	9.9	20.8	16.9	21.2	10.4
Hot water	26.7	33.8	30.4	27.1	36.1	19.8	28.8	35.1

Table 2. Fatty acids from the researched mushrooms

Name	Retention time*/I	<i>F. velutipes</i>		<i>H. capnoides</i>		<i>A. borealis</i>		<i>A. cepistipes</i>	
		Cap	Stem	Cap	Stem	Cap	Stem	Cap	Stem
		Fractional content, %				Fractional content, %			
9-Oxo-nonanoic acid	7.8/1420 (1436 for HP-5MS [12])	–	–	–	–	0.3	–	0.3	9.0
Lauric acid	9.7/1507 (1527 for HP-5MS [13])	–	–	–	–	0.3	–	0.4	–
Azelaic acid	10.1/1520 (1548 for DB-5 [14])	–	2.5	–	–	0.5	0.4	0.4	1.3
Myristic acid	14.0/1752 (1765 for HP-5MS[15])	–	–	–	–	0.3	0.3	0.4	–
Pentadecylic acid	16.0/1820 (1833 for DB-5[16])	–	–	–	–	0.6	0.8	0.4	–
Palmitoleic acid	17.5/1945(1953 for HP-5 MS[17])	3.1	4.0	1.1	1.5	8.5	2.1	5.9	–
Palmitic acid	17.9/1883 (1898 for DB-5[16])	17.0	19.5	10.8	15.2	10.6	19.6	22.8	32.5
Linoleic acid	21.0/2086 (2095 for HP-5MS[18])	30.2	27.7	57.2	76.2	47.8	64.7	34.5	40.7
Linolenic acid	21.1/2095(2101 for HP-5MS [19])	28.5	11.0	–	–	–	–	–	–
Oleic acid	21.2/2104 (2103 for HP-5MS [17])	19.4	29.3	24.5	3.8	23.8	7.3	26.9	14.4
11-octadecenoic acid	21.4/2113 (2115 for VF-5MS [20])	–	–	–	1.0	4.8	2.2	4.7	–
Stearic acid	22.1/2122 (2128 for HP-5MS [17])	1.7	2.2	1.0	2.1	1.5	1.7	1.8	1.0
Unidentified	22.8, 28.2	–	2.3	–	–	0.3	–	–	–

*for methyl esters

Table 3. “Volatile” neutral substances of MTBE extract

Name	Retention time/I	<i>F. velutipes</i>		<i>H. capnoides</i>		<i>A. borealis</i>		<i>A. cepistipes</i>	
		Cap	Stem	Cap	Stem	Cap	Stem	Cap	Stem
		Fractional content, %				Fractional content, %			
Monoglyceride	27.0–28.0	–	–	–	–	34.6	–	51.8	3.3
5 Unidentified	30.6–30.9, 35.4	–	–	–	–	–	–	2.4	7.4
Anthraergostatetraenol	33.9	51.4	36.7	2.3	1.5	–	11.0	1.6	1.7
5 Unidentified	34.0–35.8	24.3	12.5	–	2.6	–	–	2.3	2.1
Ergosta – 5,7,9,(11),22-tetraen-3 β -ol M=394	36.05/3116 (3150 for DB-5 MS[21])	–	–	–	20.7	–	–	–	2.1
Unidentified	36.2	–	2.1	–	–	–	–	–	–
Unidentified	36.3	–	–	–	–	–	–	–	2.1
Ergosterol C ₂₈ H ₄₄ O M=396	36.6/3183 (3152 for DB-5 MS [21])	24.2	18.8	18.0	15.9	6.7	16.1	1.5	5.8
Ergosta-7,22-dien-3 β -ol C ₂₈ H ₄₆ O M=398	36.8/3212 (3202 for DB-5 MS [21])	–	–	–	–	–	–	–	4.6
Ergosta-5,8,22-trien-3-ol	37.1/ 3238 (3158 for DB-5 MS [21])	–	–	–	–	–	21.8	17.8	–
Unidentified	37.2	–	–	54.2	17.4	–	–	–	–
Ergosta-14,22-dien-3-ol	37.3/3243	–	–	–	–	–	–	–	5.8
Unidentified	37.4	–	–	–	–	–	–	1.0	4.7
Ergosta-7-en-3 β -ol M=400	38.2/3246 (3220 for Methyl Silicone [22])	–	6.5	4.0	2.6	9.0	29.1	3.0	19.5
Stigmasterol	38.6/3263 (3170 for HP-5 MS[21])	–	–	–	–	–	–	–	3.4
8 Unidentified	39.2–41.4	–	15.6	11.1	21.8	14.9	–	1.5	23.7
Stigmast-5,22-dien-3-ol M=454	41.7/3414 (3244 for DB-5MS[21])	–	–	6.7	17.1	16.6	–	6.3	11.9
5 Unidentified	42.3	1.0	–	–	–	17.9	21.7	10.2	4.7

Table 4. Triglyceride content in mushrooms

	<i>F. velutipes</i>		<i>H. capnoides</i>		<i>A. borealis</i>		<i>A. cepistipes</i>	
	stems	caps	stems	caps	stems	caps	stems	caps
Triglycerides, % of dry matter	0.3	1.1	0.2	0.6	0.2	1.0	<0.1	1.1
Triglycerides yield from petroleum extract, %	33	67	50	70	75	92	20	83

The content of triglycerides of fatty acids on a high-temperature DB-HT SimDis column was determined by gas chromatography since the mushrooms contained monoglycerides of fatty acids, which are determined on a Rxi®-5 Sil MS column (Table 5).

1. *Flammulina velutipes*. The identical range of fatty acids is presented in stems and caps of *F. velutipes*. The presence of linolenic acid was noticed in the caps and stems of mushrooms. The content of linoleic acid in the stems and caps is the same and is 30% of the total acid content. The linolenic acid is mainly presented in mushroom caps (almost 30% of the total content of fatty acids). The content of oleic acid is more in the stems of mushrooms than in the caps (19% and 29%, respectively). The content of palmitic and stearic acids is the same both in the caps and in the stems. Our results on the fatty acids composition of *F. velutipes* are coincided with the earlier ones [25]. A similar series of fatty acids and with an almost identical quantitative composition is given.

The content of anthraergostatetraenol is 2.5 times higher in the caps than in the stems. *F. velutipes* contain ergosterol.

Glycerole is presented in the sugar alcohols content of *F. velutipes*. Its content is more than 60% of the total content of sugar alcohols in mushrooms caps, 30% is accounted for by the content of arabite. In the stems of mushrooms arabite already has a predominant value and accounts for 50% of the total content of sugar alcohols, 40% is glycerole. There is a small amount of mannitol (the same in the caps and stems, 3%). It is known that glycerole demonstrates cryo-protective properties, which allows *F. velutipes* to grow in cold seasons. The solution of water and glycerole freezes at -20 °C [26].

2. *Hypholoma capnoides*. Linoleic acid is the main from the fatty acids. There is more linoleic acid in the stems than in the caps (75% and 65%, respectively). Oleic acid is almost 6 times more in the caps than in the stems of mushrooms (24% and 4%, respectively). Palmitic and stearic acids are also presented. There is more palmitic and stearic acid in the stems of mushrooms.

The main part of neutral substances in caps is unidentified substance-more than 50% of neutral substances total content in caps, in the stems it is 3 times less. Ergosterol is presented both in the caps and in the stems of mushrooms in approximately the same quantity (18% – caps, 15% – stems). The stems of *H. capnoides* contain sterol – Ergosta-5,7,9,(11),22-tetraene-3 β -ol, which is 20% of the total content of sterols in the mushroom stems. It was noted that sterol Ergosta-5,7,9,(11),22-tetraen-3 β -ol is absent in the caps of *H. capnoides*.

The qualitative content of *H. capnoides* sugar alcohols was identified. The main content is accounted for by trehalose. There is 70% of trehalose in caps from the total content of sugar alcohols, and the trehalose content in the stems is about 65%. Mannitol is revealed both in the caps and in the stems (in the caps is a little more, 17 and 14%, respectively).

Table 5. Sugar alcohol from the researched mushrooms

Name	Retention time/I	<i>F. velutipes</i>		<i>H. capnoides</i>		<i>A. borealis</i>		<i>A. cepistipes</i>	
		Cap	Stem	Cap	Stem	Cap	Stem	Cap	Stem
		Fractional content, %				Fractional content, %			
Glycerol	5.0/780	63.7	42.0	3.1	0.3	3.9	1.4	1.8	1.2
Threitol	9.3/1452	0.08	0.05	1.0	1.7	50.7	54.2	16.6	37.1
Erythritol	9.5/1462 (1535 for HP-5[23])	0.08	0.05	–	–	0.7	0.5	0.6	0.6
Ribitol	13.7/1729 (1766 for HP-5[23])	0.9	1.2	–	–	–	–	0.1	–
Arabitol	14.2/1735 (1760 for HP-5[23])	31.2	53.1	3.8	0.9	0.5	0.8	1.2	0.8
α -Glucose	17.4/1894	–	–	1.4	7.4	–	–	0.2	0.4
Mannitol	18.1/1944 (1975 for HP-5[23])	3.5	3.5	17.4	14.2	42.5	40.4	76.8	52.5
β -Glucose	19.3/1989 (2031 for HP-5[23])	–	–	1.5	9.2	–	–	–	0.3
Trehalose	31.4/2776 (2816 for DB-5MS[24])	0.5	–	71.6	66.2	1.4	2.5	2.5	7.0

The research on the comparing of the chemical composition of the stems and caps of mushrooms is presented in the work [27]. Our results regarding the content of trehalose in the stems and caps of *H. capnoides* are not coincided with the results [27]. The authors of this paper assert that the stems of *H. capnoides* contain more trehalose than the caps.

3. *Armillaria borealis*. Linoleic acid is presented in the stems and caps of *A. borealis* and it has a predominant content compared to other acids (47% – in mushroom caps, 65% – in mushroom stems). Palmitic acid in the stems is 9% more than in the caps, and oleic acid is 3.5 times more in the caps than in the stems. There is a small content of 11-octadecenic and stearic acids, both in the caps and in the stems of mushrooms (approximately in the same ratio).

Mono-glycerides are presented in the caps of *A. borealis* and absent in the stems of mushrooms. Sterol Ergosta-5,8,22-triene-3-ol is presented in the stems of mushrooms and it is more than 20% of the total content of sterols in the stems of *A. borealis*. Ergosta-5,8,22-triene-3-ol is absent in mushroom caps. Ergosterol is presented in small amounts (more than 6% in the caps, more than 16% in the stems). One of the known sterols, ergost – 7-en-3 β -ol, is presented in this mushroom. The content of ergost-7-en-3 β -ol sterol in the stems is 3 times higher than in the caps of mushrooms.

Threitol and mannitol are the main sugar alcohols of these mushrooms. The content of threitol in the caps and stems of mushrooms is approximately the same and is 50% of the total content of sugar alcohols. Mannitol (40%) is presented both in the caps and in the stems of mushrooms. Trehalose present, in the stems is a little more than in the caps (2.5% and 1.5%, respectively).

4. *Armillaria cepistipes*. The main component of neutral substances in the caps of *A. cepistipes*, determined by the GLC method, are monoglycerides, more than 50% of the total content of "volatile" neutral substances. The content of ergosterol in the mushroom caps is 1.5%, when its content in the stems is 3 times higher (more than 5%). The mushrooms caps contain sterol ergost-5,8,22-triene-3-ol (18% of the total content of sterols), and this sterol is absent in the mushrooms stems.

Mannitol and threitol are the main content of sugar alcohols of *A. cepistipes* caps and stems. The ratio of mannitol and threitol in mushroom caps is 75 and 15%, respectively. The content of these two sugar alcohols in the stems of mushrooms is slightly different, but also mannitol is predominated (55 and 35%). There is also trehalose, in the stems 2.5 times more than in the caps.

5. *Triglycerides*. Most triglycerides were found in the mushrooms caps. In the caps of *A. borealis* there are slightly more triglycerides than in the stems (1.0 and 0.2%, respectively). In the caps of *A. cepistipes* there are many times more triglycerides than in the stem, about 6 times (0.05 and 0.6%, respectively).

6. *Heavy metals (Hg, Cd, Fe)*. The analysis of the heavy metals was carried out in mushrooms after isopropyl alcohol extraction in order to assess their quantitative content (Table 6).

The highest iron content is observed in the stems of *H. capnoides*, in the caps it is an order of magnitude less. The iron content is about the same in the caps and stems of *A. borealis*. The caps and stems of *F. velutipes* have approximately the same iron content, but in the stems it is slightly larger. The highest content of cadmium is observed in the caps of *A. cepistipes*, and the highest content of mercury is observed in the caps of *A. borealis*. *A. borealis* contains more heavy metals almost as well as *P. squarrosa* [9]. *A. borealis* and *P. squarrosa* were collected from birches trees, unlike other mushrooms.

Table 6. The heavy metals content in mushrooms

Sample	Hg mg/kg	Cd mg/kg	Fe mg/kg
<i>F. velutipes</i>			
stems	0.07	0.05	916.1
caps	0.04	0.03	852.9
<i>H. capnoides</i>			
stems	0.18	0.03	1320.8
caps	0.18	0.06	310.5
<i>A. borealis</i>			
stems	0.34	0.04	208.3
caps	0.60	0.23	191.7
<i>A. cepistipes</i>			
stems	0.04	0.25	210.8
caps	0.04	0.67	221.3

Conclusions

The main differences in the researched mushrooms are observed in the content of sugar alcohols and carbohydrates. *F. velutipes* mainly contains glycerol, *A. borealis* and *A. cepistipes* – threitol, *H. capnoides* – trehalose. As for *L. edodes* and *P. squarrosa* the main sterol is ergosterol [9], but in the *A. borealis*, *A. cepistipes*, *F. velutipes* and *H. capnoides* ergosterol is not a predominant substance. No differences were found among the acids, the main fatty acid is linolenic acid (regarding acid fractions) and its content is approximately the same in all parts of the mushrooms with the exception of *H. capnoides* stems – almost 2 times more than in the other mushrooms. Monoglycerides were found in mushrooms of the same genus: *A. cepistipes* and *A. borealis*, and triglycerides were found in all studied mushrooms. Also we can conclude that heavy metals are accumulated mostly in the caps of the researched mushrooms. The most mercury and cadmium contains the *A. borealis*.

References

1. Fedorov A. *Zhizn' rastenii v 6-ti t.* [Plant life in 6 volumes]. Moscow, 1976, vol. 2, 546 p. (in Russ.).
2. Vavrish P.O., Gorovoy L.F. *Gribi v lesu i na stole.* [Mushrooms in the forest and on the table]. Kiev, 1993, 207 p. (in Russ.).
3. Antonin V. *Micological Progress*, 2009, vol. 8, no. 3, pp. 259–271. DOI: 10.1007/s11557-009-0597-1.
4. Banerjee D., Das K., Banerjee R., Pateiro M., Nanda P., Gadekar Y., Biswas S., McClements D., Lorenzo J. *Foods*, 2020, vol. 9, pp. 1–15. DOI: 10.3390/foods9040432.
5. Marxmüller H. *Bulletin de la société mycologique*, 1982, vol. 98, pp. 87–124. DOI: 10.1007/s11557-009-0597-1.
6. Hu Y.-N., Sung T.-J., Chou C.-H., Liu K.-L., Hsieh L.-P., Hsieh C.-W. *Antioxidants*, 2019, vol. 8, pp. 1–15. DOI: 10.3390/antiox8080298.
7. Morozova E.N. *Biokhimiya*, 1982, vol. 47, no. 7, pp. 1181–1185. (in Russ.).
8. Psurtseva N.V. *Mikologiya i fitopatologiya*, 1982, vol. 16, no. 6, pp. 518–521. (in Russ.).
9. Bakanov V.V., Vedernikov D.N., Khabarova L.S. *Chemistry of plant raw materials*, 2020, vol. 3, pp. 67–72. DOI: 10.14258/jcprm.2020037277.
10. Gładkowski W., Chojnacka A., Kielbowicz G., Trziszka T., Wawrzeńczyk C. *Journal of the American Oil Chemists' Society*, 2012, vol. 89, pp. 179–182. DOI: 10.1007/S11746-011-1893-X.
11. Muhutdinov R.R., Pilipenko T.V., Kruchina-Bogdanov I.V. *Vestnik Yuzhnoural'skogo gosudarstvennogo universiteta*, 2019, vol. 7, no. 4, pp. 75–84. (in Russ.).
12. Hadaruga N., Hadaruga D., Paunescu V., Tatu C., Ordod V., Bandur G., Lupea A. *Food Chemistry*, 2006, vol. 99, no. 3, pp. 500–508. DOI: 10.1016/J.FOODCHEM.2005.08.012.
13. Alissandrakis E., Tarantilis P., Harizanis P., Polissiou M. *Agricultural and food chemistry*, 2007, vol. 55, no. 20, pp. 8152–8157. DOI: 10.1021/jf071442y.
14. Rostad C., Pereira W. *J. High Resolut. Chromatogr.*, 1986, vol. 9, no. 6, pp. 328–334. DOI: 10.1002/JHRC.1240090603.
15. Roussis V., Tsoukatou M., Petrakis P., Chinou L., Skoula M., Harborne J. *Biochem. Syst. Ecol.*, 2000, vol. 28, pp. 163–175. DOI: 10.1016/S0305-1978(99)00046-0.
16. Silva U.F., Borba E.L., Semir J., Marsaioli A.J. *Phytochemistry*, 1999, vol. 50, no. 1, pp. 31–34. DOI: 10.1016/S0031-9422(98)00459-2.
17. Pino J., Mesa J., Munoz Y., Marti P., Marbot R. *J. Agric. Food Chem.*, 2005, vol. 53, pp. 2213–2223. DOI: 10.1021/JF051106M.
18. Zeng Y.X., Zhao C.X., Liang Y.Z., Yang H., Fang H.Z., Yi L.Z., Zeng Z.D. *Anal. Chim. Acta*, 2007, vol. 595, pp. 328–339. DOI: 10.1016/j.aca.2006.12.022.
19. Zhao C., Zeng Y., Wan M., Li R., Liang Y., Li C., Zeng Z., Chau F.T. *J. Separation Science*, 2009, vol. 32, no. 4, pp. 660–670. DOI: 10.1002/jssc.200800484.
20. Tret'yakov K.V. *Retention Data*. NIST Mass Spectrometry Data Center, 2007.
21. Radulovic S., Dordevic D. *Serbian Chemical Society*, 2011, vol. 76, no. 11, pp. 1471–1483. DOI: 10.2298/JSC110206128R.
22. Shlyakhov A.F. *Gazovaya khromotografiya v organicheskoi geokhimi.* [Gas Chromatography in Organic Geochemistry]. Moscow, 1984, 222 p. (in Russ.).
23. Madaj J., Wisniewski A., Skorupowa E., Sokolowski J. *J. Chromatography A*, 1993, vol. 655, no. 2, pp. 267–273. DOI: 10.1016/0021-9673(93)83232-H.
24. Medeiros P.M., Simoneit B.R.T. *Chromatography A*, 2007, vol. 1141, no. 2, pp. 271–278. DOI: 10.1016/J.CHROMA.2006.12.017.
25. Ergönül P., Akata I., Kalyoncu F., Ergönül B. *The scientific world journal*, 2013, pp. 1–4. DOI: 10.1155/2013/163964.
26. Agranenko V.A., Fedorova L.I. *Zamorozhennaya krov' i eyo klinicheskoe primeneniye.* [Frozen blood and its clinical applications]. Moscow, 1983, 96 p. (in Russ.).
27. Alanne A.-L., Issakainen J., Pihlaja K., Jokioja J., Sinkkonen J. *Zeitschrift für Naturforschung C*, 2019, vol. 74, no. 7–8, pp. 201–210. DOI: 10.1515/znc-2018-0214.

Received February 10, 2022

Revised May 9, 2022

Accepted May 10, 2022