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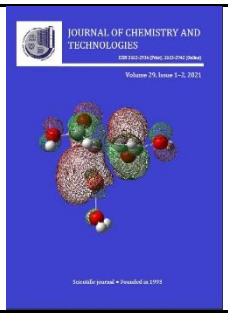

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ANALYSIS OF CHLORODIFLUOROMETHANE EXTRACT OF BAY LEAF BY GAS CHROMATOGRAPHY WITH DIFFERENT KINDS OF DETECTION

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Abstract

Promising technologies of food additives are technologies that allow obtaining biologically active substances in the process of biosynthesis and extraction technologies of processing the vegetable raw materials. The aim of our work was to determine the composition of chlorodifluoromethane extract of the bay leaf using gas chromatography with mass spectrometric and flame ionization detectors. The results of the analysis of chlorodifluoromethane (Freon R22) extract of bay leaf by gas chromatography with mass spectrometric (MS) and flame ionization detection (FID) are presented. The 93 components were found, 49 of which were identified. It was established that the composition of chlorodifluoromethane extract of bay leaf includes terpenes (25.07 %), terpenoids (40.50 %), fatty acids (7.93 %), aromatic hydrocarbons (0.53 %), alkanes (0.22 %). The predominant compounds were 1,8-cineole and camphene. The presence of significant amounts of sabinen and linolenic acid was shown. These components can be used as markers for quality control and stability studies of food products made using chlorodifluoromethane bay leaf extract.

Keywords: bay leaf; extract; chlorodifluoromethane; freon extraction; gas chromatography.

АНАЛІЗ ДИФТОРХЛОРМЕТАНОВОГО ЕКСТРАКТУ ЛАВРОВОГО ЛИСТА МЕТОДОМ ГАЗОВОЇ ХРОМАТОГРАФІЇ З РІЗНИМИ ВИДАМИ ДЕТЕКТУВАННЯ

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Анотація

Наведено результати аналізу дифторхлорметанового (фреонового 22) екстракту лаврового листа методом газової хроматографії з мас-спектрометричним (МС) і полуменево-іонізаційним детектуванням (ПІД). Виявлено 93 компоненти, 49 з яких ідентифіковано. Встановлено, що до складу дифторхлорметанового екстракту лаврового листа входять терпени (25.07 %), терпеноїди (40.50 %), жирні кислоти (7.93 %), ароматичні вуглеводні (0.53 %), алкани (0.22 %). Домінуючими сполуками були 1,8-цинеол та камфен.

Ключові слова: лавровий лист; екстракт; дифторхлорметан; фреонова екстракція; газова хроматографія.

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АНАЛИЗ ДИФТОРХЛОРМЕТАНОВОГО ЭКСТРАКТА ЛАВРОВОГО ЛИСТА МЕТОДОМ ГАЗОВОЙ ХРОМАТОГРАФИИ С РАЗНЫМИ ВИДАМИ ДЕТЕКТИРОВАНИЯ

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Аннотация

Приведены результаты анализа дифторхлорметанового (фреонового 22) экстракта лаврового листа методом газовой хроматографии с масс-спектрометрическим (МС) и пламенно-ионизационным детектированием (ПИД). Выявлено 93 компонента, 49 из которых идетнифицированы. Установлено, что в состав дифторхлорметанового экстракта лаврового листа входят терпени (25.07 %), терпеноиды (40.50 %), жирные кислоты (7.93 %), ароматические углеводы (0.53 %), алканы (0.22 %). Доминирующими соединениями являются 1,8-цинеол и камфен.

Ключевые слова: лавровый лист; экстракт; дифторхлорметан; фреоновая екстракция; газовая хроматография.

Introduction

Modern production of food additives is characterized by a steady increase in the use of biologically active raw materials of vegetable and animal origin. Promising technologies of food additives are technologies that allow obtaining biologically active substances in the process of biosynthesis and extraction technologies of processing the vegetable raw materials.

Currently, the main technologies for the production of liquid food additives from vegetable raw materials are the steam distillation and extraction with hexane, petroleum ether, alcohol, acetone, perchlorethylene, etc. [1]. All these methods have well-known drawbacks. In particular, during the steam distillation, there is a significant destruction of the molecular thermolabile complexes due to hydrolysis and high temperature during this process, which reduces the biological value of such extracts. The use of organic solvents can also cause degradation of the final product by the reactive compounds of such solvents. In addition, the removal of organic solvents from extract leads to evaporation of the most volatile compounds and this is quite difficult, so almost always the final product contains the residues of these solvents. Moreover, the removal of organic solvent reduces the biological value of such extracts. A promising way for solving the problem of improving the quality of plant extracts is to use cryotechnology.

One of the rapidly evolving cryobiotechnologies is the extraction of lipid fractions from the plant and animal raw material using liquefied gases. Recently, research has been actively undertaken on the production of freon extracts from various vegetable raw materials and their use in food technology [2–4]. Interest in freon extraction is associated with the ability to obtain extracts that retain the taste and aromatic properties of the feedstock, as well as the biological activity of the most important ingredients. In this case, due to low boiling point, the freons are completely removed from the final product, while the chemical inertness does not affect the chemical composition of the odor constituents [1; 5; 6].

A number of studies have demonstrated the feasibility of using chlorodifluoromethane (Freon R22) as an extractant, due to its physicochemical properties. Among the freons used as extractants, chlorodifluoromethane has the lowest molecular weight, minimum surface tension and dynamic viscosity. Due to these facts, chlorodifluoromethane moistens better the particles of plant material and penetrates quickly into the cells [7].

It was proved that chlorodifluoromethane is the optimal extractant of lipophilic complexes from the medicinal plant raw materials [8]. A technology of extraction with difluorochloromethane for producing the lipophilic fraction of the medicinal Marigold and Echinacea purpurea has been developed [9]. It is shown that the sea buckthorn oil extracted with chlorodifluoromethane contains an increased amount of dilaurin, distearin, trilaurin, trilinolein, myristic and palmitic acids, in comparison with oil obtained by other methods such as centrifugation and enzymatic hydrolysis [10]. Extraction with chlorodifluoromethane of a mixture of different types of the medicinal raw materials yielded an oil extract with hypocholesterolemic action [11]. The expediency of using chlorodifluoromethane in the composition of the extractant in the technology of obtaining lipophilic complexes from rose hips and the amount of alkaloids from barberry root

has been proved [12]. The prospectivity of using the liquefied chlorodifluoromethane for the extraction of the lipophilic complexes from the overground part of yellow bedsraw is proved [13]. It was shown that this extract exhibits antimicrobial activity [14].

Scientists are actively developing new technologies for food products using freon extracts from the vegetable raw materials. Thus, the replacement of natural ground spices (black pepper, caraway seeds) with aliquots of their freon extracts in smoked sausage technology has improved the sensory properties and stabilized the color characteristics of the product during storage at 0–4 °C for 30 days [15; 16]. Addition of freon extract from the mixture of carrot and mustard seeds, rice flour to vegetable seasoning consisting of mashed salad, spinach, sorrel, green parsley, green dill, garlic, sweet pepper, red baking pepper, sugar and salt leads to obtain more balanced composition of basic nutrients and improved organoleptic properties [17].

However, the use of freon extracts in food technology is not possible without studying their composition and properties, which will predict the properties of the final product. For the identification and determination of volatile organic compounds in the mixture, the most convenient and commonly used method is gas chromatography with mass spectrometric detection (GC-MS) [18–21]. It allows separating volatile organic compounds basing on the difference in their physical and chemical properties [22]. The advantages of the method are shorter time and absence of reagents for sample preparation, the ability of determination of the approximate components percentage ratio in the sample using the method of internal normalization [23]. However, the method allows only the approximate estimation of the relative concentration of substances [22; 23]. In order to obtain more accurate estimation of the volatile organic compounds content with the internal normalization method, it is preferable to use the method of gas chromatography with flame ionization detection (GC-FID) [18; 24]. To determine the concentrations of the components of extracts accurately, it is necessary to have standards of corresponding substances for the calibration of FID or MS detectors.

Thus, the aim of our work was to determine the composition of chlorodifluoromethane extract of the bay leaf using gas chromatography with mass spectrometric and flame ionization detectors.

Experimental

*Materials***.** In this research the dried leaves of noble laurel, harvested in Georgia in 2019 (5 % humidity) as a raw materials, and chloro-difluoromethane (Freon R22) produced by Sanmei (original Japan TM) as an extractant, were used. Dichloromethane (reagent grade), helium gas (compressed) with a purity of 99.999 %, nitrogen, extra-pure grade – in accordance with GOST 9293 were used for GC analysis.

*Preparation of the extract***.** In order to obtain chlorodifluoromethane extract, the leaves of the laurel were ground to a particle size of 220–280 microns, then were loaded in a special filter bags into two parallel-connected extractors at a facility developed at the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine. The extraction was carried out with three-stage liquefied chlorodifluoromethane at the temperature of 25 ± 2°C, pressure of 1.5 ± 0.1 MPa and the ratio of raw material: extractant 1:8 for 10 hours. After the end of the process the extract was poured into the evaporator, where the evaporation and recovery of chlorodifluoromethane were carried out. The remaining extract was poured into a receiving container. In order to remove the precipitate, sedimentation of the final product was carried out. The installation was evacuated to recover the extractant back to the extractor. The total duration of the process was 30 hours. The yield of the final product was 5 % of the initial amount of dried bay leaves.

Investigation Methods. Analysis of chlorodifluoromethane extract of bay leaf by gas chromatography was performed on the basis of the Department of Luminescent Materials and Dyes named after B.M. Krasovytskiy SSI "Institute for Single Crystals" of NAS of Ukraine. Studies of qualitative and quantitative composition of the extract were performed by gas chromatography with different types of detection (mass spectrometric and flame ionization).

*Gas Chromatography/Mass Spectrometry (GC/MS) Analysis***.** The experiment was carried out on Agilent 7890A gas chromatography (GC) System with Agilent 5975C Inert MS mass-detector. HP-5ms ((5 % – phenyl) – methylpoly-siloxane) capillary column with 30 m length, 0.25 mm internal diameter and 0.25 μm stationary phase thickness was used. Helium was used as carrier gas. The flow rate of the carrier gas was 1 ml/min. Injector temperature was set to 250 °C, split ratio – 10 : 1. Oven temperature program was the following:

initial temperature – 40 °C, increasing rate 5 °C/min up to 270 °C. Solution of bay leaf extract in dichloromethane (24 mg/ml) was prepared. Injection volume – 1 µl. The mass scanning range was from 35 to 500 m/z. The qualitative estimation of bay leaf extract components was based on a comparison of extract components mass spectra with the corresponding data of the NIST-08 mass spectral library (National Institute of Standards and Technology, USA). Results with a minimum similarity index of 80 % were selected. An internal normalization method was used to evaluate the content of the bay leaf extract components. The total analysis time was 46 minutes.

Gas Chromatography / Flame-Ionization Detection (GC/FID) Analysis. The quantitative analysis of bay leaf extract was performed on Agilent 7890A GC System with flame ionization detector (GC-FID). HP-5ms ((5 % – phenyl) – methylpolysiloxane) capillary column with 30 m length, 0.32 mm internal diameter and 0.25 µm stationary phase thickness was used. Nitrogen was used as carrier gas. The flow rate of the carrier gas was 1 ml/min. Injector temperature was 300 °C, split ratio – 50 : 1. Oven temperature program was: initial temperature – 40 °C, increasing rate 5 °C/min up to 270 °C, detector temperature 300 °C. Solution of bay leaf extract in dichloromethane (24 mg/ml) was prepared. Injection volume – 1 µl. The quantitative content of components of the bay leaf extract was evaluated by internal normalization and comparison of signals on chromatogram obtained by GC-MS. The total analysis time was 46 minutes.

Results and Discussion

Chlorodifluoromethane bay leaf extract was a green, viscous liquid, with distinct laurel-like taste and odor, without extraneous odor and taste. Chromatograms of chlorodifluoromethane bay leaf extract obtained by GC-MS and GC-FID are shown in Figures 1 and 2, respectively. On the chromatograms obtained it is noticed some slight differences in the components retention time values. These differences are caused by the differences in the internal diameter of the columns and properties of carrier gases, but they don't effect on the order of components on chromatograms

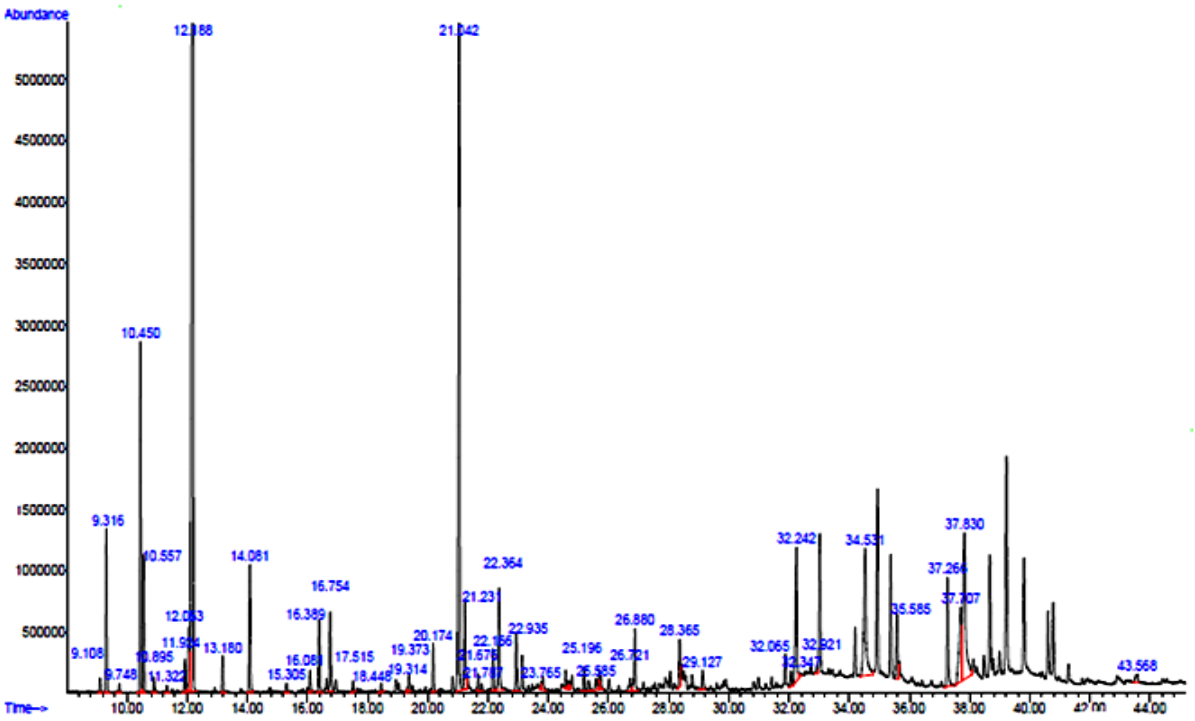

Fig. 1. Chromatogram of chlorodifluoromethane bay leaf extract obtained by GC-MS

According to the data obtained (see the table), chlorodifluoromethane bay leaf extract contains at least 93 components, 49 of which were identified by their mass-spectra. There are two very intense peaks on the chromatograms with a retention time of 12.188 (11.672) and 21.042 (20.335) minutes, which were corresponded to 1,8-cineole and camphene, respectively. These compounds were dominant in the analyzed sample.

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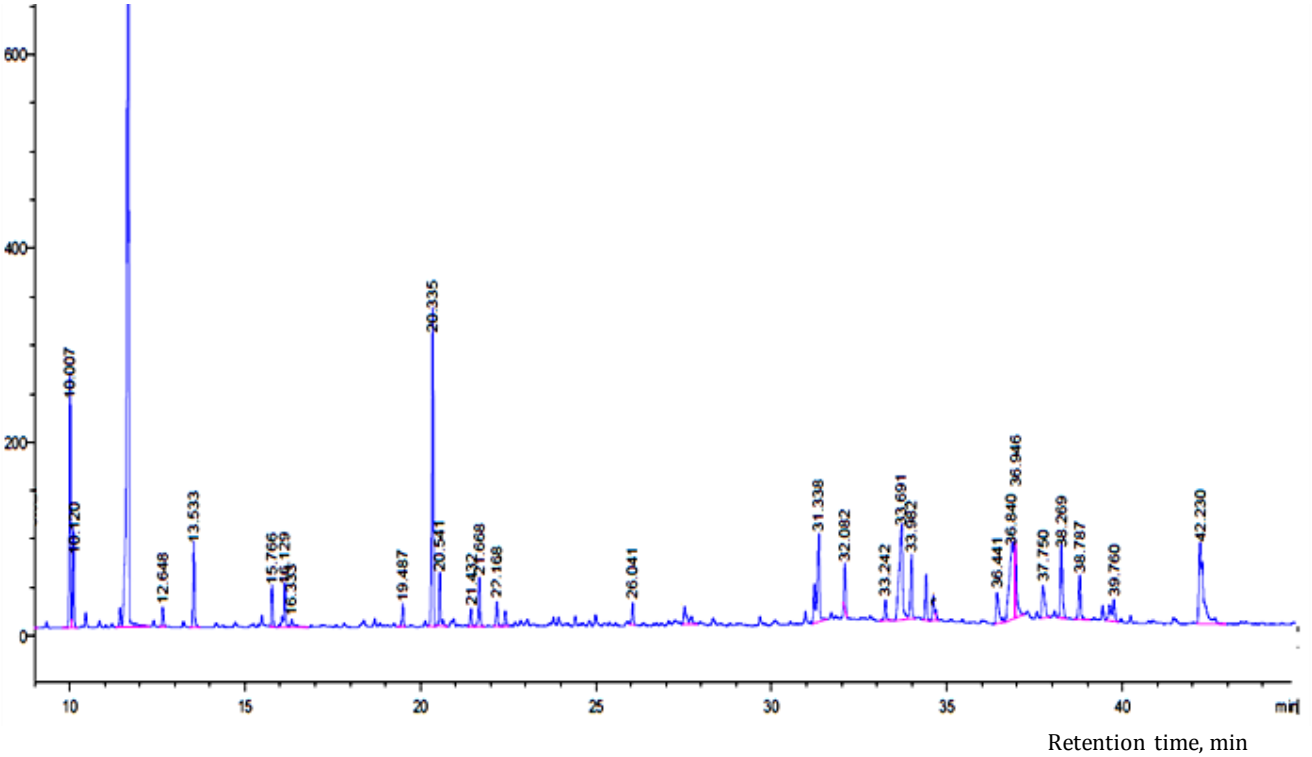


Fig. 2. Chromatogram of chlorodifluoromethane bay leaf extract obtained by GC-FID

Table

Composition of volatile substances from chlorodifluoromethane bay leaf extract

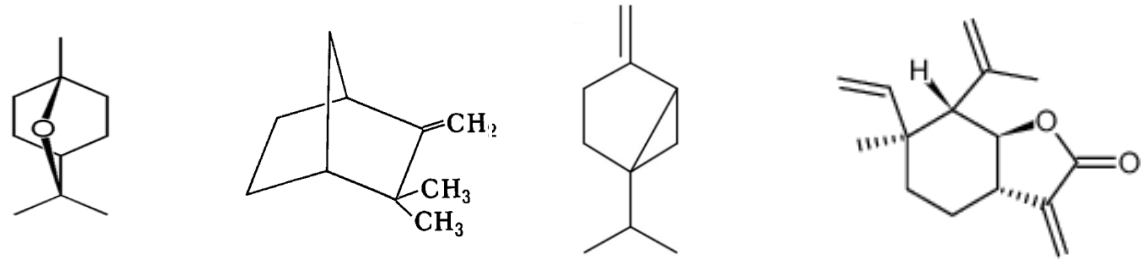
№	Name of the compound	Molecular formula	Molecular weight, g/mol	Retention time, min		Area,%
				MSD	FID	FID
1	2	3	4	5	6	7
1	α-Thujene	$C_{10}H_{16}$	136	9.108	8.739	0.23
2	α-Pinene	$C_{10}H_{16}$	136	9.316	8.928	2.09
3	Camphene	$C_{10}H_{16}$	136	9.748	9.338	0.12
4	**Sabinene**	$C_{10}H_{16}$	136	**10.450**	**10.007**	**4.83**
5	β-Pinene	$C_{10}H_{16}$	136	10.557	10.105	1.88
6	β-Myrcene	$C_{10}H_{16}$	136	10.895	10.452	0.28
7	α-Phellandrene	$C_{10}H_{16}$	136	11.324	10.850	0.16
8	δ-3-Carene	$C_{10}H_{16}$	136	–	11.203	0.09
9	*o*-Cymol	$C_{10}H_{14}$	134	11.924	11.432	0.42
10	DL-Limonene	$C_{10}H_{16}$	136	12.063	11.556	1.21
11	**1,8-Cineole**	$C_{10}H_{18}O$	**154**	**12.188**	**11.672**	**27.83**
12	γ-Terpinene	$C_{10}H_{16}$	136	–	12.391	0.13
13	5-Isopropyl-2-methylbicyclo [3.1.0.]hexan-2-ol	$C_{10}H_{18}O$	154	13.180	12.645	0.44
14	α-Terpinolene	$C_{10}H_{16}$	136	–	13.239	0.14
15	(±)-Linalool	$C_{10}H_{18}O$	154	14.081	13.533	1.88
16	Menta-1,4,8-triene	$C_{10}H_{14}$	134	–	14.175	0.11
17	Sabinol	$C_{10}H_{16}O$	152	15.305	14.714	0.21
18	Sabina ketone	$C_9H_{14}O$	138	–	15.237	0.15
19	4-Terpineol	$C_{10}H_{18}O$	154	16.389	15.766	0.93
20	α-Terpineol	$C_{10}H_{18}O$	154	16.754	16.049	0.28
21	Methylchavicol	$C_{10}H_{12}O$	164	–	16.130	1.01
22	γ-Terpinene	$C_{10}H_{16}$	136	17.515	–	–
23	δ-3-Carene	$C_{10}H_{16}$	136	18.448	–	–
24	4-Thujen-2alpha-yl acetate	$C_{12}H_{18}O_2$	194	19.033	18.376	0.27
25	Bornyl acetate	$C_{12}H_{20}O_2$	196	19.373	18.690	0.20

Table (continued)

26	1-Methylene-4-(1-methylethenyl)-cyclohexane	$C_{10}H_{16}$	136	20.174	19.489	0.52
27	**Camphene**	$C_{10}H_{16}$	136	**21.042**	**20.335**	**7.65**
28	*p*-Eugenol	$C_{10}H_{12}O_2$	164	21.231	20.541	1.19
29	α-Ylangene	$C_{15}H_{24}$	204	21.676	–	–
30	α-Copaene	$C_{15}H_{24}$	204	21.791	20.939	0.31
31	*β*-Elemene	$C_{15}H_{24}$	204	22.166	21.433	0.45
32	Methyleugenol	$C_{11}H_{14}O_2$	178	22.364	21.669	1.16
33	*β*-Caryophyllene	$C_{15}H_{24}$	204	22.935	22.168	0.64
34	3,7-Guaiadiene	$C_{15}H_{24}$	204	–	23.470	0.11
35	1, 5, 9, 9-Tetramethyl-1, 4, 7-cycloundecatriene	$C_{15}H_{24}$	204	23.769	–	–
36	*β*-Guaiene	$C_{15}H_{24}$	204	23.823	23.040	0.19
37	*β*-Selinene	$C_{15}H_{24}$	204	24.574	23.784	0.17
38	Isohomogenol	$C_{11}H_{14}O_2$	178	24.650	–	–
39	Valencene	$C_{15}H_{24}$	204	24.718	23.925	0.24
40	*γ*-Cadinene	$C_{15}H_{24}$	204	25.196	24.403	0.24
41	*γ*-Muurolene	$C_{15}H_{24}$	204	25.338	–	–
42	α-Copaene	$C_{15}H_{24}$	204	25.585	24.808	0.18
43	*α* -Bisabolene	$C_{15}H_{24}$	204	25.753	24.984	0.37
44	Spathulenol	$C_{15}H_{24}O$	220	26.721	25.902	0.17
45	Caryophyllene oxide	$C_{15}H_{24}$	204	26.880	26.043	0.61
46	*β*-Eudesmol	$C_{15}H_{26}O$	222	28.365	27.522	0.66
47	Neophytadiene	$C_{20}H_{38}$	279	32.065	31.216	0.95
48	**Dehydrosaussurea lacton**	$C_{15}H_{20}O_2$	**232**	**32.242**	**31.345**	**2.73**
49	Costunlide	$C_{15}H_{20}O_2$	232	32.921	31.702	0.16
50	**Palmitinic acid**	$C_{16}H_{32}O_2$	**256**	**34.531**	**33.707**	**2.38**
51	Eremanthin	$C_{15}H_{18}O_2$	230	35.584	34.620	0.62
52	Neophytadiene	$C_{20}H_{38}$	279	37.266	36.810	1.89
53	**Linoleic acid**	$C_{18}H_{32}O_2$	**280**	**37.707**	**36.869**	**2.30**
54	**Linolenic acid**	$C_{18}H_{30}O_2$	**278**	**37.829**	**36.952**	**3.25**
55	Octadecane	$C_{18}H_{38}$	255	43.571	42.638	0.22
	Unidentified compounds					25.75
	Total					100

∗ components highlighted with bold text are with content of more than 2.0 %.

According to the table, the main components, with content of more than 2.0 % were the following substances: 1,8-cineole, camphene, sabinene, dehydrosaussurea lacton, palmitinic acid, linoleic acid, linolenic acid, and three unidentified compounds containing 2.34–2.56 % (Fig. 3).



1,8-cineole Camphene sabinene dehydrosaussurea lacton

$CH_3—(CH_2)_{14}—COOH$

palmitinic acid

$CH_3—(CH_2)_4—CH=CH—CH_2—CH=CH—(CH_2)_7—COOH$

linoleic acid

$CH_3—CH_2—CH=CH—CH_2—CH=CH—\ CH_2—CH=CH—(CH_2)_7—COOH$

linolenic acid

Fig. 3. Chemical structure of major compounds of chlorodifluoromethane bay leaf extract

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Chromatographic studies of the bay leaf extract make it possible to evaluate its composition: terpenes – 25.07 %, terpenoids – 40.5 %, fatty acids – 7.93 %, aromatic hydrocarbons – 0.53 %, alkanes – 0.22 % (Fig. 4). The total content of unidentified substances was 25.75 % of the total number of identified compounds.

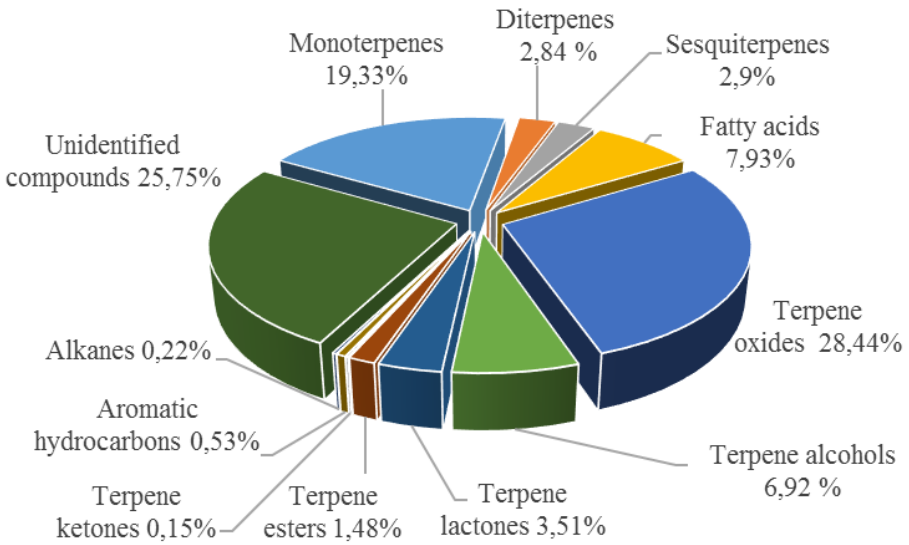

Fig. 4 Content of different classes of compounds in chlorodifluoromethane bay leaf extract (by gas chromatography analysis)

The only one representative of identified acyclic monoterpenes was *β*-myrcene. The compositions of monocyclic monoterpenes were very diverse. The compound with the highest content of 1.21 % was limonene. Among the bicyclic compounds camphene with a content of 7.77 % was dominant. The only representative of diterpenes was neophytadiene with a content of 2.84 %. Ten compounds of the chlorodifluoromethane bay leaf extract were sesquiterpenes. Their total content was 2.9 %.

Oxygen-containing compounds are known to have a greater effect on the aroma of essential oils than monoterpenes [25]. In the chlorodifluoromethane bay leaf extract 16 oxygen-containing terpene compounds (oxides, alcohols, lactones, esters, ketones) were identified, the total amount of these compounds being 40.5 % of the total content of the components. Therefore, the bay leaf extract obtained with chlorodifluoromethane extraction has a good aroma. As it was shown by the results of chromatographic analysis, the dominant terpene compounds were oxides – 1.8-cineole and caryophyllene oxide. Terpene lactones were represented by three compounds – dehydrosaussurea lacton, costunlide, eremanthin. Two terpene esters – bornyl acetate and 4-thujen-2alpha-yl acetate were representatives of esters, one – methylchavicol is a representative of ethers. It was found that the chlorodifluoromethane extract contained one terpene ketone – sabina ketone (0.15 %). The extract contained small amount of aromatic hydrocarbons such as *o*-cymol (0.42 %), menta-1,4,8-triene (0.11 %). The only alkane representative was octadecane.

Important components of the extract were fatty acids, this class was represented by palmitinic (2.38 %), linoleic (2.30 %) and linolenic (3.25 %) acids.

The chemical profile of the bay leaf extract obtained by chlorodifluoromethane extraction was different from that of the CO_2 extract [26]. It was found that 32 components were selectively extracted with chlorodifluoro-methane, including palmitinic, linoleic and linolenic acids. The 17 components were determined in both samples, namely: 1,8-cineole, linalool, terpinene-4-ol, *α*-terpineol, 4-thujen-2alpha-yl acetate, bornylacetate, eugenol, *β*-elemene, methylelegenol, *β*-caryophyllene, *β*-selinene, *γ*-cadinene, spathulenol, caryophyllene oxide, *β*-eudesmol, dehydrosaussurea lacton, eremanthin. A significant difference was that in the chlorodifluoromethane extract the predominant compounds were 1,8-cineole and camphene, and in the CO_2 extract – methyllinoleate, α-terpenyl acetate, linalool, methyleugenol.

Comparative analysis of the chemical composition of chlorodifluoromethane bay leaf extract with steam distillation extract has been performed [26]. It was found that 19 compounds were identified in both samples. Their total

content in chlorodifluoromethane extract was 51.48 %, in the extract obtained by steam distillation – 78.69 %. 29 compounds were selectively extracted with chlorodifluoromethane, including fatty acids. The compound with the maximum content in both cases was 1.8-cineole, while other dominant compounds were different. For the steam distillation extract, these were linalool and α-terpenyl acetate.

Thus, chlorodifluoromethane is able to extract a wide range of non-polar substances (monoterpenes, sesquiterpenes, terpenoids, fatty acids) from the bay leaf. The results show that due to its chemical composition, chlorodifluoromethane extract of bay leaf can be used in food technology as a flavoring, preservative and enrichment additive.

Conclusions

The chlorodifluoromethane extract of bay leaf was analyzed by gas chromatography with mass spectrometric and flame-ionization detection. The 93 components were found, 49 of which were identified. The total content of unidentified compounds was 25.75 % of the total number of identified compounds.

It is established that the composition of chlorodifluoromethane bay leaf extract includes terpenes (25.07 %), terpenoids (40.5 %), fatty acids (7.93 %), aromatic hydrocarbons (0.53 %), alkanes (0.22 %).

It was determined that the predominant compounds were 1,8-cineole and camphene. The presence of significant amounts of sabinen and linolenic acid was shown. These components can be used as markers for quality control and stability studies of food products made using chlorodifluoromethane bay leaf extract.

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