

**G. N. Ilyina**, PhD student (BSTU);

**S. A. Lamotkin**, PhD (Chemistry), assistant professor (BSTU);

**K. P. Kolnogorov**, PhD (Engineering), assistant lecturer (BSTU);

**E. D. Skakovskiy**, PhD (Chemistry), assistant professor (POCI Belarus NAS)

## IDENTIFICATION OF THE PLANT OIL COMPOSITION BY CHROMATOGRAPHIC AND SPECTRAL METHODS

This article presents the results of research by chromatographic and spectroscopic methods of fatty acid composition of vegetable oils which sold in the trading network of the Republic of Belarus, it also presents an assessment of their quality characteristics from the standpoint of modern views on food hygiene. It was found, that the vegetable oils include both saturated and unsaturated fatty acids with one or two double bonds. Two samples of sunflower oil was found in the analysis that do not meet the requirements of the quantitative content of oleic and linoleic acids. Quality counterfeits were also found among the tested olive oils. Fatty acid composition must be controlled continuously during production and use of vegetable oils, and especially mixtures thereof. The combination of spectroscopic and chromatographic methods allows obtaining detailed picture of analysis of the fatty acid content in oils.

**Introduction.** Fatty foods are traditionally considered to be the basic products forming the diet of the majority of the population. With proper selection and consumption, fats play an important role in a healthy diet [1].

According to the recommendations of the World Health Organization fat intake should provide caloric value of food by 15–30%, while the content of saturated fatty acids in the diet should not be more than 10% of total calories, and the content of trans-fatty acids (FA) – not more than 1% of total calories. It is extremely important the presence and ratio of polyunsaturated fatty acids (PUFA), omega-6 and omega-3 in fatty products.

Dietary requirements of the population in the Republic of Belarus are harmonized with international principles of healthy eating and identified by sanitary norms and rules (Decree of the Ministry of Health of the Republic of Belarus № 180 from November 20, 2012). Herewith, one of the solutions to the problem of producing of oil and fat production meeting modern requirements of food hygiene, is the production of combined fat products, which include animal fats as well as various types of vegetable oils. Creation of combined fatty products will enable significantly enrich food with polyunsaturated fatty acids while reducing the level of cholesterol, saturated fatty acids and energy value.

Such production makes very stringent requirements to the methods and techniques of controlling the composition of the components used. At the same time traditional physical-chemical parameters such as acid value, iodine value, peroxide value and other gross-characteristics defined by proper methods of chemical analysis are not sufficient today to solve technological issues, to assess the quality of fatty foods, to identify their naturalness, to determine authenticity of oil grades etc. For this purpose, according to the literature, is currently

widely used techniques such as NMR spectrum, and chromatography [2, 3].

The proof of an urgent need in the transition to use modern control methods is that in recent years in our country national standards harmonized with the international standards ISO are actively implemented: STB ISO 15304-2007 “Animal and vegetable fats and oils. Determination of the content of transisomers of fatty acids in vegetable oils by gas chromatography”, STB ISO 5509-2007 “Animal and vegetable fats and oils. Techniques for producing methyl ether of fatty acids”.

In view of the aforesaid, the aim of this work was to study the fatty acid composition of vegetable oils sold in shops of Belarus by chromatographic and spectroscopic methods as well as the assessment of their quality from the point of view of modern views on food hygiene.

**Main part.** The sunflower oils were taken for analysis:

1 – “Zolotaya kaplya”, the Republic of Belarus; 2 – “Masloviya», Ukraine; 3 – “Zolotaya semchka”, Russia; 4 – “Oleina”, Ukraine; 5 – “Slaviya”, Ukraine.

The olive oils were taken for analysis as well: 1 – Lioxarpi, Greece; 2 – CarleOne, Italy; 3 – Maestro de Oliva, Spaine; 4 – Grande Oliva, Italy; 5 – Carbonell, Spaine; 6 – Carapelli, Italy.

0.2ml of vegetable oil was dissolved in 0.3 ml  $\text{CDCl}_3$  and placed in a 5-mm vial for recording NMR spectra.

Recording of spectra was carried out in a quantitative mode on a spectrometer AVANCE-500 with an operating frequency of 500 MHz for  $^1\text{H}$  nuclei and 125 MHz for  $^{13}\text{C}$  nuclei.

For identification signal of vegetable oils and margarine components spectra of  $^1\text{H}$  and  $^{13}\text{C}$  NMR of the following fatty acids were recorded: linoleic, oleic, linolenic, palmitinic and stearic. Analysis of the methyl ethers of fat acids was carried out with

a gas chromatograph "Crystal Chromatec 5000.1" with a capillary column with an internal diameter of 0.25 mm and a length of 100 m, fixed liquid phase cyanopolisiloksan. For complete separation of methyl ethers of fatty acids, mode sharing with programming temperature was selected (isothermal at 140°C for 4 minutes, then programmable rising temperature rate of 3°C / min up to 180°C at exposures at this temperature for 40 min, then programmable temperature rise by 3°C / min up to 240°C at exposures at final temperature for 25 minutes; evaporator temperature is 250°C; analysis time is 102.3 min). Identification of separate components of oils was carried out by Restek 35077 and Restek 35079 standards.

Preparation of methyl ethers of fat acids was performed by a standard technique.

Fig. 1 shows  $^1\text{H}$  NMR spectrum in  $\text{CDCl}_3$  of sunflower oil at 500 MHz.

Fig. 1 shows that the spectrum consists of a series of multiplets. The chemical shifts of the proton signals of the compounds were determined from the signal of chloroform ( $\text{CHCl}_3$ ,  $\delta = 7.26$  m. f.), which presents as an impurity in the deuterated solvent. One can be observed the signals of the olefinic proton and methylene proton of the glycerol residue in the area of 5.2–5.4 m. f.; 4.1–4.3 m. f. – absorption region of methylene protons of glycerin; 2.6–2.8 m. f. – absorb methylene protons  $\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}$ - residues of linoleic and linolenic acids; about 2.3 m. f. – absorption region of all methylene protons, located next to carboxyl group; about 2.0 m. f. – absorption region of all

methylene protons next to double bonds; about 1.6 m. f. – region of other methylene protons; 1.2–1.4 m. f. – absorption region of all the rest methylene protons; about 0.95 m. f. – absorption region of all methylene protons of linolenic acid located near double bond; 0.8–0.9 m. f. – absorb all methylene protons except linolenic. The  $^{13}\text{C}$  NMR spectrum shown in Fig. 2 is more informative.

$^{13}\text{C}$  NMR spectrum shown in Fig. 2, comprises the following groups of signals: in the region of approximately 173 m. f. they absorb carbon atoms of the carboxyl groups; the absorption region of the double bonds is in the range of 127–132 m. f., with a range of approximately 130.4–130.5 m. f. absorb C-13 and C-14 carbons of erucic acid; carbons of the glycerol residue take up about 69 m. f. ( $\text{CH}$ ) and about 62 m. f. ( $\text{CH}_2$ ); methylene carbon atoms of molecular chains appear in 21–34 m. f., and the methyl carbon – about 14 m. f.

Based on the data presented in Fig. 2 it is shown that the olefinic carbon atoms of different fatty acids have distinct chemical shifts and separate out signals which greatly facilitates the analysis of vegetable oils.

Spectral data for the identification of separate components are in good agreement with the results of G. Vlahov, which in work [3] studied in detail the composition of olive oils using  $^1\text{H}$  NMR and  $^{13}\text{C}$ .

In work [4] it is shown that the results obtained by NMR spectroscopy correlate well with the results obtained by gas chromatography. At the same time the spectral data can be used for quantification of the fatty acids in oils.

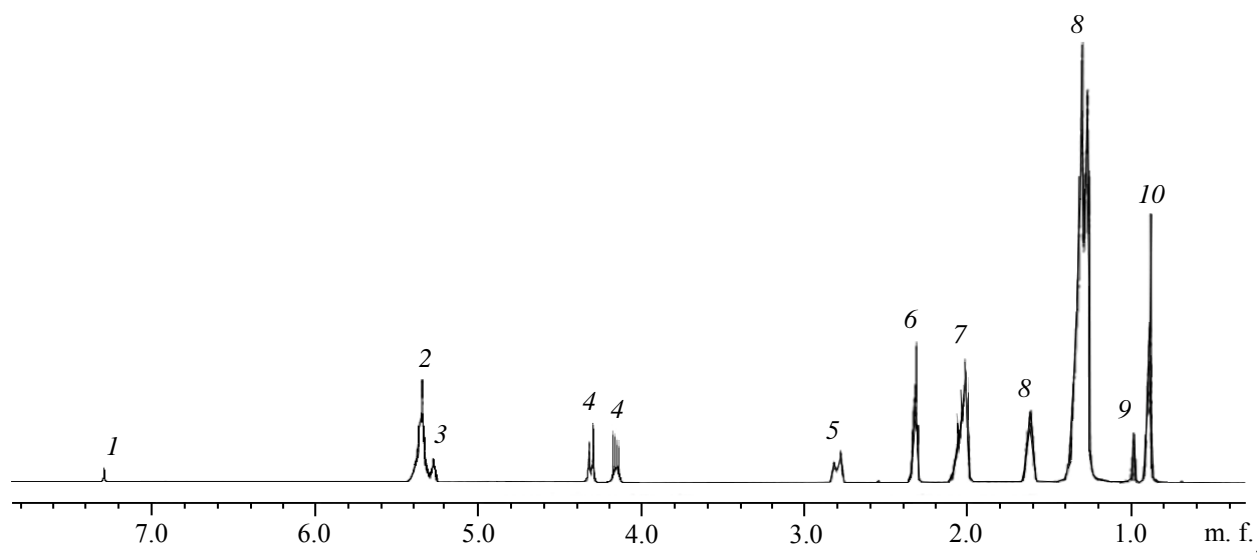


Fig. 1.  $^1\text{H}$  NMR spectrum of the sunflower oil solution  $\text{CDCl}_3$ :

- 1 –  $\text{CHCl}_3$  in  $\text{CDCl}_3$  (used solvent); 2 – H atoms of the double bonds;  
 3 –  $\text{CH}$ -glycerin groups; 4 –  $\text{CH}_2$ -glycerin groups; 5 –  $\text{CH}_2$ -groups, located between double bonds;  
 6 –  $\text{CH}_2$ - groups, adjacent to group  $\text{COOH}$ ;  
 7 –  $\text{CH}_2$ -groups adjacent to double bonds; 8 – groups  $(\text{CH}_2)_n$ ;  
 9 – H atoms of double bonds of linoleic acid; 10 – all  $\text{CH}_3$ -groups

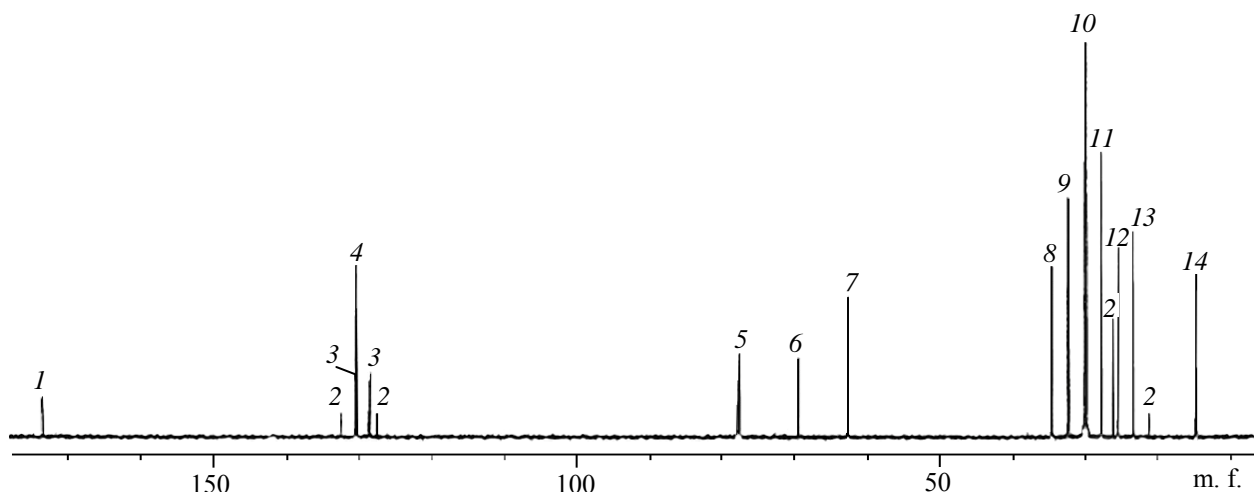


Fig. 2.  $^{13}\text{C}$  NMR spectrum of the solution of sunflower oil solution in  $\text{CDCl}_3$ :

- 1 – carboxyl groups; 2 – characteristic signal of linolenic acid;  
 3 – characteristic signal of linoleic acid; 4 – characteristic signal of oleic acid;  
 5 – characteristic signal of chloroform (solvent); 6 – CH-group of glycerin residues;  
 7 –  $\text{CH}_2$ - group of glycerin residues; 8 – atoms  $\text{C}_2$  of all acids;  
 9 – atoms  $\text{C}_{16}$  of stearic, linolenic, oleic acids; 10 – groups  $(\text{CH}_2)_n$  of all acids;  
 11 – atoms  $\text{C}_8$  of oleic, linoleic, linolenic acids; 12 – atoms  $\text{C}_3$  of all acids;  
 13 – atoms  $\text{C}_{17}$  of stearic, linolenic, oleic acids; 14 – atoms  $\text{C}_{18}$  of all acids

During the analysis of the spectra and chromatographic analysis about 30 fatty acids were identified, the total mass fraction ranging from 98.23 to 98.99%. The qualitative composition of oils for different samples is stable.

Tables 1 and 2 show the qualitative and quantitative composition of the fatty acid content of the studied oils and fatty acid composition in vegetable oils regulated by standard on the basis of the obtained data.

From the data we can conclude that vegetable oils contain both saturated and unsaturated fatty acids with 1 and 2 double bonds. It should be noted that among the investigated sunflower oils there were two samples (No. 1 and 3) and the quantitative content of oleic and linoleic acids doesn't not meet the standard regulated. Among the studied olive oils were also found quality falsification. Samples No. 1 and 2 don't meet requirements of standard.

Table 1

Fatty acid composition of sunflower oil (wt %)

Common name FA	No. 1	No. 2	No. 3	No. 4	No. 5	Standard
Myristinic	0.04	0.1	0.07	0.06	0.07	–
Palmitinic	13.24	6.24	11.53	6.17	6.8	3–7
Palmitoleic	0.95	0.2	0.8	0.1	0.11	–
Margarine	0.09	–	0.06	–	–	–
Stearic	2.76	2.98	3.01	3.25	0.02	1–3
Oleic	68.73	40.09	72.25	29.7	27.03	14–44
Vaccine	2.06	0.89	1.84	0.68	0.42	–
Linoleic	10.07	43.8	8.11	58.28	64.06	42–70
Peanut	0.7	0.37	1.08	0.22	0.19	–
Cis-11-akozanic	0.22	2.7	0.24	–	–	–
$\alpha$ -Linolenic	0.49	–	0.57	0.14	0.19	–
Behenic	0.07	0.53	0.08	0.66	0.65	–
Unidentified compounds	0.58	2.1	0.34	0.74	0.46	–

Table 2

**Fatty acid composition of olive oils (wt %)**

Common name FA	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Standard
Myristinic	0.08	0.07	0.01	0.02	0.01	0.01	0–1.5
Palmitinic	6.53	6.58	10.82	10.47	10.06	10.8	6–16
Palmitoleic	0.08	0.15	0.73	0.75	0.62	0.7	–
Margarine	–	0.04	–	0.07	0.07	0.06	–
Stearic	4.27	3.44	3.35	2.78	2.19	2.42	1–3.5
Oleic	22.68	32.02	74.33	74.48	76.68	74.91	62–85
Vaccine	0.23	0.73	1.37	1.89	1.82	1.87	–
Linoleic	64.09	53.96	7.45	5.87	5.24	6.04	3–15
Peanut	0.26	0.08	0.54	0.4	0.37	0.42	–
Cis-11-akozanic	0.07	0.17	0.5	0.25	0.24	0.27	–
$\alpha$ -Linolenic	–	0.1	–	0.63	0.49	0.63	–
Behenic	0.71	0.64	0.09	0.36	0.16	0.12	–
Arachidonic	–	–	0.21	0.7	0.5	0.66	–
Unidentified compounds	0.8	2.02	0.6	1.33	1.55	1.09	–

**Conclusion.** Thus, it was found that the production and use of plant oils and especially their mixtures must constantly monitor the fatty acid composition. The combination of spectroscopic and chromatographic methods allows detailing pattern analysis of fatty acid content in oils.

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