УДК 543.429.23 : 547.426.23

E. D. Skakovskiy[1], L. Yu. Tychinskaya[1], S. N. Shish[2], A. G. Shutova[2], S. A. Lamotkin[3], E. V. Kryvonosova[3]

[1]Institute of Physical Organic Chemistry of the National Academy of Sciences of Belarus
[2]Central Botanical Garden of the National Academy of Sciences of Belarus
[3]Belarusian State Technological University

NMR ANALYSIS OF CHLOROFORM EXTRACTS OF *NIGELLA* SEEDS

A comparative NMR analysis of chloroform extracts of three species of the *Nigella* family (*Nigella damascena* L., *Nigella sativa* L., *Nigella orientalis* L.) has been carried out and the influence of seeds grinding on the extract composition has been investigated. The seeds were obtained from plants cultivated on the experimental site of the Department of Plant Biochemistry and Biotechnology of the Central Botanical Garden NAS of Belarus.

Fatty acid composition of the extract was evaluated; linoleic and oleic acids are the most abundant among triglycerides. The presence of *p*-cymene and thymoquinone in the extracts was proven. A severe grinding of the seeds results in the extraction of additional compounds from the seed shell, and decomposition of triacylglycerols.

The difference of fatty acids composition of three different Nigella species has been shown. The specificity of *Nigella damascena* is the highest content of eicosadienoic acid in the seeds extracts, while *Nigella orientalis* contains the highest amount of linoleic acid. *Nigella sativa* is the leader in *p*-cymene and thymoquinone accumulation.

Key words: seeds, *Nigella sativa*, *Nigella damascena*, *Nigella orientalis*, nuclear magnetic resonance, chloroform extracts, fatty acids, thymoquinone.

Introduction. Plants of the genus Nigella (*Nigella* L.) is an annual herbaceous plant of buttercup family (*Ranunculaceae*), it is up to 0.7 metre height, it grows in Western Europe, Northern and Western Africa, Southeastern and Western Asia. The most common types are Nigella Damascus (*Nigella damascena* L.), cultivated Nigella (*Nigella sativa* L.), as well as the eastern Nigella (*Nigella orientalis* L.) [1].

Since ancient times, Nigella has been used as an important medicinal plant and for seasoning vegetables, legumes and various types of products for cooking [2]. In modern medicine a decoction of the seeds black cumin is used as antiflatulent, somnificient, alleviant remedy for toothache; it is used in the treatment of pancreatitis, hepatitis, cholecystitis. Extracts of black cumin seeds have hepatoprotective and antioxidant properties [3].

The antioxidant properties of black cumin protect the gastric mucosa from the damaging effects of alcohol and other aggressive agents [4]. It is believed that Nigella is a cure for all diseases except death [2]. The high biological activity of the plant is due to the presence therein oils. There are acylglycerides and essential oils in its different vegetative organs [2, 5].

Their composition has been studied mainly with chromatographic methods, which, despite their versatility, have several disadvantages. Firstly, different classes of compounds require different columns. Secondly, there is no distinguishing of the peaks in all cases for the compounds; and, thirdly, it is necessary to have compounds which are to be analyzed in the oils or the relevant database. Furthermore, in the case of fatty acids of triacylglycerides preliminary sample preparation is needed; it is obtaining of methylene ethers of these acids. In this regard, the NMR method has several advantages and it is increasingly being used for analysis of oils and acylglycerides.

The purpose of this work is comparative NMR analysis of chloroform extracts of different types of black cumin seeds, as well as to study the influence of different types of grinding the seeds before extracting the composition components.

Main part. *N. orientalis*, *N. damascena* and *N. sativa* were taken for the analysis of seeds. They were obtained from plants grown in 2014 cultivated on the experimental plot of the Department of Plant Biochemistry and Biotechnology of the Central Botanical Garden NAS of Belarus. Before extraction the seeds were ground in one of the two ways. In the first one they are triturated in an agate mortar, the second one is the seeds were ground in a coffee grinder for 90 seconds. At the same time the samples were significantly heated. Then, 50 mg of ground seeds filled with 1 ml of deuterated chloroform ($CDCI_3$) and were kept in a covered container for 12 hours.

Before recording the NMR spectra of the solutions were filtered. Extracts were prepared and spectra were recorded for several samples of each type. For the quantitative determination of oil extraction is performed with 1 g of seed. The oil content in seeds *N. orientalis* was 17.0%, *N. damascena* – 13.4% and *N. sativa* – 15.1%. The relative measurement error was 5%.

NMR spectra of the $CHCI_3$ solutions were recorded with AVANCE-500 spectrometer (Bruker) with an operating frequency of 500 and 126 MHz for ^{1}H and ^{13}C nuclei, respectively.

Recording was carried out at a temperature of 293 K in 5 mm standard ampoules. The accumulation of signals for proton spectra was carried out for 10 min and carbon – for 12 hours. As an internal standard in the case of IP-cores ^{1}H signal $CNCI_3$ (admixture amount in $CDCI_3$, δ = 7.27 ppm) was used. For ^{13}C nuclei dissolve signal is (δ = 77.7 ppm). All the experimental data were obtained and processed with the software package XWIN – NMR 3.5.

Fig. 1 shows the NMR spectra of the chloroform extract of black cumin seed sowing (first grinding method): In the ^{1}H, ^{13}C used in the ^{13}C (the area of the double bonds).

Apart from these signals belonging to protons of triglycerides, there are signals of other compounds. The presence of *p*-cymene is identified, its signals are δSN (aromatic) = 7.12; 7.13 ppm, Methine proton is at 2.88 ppm, and protons and methyl groups with chemical shifts at 2.33 and 1.2 ppm. Apparently, this compound has been extracted from the seed coat. In addition to p-cymene there is a significant amount of thymoquinone in the extrat; there are olefin protons at 6.51 and 6.58 ppm, methine proton is at 3.01 ppm, and methyl protons are at 1.12; 2.03 ppm.

It should be noted that thymoquinone is capable of inhibiting oxidation processes in leukocytes and membrane lipids, it also possesses anticonvulsant effect.

Proton spectra of extracts of black cumin seeds and eastern Damascus are considered to be in a similar range. However, *p*-cymene and thymoquinone are not found in considerable quantities.

According to the identified compounds carbon spectrum extract of *N. sativa* correlates well with the proton spectrum. Thus, the carbon atoms of the carboxyl groups absorb triacylglycerides at 173.53 and 173.54 ppm, In the area 128–131 ppm there are resonances of olefinic C atoms, methine carbon in the glycerol area absorbs at 69.57 ppm, and the carbon atoms of the CH_2 groups – at 62.79 ppm, methylene carbon aliphatic chains of fatty acids resonate at 23–38 ppm, and the methyl carbons – at 14.76 ppm Furthermore, there is absorption of *p*-cymene: aromatic hydrocarbons – at 126.95; 129.63; 135.79 and 146.52 ppm, methine carbon – at 34.40 ppm, and the methyl carbons – at 21.64 and 24.80 ppm. From the signals of thymoquinone in this case it is clearly observed absorption of carbon atoms of methyl groups at 22.06 ppm.

Using ^{13}C (Fig. 1, *c*) one can estimate the relative amount of unsaturated fatty acids as well as their distribution in triacylglycerids. Thus, olefinic C-atoms of fatty acids have the following chemical shifts: oleic – 130.36; 130.39; 130.69; 130.71 ppm, linoleic – 128.57; 128.58; 128.75; 128.77; 130.67; 130.69; 130.91 ppm. All signals are doublets (excluding the last one), the difference in the chemical shifts is due to the point of attaching acid residues to the end or central hydroxyl groups of glycerol. A comparison of the integrated intensities of these lines in the doublets helps to evaluate the preferred acid residues to glycerol. If it is not available, the ratio is 2 : 1.

It should be noted that there is a significant amount of olefin carbon in the absorption area with four singlets of the same intensity δ = 128.61; 128.66; 130.81 and 130.89 ppm. According to [2], they appear to be eicosadienoic acid (C 20 : 2, n 11, 13). As the observed lines do not have doublet structure, the mentioned acid extract is likely to be present in free form, not being a part of the triacylglycerides.

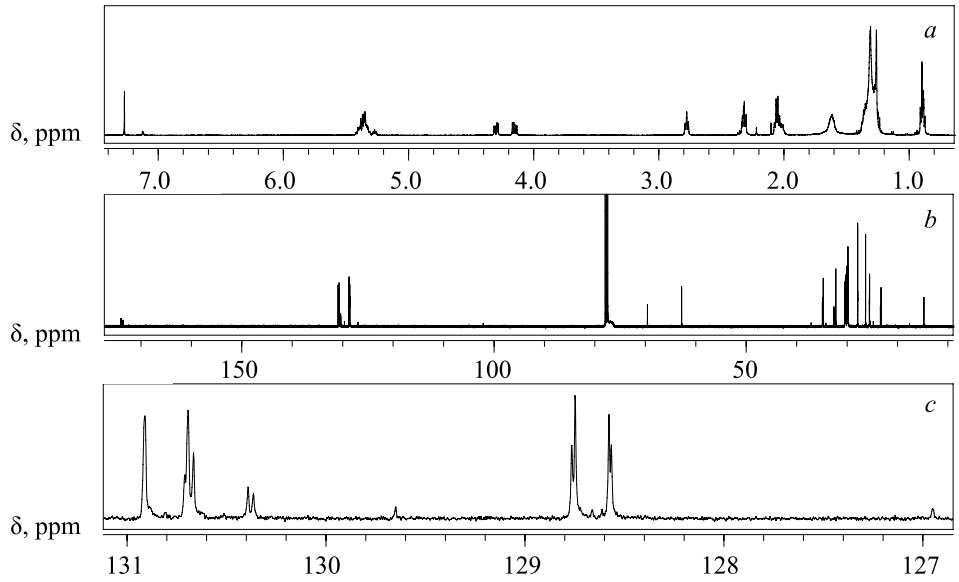

Fig. 1. NMR spectra $CDCl_3$-black cumin seed sowing seed extract:
a – ^{1}H; *b* – ^{13}C; *c* – ^{13}C (the area of double bonds)

The content of substances, expressed in mole percent, is given in Table 1.

Table 1

The components contents in the chloroform extract of seeds of various species of black cumin (%)

Compound	*N. orientalis*	*N. damascena*	*N. sativa*
Linoleic acid	60.7	50.8	53.4
Oleic acid	12.4	33	20.9
Eicosadienoic acid	3.9	4.2	2.1
Saturated acids	20.2	6.3	7.7
p-cymene	–	1.6	8.5
Thymoquinone	–	–	4.3

Table 1 shows that the highest number of linoleic acid contained in the extract of black cumin eastern (60.7%), but if we consider only triacylglycerides, then this indicator (the content of linoleic acid in fats) is higher in the seeding Nigella exceeding the eastern one (61.6%). It is believed that the most useful acid out of the list is eicosadienoic acid. It is present in the Nigella Damascus the most.

It should be noted that *p*-cymene is found in considerable quantities as well as triacylglycerides. In the *N. sativa* it is 8.5%. In the thymoquinone – 4.3%.

Table 2 shows the ratio of linoleic and oleic acids, attached to glycerin lateral hydroxyl groups to the content of these acids attached to the central hydroxyl group of glycerin.

These figures show that both unsaturated acids prefer to be in the central position of glycerin. This is especially true for oleic acid. Therefore, unsaturated acids attach mostly to lateral hydroxyl groups.

NMR spectra of the chloroform extract of the black cumin seed ground in a coffee grinder are shown in Fig. 2.

Table 2

The ratio of unsaturated fatty acids that are attached to the side hydroxyl groups of glycerol, identical to the contents of acids, attached to the central hydroxyl group of glycerol from various oil seeds of the black cumin seed

Acids	*N. orientalis*	*N. damascena*	*N. sativa*
Linoleic acid	1.54	1.48	1.62
Oleic acid	1.20	1.23	1.22

It should be noted that the proton spectra (Fig. 1, *a* and 2, *a*) of different extracts greatly differ. So, in the spectrum of seed extract, ground in a coffee grinder, along with the considered ones, there are many additional lines that can logically be attributed to the connecting lines efficiently extracted from the seed coat. In the absorption of glyceryl protons new lines are observed, witnessing the decomposition of triacylglycerides and formation of diacylglycerides. Moreover, the integral intensity of methylene protons (δ = 2.78 ppm), eicosadienoic and linoleic acids decreased due to either polymerization or to oxidation processes.

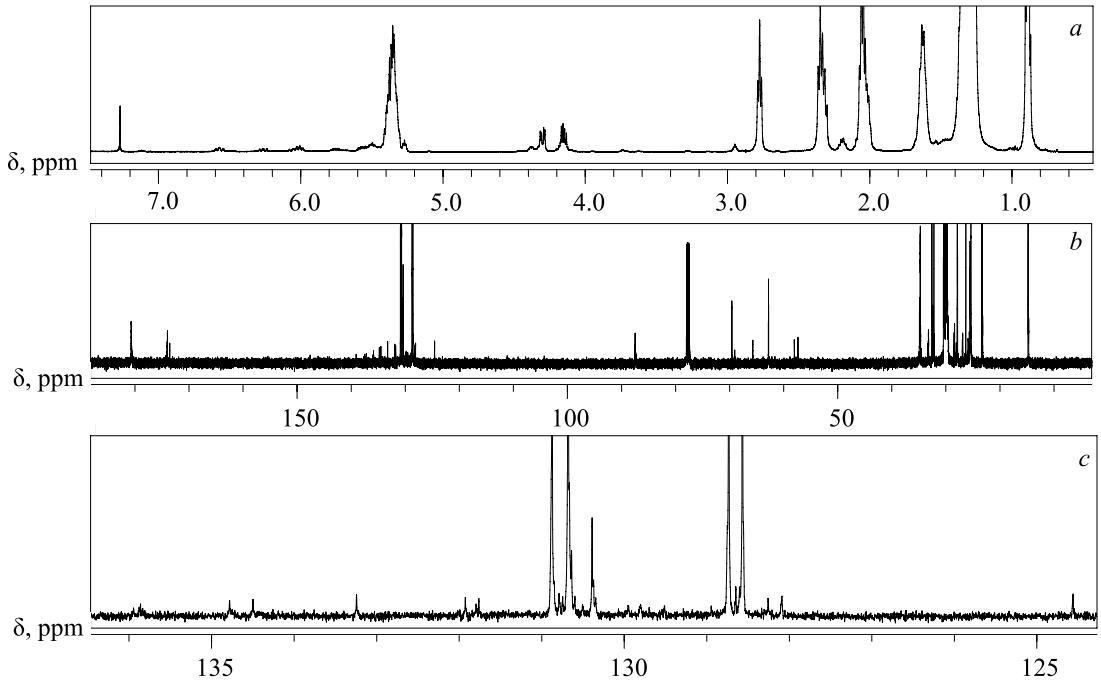

Fig. 2. NMR spectra $CDCl_3$-extract of the black cumin seed (second method of grinding): *a* – ^1H; *b* – ^{13}C; *c* – ^{13}C (area of double bonds)

The carbon spectrum (Fig. 2, *b*) correlates well with the proton one. Here, with δ = 180.68 ppm there is an intensive singlet belonging to the carboxyl group of free fatty acids. In the glycerol carbons there are two lines δSN = 68.99 ppm and δSN2 = 65.7 ppm, corresponding to Sn-1.3-diacylglycerol in an amount of ~11% of the content of triacylglycerols [6]. Sn-1.2-diacylglycerol are found in appreciable amounts. Analysis of the olefinic portion of the spectrum (Fig. 2, *c*) shows that it is markedly different from that (Fig. 1, *c*) of the first extract. This is manifested in the fact that the signals in this case are predominantly singlets, in the previous case – doublets. Thus, along with the additional signals after thoroughly grinding seeds that are associated with a better extraction of substances from seed shells, degradation of triacylglycerides and formation of diacylglycerides and free fatty acids is observed. A similar phenomenon can be observed in the analysis of extracts of cereals and flour from them [7].

Conclusion. NMR analysis of chloroform extracts of seeds of various species of black cumin allowed us to establish their fatty acid composition, to detect the presence of *p*-cymene and thymoquinone. It is shown that linoleic and oleic acid in triacylglycerides molecules mainly occupy the central position. It is found that careful grinding of seeds leads to other substances extraction, apparently belonging to the shell, and to the destruction of triacylglycerides.

References

1. Gogue D. O., Kholodova V. P., Kuznetsov V. V. Influence of the salinity stress on growth and some physiological characteristics of plants *Nigella* species. *Vestnik RUDN. Seriya agronomiya i zhivotnovodstvo* [Bulletin of People's Friendship University of Russia. Ser. Agriculture and Livestock], 2013, no. 2, pp. 12–19 (in Russian).

2. Aftab A. K., Mahesar S. A., Khaskheli A. R., Sherazi S. T. H., Sofia Q., Zaki K. Gas chromatographic coupled mass spectroscopic study of fatty acids conposicion of *Nigella sativa* L. (Kalonji) vil comercially available in Pakistann. *International Food Research Journal*, 2014, vol. 21, no. 4, pp. 1533–1537.

3. Lebeda A. F., Dzhurenko N. I., Isaykina A. P., Sobko V. G. *Lekarstvennye rasteniya* [Drug Plants]. Moscow, AST-Press Kniga Publ., 2004, 907 p.

4. Nurmagomedova P. M., Omarieva M. G. [Review articles. Properties of *Nigella sativa*] *Nigella sativa*. *Materialy II Mezhdunar. nauch. konf. (Meditsina i zdravookhranenie)* [Materials of the II International Scientific Conference (Medicine and Health)]. Ufa, 2014. pp. 62–65 (in Russian).

5. Gogue D. O., Sidorov R. A., Tsydendambiev V. D., Kholodova V. P., Kuznetsov V. V. Fatty acid lipid composition of vegetations organs of *Nigella* at different salinity of media. *Izv. RAN. Seriya biol*. [Proceedings of Russian Academy of Scienses. Series biol.], 2014, no. 3, pp. 264–270 (in Russian).

6. Sacchi R., Addeo F., Paolillo L. H and C NMR of Virgin Olive Oil. An Overview. *Magnetic Resonance in chemistry*, 1997, vol. 35, pp. 133–145.

7. Gaydukevich O. A., Skakovskiy E. D., Tychinskaya L. Yu., Kulakova A. N., Lamotkin S. A. Analysis of gramineous crops extracts and flour form them by NMR. *Trudy BGTU* [Proceedings of BSTU], series IV, Chemistry and Organic Substances Technology, 2007, issue XV, pp. 235–239 (in Russian).

Information about the authors

Skakovskiy Evgeniy Dominikovich – Ph. D. Chemistry, Head of the Laboratory of Physico-chemical Methods of Research. Institute of Physical Organic Chemistry of the National Academy of Scienses of Belarus (13, Surganova str., 220072, Minsk, Republic of Belarus). E-mail: sed@ifoch.bas-net.by

Tychinskaya Lyudmila Yul'yevna – Ph. D. Chemistry, leading researcher, Laboratory of Physico-chemical Methods of Research. Institute of Physical Organic Chemistry of the National Academy of Sciences of Belarus (13, Surganova str., 220072, Minsk, Republic of Belarus). E-mail: sed@ifoch.bas-net.by

Shish Svetlana Nikolaevna – graduate student, junior researcher, Department of Biochemistry and Plant Biotechnology. Central Botanical Garden of the National Academy of Sciences of Belarus (2b, Surganova str., 220012, Minsk, Republic of Belarus). E-mail: cazonovacv@mail.ru

Shutova Anna Genad'yevna – Ph. D. Biology, leading researcher. Central Botanical Garden of the National Academy of Sciences of Belarus (2b, Surganova str., 220012, Minsk, Republic of Belarus). E-mail: anna_shutova@mail.ru

Lamotkin Sergey Aleksandrovich – Ph. D. Engineering, associate professor, Department of Physical-chemical Methods of Products Certification. Belarusian State Technological University (13a, Sverdlova str., 220006, Minsk, Republic of Belarus). E-mail: jossby@rambler.ru

Kryvonosova Elena Valeryevna – senior lecturer, Department of Technical Translation and Media-communications. Belarusian State Technological University (13a, Sverdlova str., 220006, Minsk, Republic of Belarus). E-mail: ekrivonosova@tut.by

Received 20.02.2015