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DETERMINATION OF FATTY ACID PROFILE OF SUNFLOWER OIL SAMPLES BY NMR ^{1}H SPECTROSCOPY

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Abstract: *Sunflower oil with a high content of oleic acid (Omega-9) and a sufficiently low content of polyunsaturated linoleic acid (Omega-6) is characterized by a lower nutritional value but greater chemical stability at high temperatures and in the presence of oxidizing agents, therefore, it has several advantages for the food and chemical industries, and also as a raw material for the production of biofuels. The determination of the TAG composition of oil is very important, because due to selection there is a large number of sunflower varieties. The spectra of oil samples extracted from seeds of various sunflower varieties were investigated by NMR ^{1}H spectroscopy to determine fatty acids composition. This method based on estimation and comparison the proton integral intensities of allylic and bis-allylic CH_2 groups with intensity of glycerol protons that allows to determine the amounts of these unsaturated fatty acids. Each oil sample obtained has its individual TAG profile determining its physicochemical properties and nutritional value. Method ^{1}H-NMR spectroscopy is rapid and non-destructive, so it is perspective for determination of oil fatty acid composition.*

Keywords: *Sunflower oil, Fatty Acid, Oleic Acid, Linoleic Acid, NMR Spectroscopy, Fatty Acid Profile.*

INTRODUCTION

Recently, sunflower varieties with a high content of oleic acid (more than 82%) have been becoming more popular with producers, traders and agricultural processors. According to published data, the seeds of ordinary varieties of sunflower contain only 27-40% of this acid. Oil with a high content of oleic acid (Omega-9) and a relatively low content of linoleic acid (Omega-6) is characterized by less nutritional value, but more technological one, since it more chemically inert under high temperatures and in the presence of oxidants and can be stored almost four times longer saving its properties compared to oils with a lower percentage of oleates (Lee, 2000). Further, oils with a high content of oleates are perspective sources for obtaining biofuel (biodiesel) in regions where the cultivation of rapeseed is unprofitable (Holt, 2016). Sunflower oil with a high content of oleic acid has become a serious competitor to olive oil in the world market, since it is much cheaper and not inferior to olive oil in the chemical composition, and, moreover, in some cases surpasses it. The growing popularity of sunflower hybrids with a high content of oleic acid causes of an increase

in acreage for cultivation them. That contributes to cross-pollination of hybrid and non-hybrid of sunflower varieties, therefore the fatty-acid profile of sunflower oil depends not only on the plant species, but also on the region where they are grown. The fatty acid profile of oils is the major factor influencing their chemical and physical properties and subsequently their various applications (Atanasova, 2016). Thus, the issue of express method to determine the fatty-acid composition of sunflower oil to use sunflower raw materials rationally is acute on the agenda. Due to their chemical composition sunflower lipids are very susceptible to oxidative processes owing to their degree of unsaturation, giving rise to the development of off-flavour and a decrease of nutritional quality and safety. Especially the more unsaturated fatty acids with bis-allylic methylene groups are susceptible to oxidation. One of the express and non-destructive methods for determination of fatty acid profile and oxidation products is NMR ^{1}H spectroscopy providing a straightforward approach to quantitative analysis of oils and enabling a simultaneous detection of different oxidation products in one single analysis. The common unsaturated fatty acids such as oleic and linoleic, in an oil can be quantified using NMR ^{1}H. This method utilizes the area per proton (determined by integration) and gives equations for determining the amounts of the unsaturated fatty acids. Up to now, NMR is not widely used in food control and in food industry.

The aim of this work was to study the triacylglycerols composition of some sunflower oil samples by NMR ^{1}H spectroscopy. Samples of oil obtained from seeds of different sunflower varieties, NMR ^{1}H spectroscopy, computer program ADVASP Analyzer for processing spectra data, a method for quantitative comparison of the integrated intensities of typical signals of groups containing hydrogen atoms in NMR ^{1}H spectra. Deuterated chloroform ($CDCl_3$) that is probably the most commonly used solvent in NMR experiments on fatty compounds and was used here to obtain the spectra depicted. The spectra of prepared solutions were recorded on a Varian VXR-300 spectrometer.

EXPOSITION

Some of shown spectra may contain minor impurities, which are visible in the spectra. The impurities and their signals are not discussed. All spectra were obtained at 300 MHz. For sake of consistency, the integration value of the secondary CH_2 protons of glycerol moiety in the triacylglycerol molecule was chosen as reference in most spectra and assigned the value that is a multiple of 4.00. Usually, the spectral range of 0-6 ppm is shown since it covers the range of chemical shifts in most fatty compounds. Besides serving as solvent, $CDCl_3$ is also the reference material for chemical shift values with residual chloroform giving a peak at 7.29 ppm, although the exact shift values of the same peaks can vary slightly from spectrum to spectrum. In accordance with IUPAC nomenclature, carbon atoms are counted from the carbon carrying the carboxylic acid or ester group in the fatty acid chain, with this carbon being C1 (see Fig. 1 and Fig. 2) The presence of few quantity of phospholipids in the oil does not significantly affect the results of interpretation of spectra.

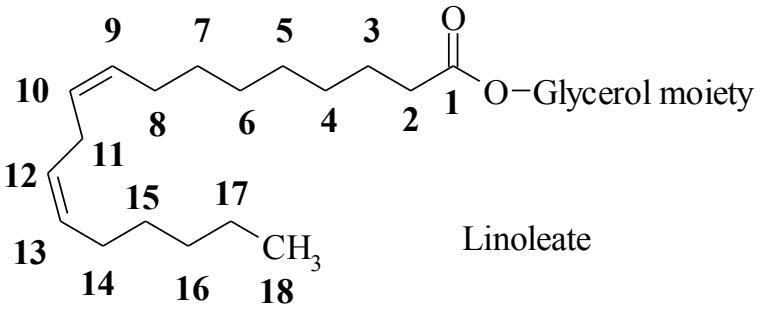

Fig. 1. General structure of linoleate

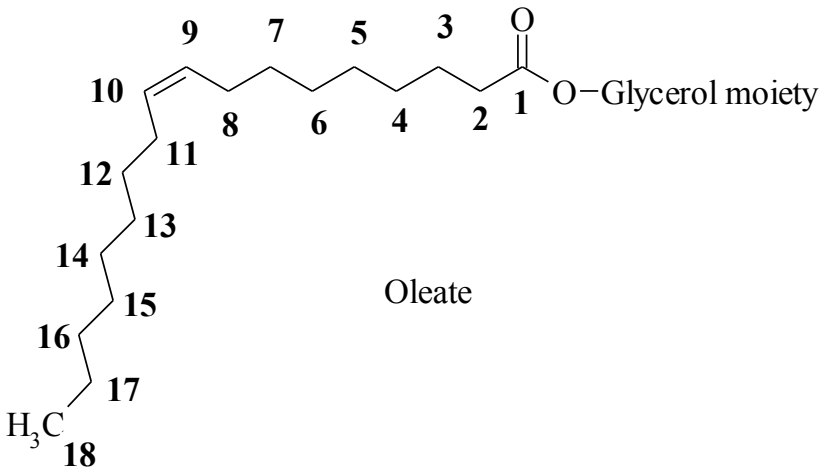

Fig. 2. General structure of oleate

Sunflower oil consists of 99.9% triacylglycerols and about 90% of them are represented by esters of oleic (Omega-9) and linoleic (Omega-6) acids. From consideration of NMR spectra in the literature various resonances can be assigned to specific chemical groups (see Table 1).

Table 1. Chemical shifts of protons in NMR spectra

Signal	Functional group	Chemical shift (ppm) of protons in oleic and linoleic esters of glycerin
1	$–CH_3$	0.96 – 0.82 (dd)
2	$–CH_2$-	1.43 – 1.16 (m)
3	$–CH_2$-C-CO_2	1.70 – 1.51 (m)
4	$–CH_2$-CO_2-	2.11 – 1.91 (m)
5	–C-CH_2-C=C-	3.38 – 2.21 (m)
6	–C=C-CH_2-C=C-	2.83 – 2.73 (t)
7	–C-CH_2-O-CO-C	4.21 – 4.08 (dd)
8	–C-CH_2-O-CO-C	4.36 – 4.22 (dd)
9	–CH(-C-O-CO-C-)$_2$ + C-HC=CH-C	5.43 – 5.13 (m)

Signal multiplicity: s, single; d, doublet; t, triplet; m, multiplet

The set of peaks at δ5.2 – 5.5 ppm arises largely from the ^{1}H nuclei attached to carbons involved in a double bond, usually referred to as olefinic. Signals at δ2.7 ppm arise from bis-allylic protons from the $–CH_2–$ group located between pairs of unsaturated bonds (see Fig. 3).

Peaks of allylic protons at C8, C11 for oleic acid C8, C14 for linoleic acid are located at about 2.05 ppm. In accordance with increasing in the percentage of linoleic acid in sunflower oil, the theoretical integrated values of the olefinic, allilyc and bis-allylic protons also increase while that of the high peak of CH2 group located at about 1.43 – 1.16 ppm decreases.

However, changes in the integrated intensity of proton signals at 1.43-1.16 ppm can not be used to estimate the number of CH_2 groups of fatty acid residues in the triacylgicerols, since signals of aliphatic solvents often used for extraction of oil from seeds are located in the same region.

To quantative determination unsaturated fragments of oleic and linoleic acid molecules , the integrated intensities of vinylic (H_v), allylic (H_a) and bis-allylic hydrogen (H_b) atoms in ^{1}H NMR spectra were used (see Fig. 3).

The ratio of the integral intensities of the signals of the hydrogen atoms of allylic (H_a), bis-allylic (H_b) groups and the tertiary hydrogen atom of the glycerol residue (H_g) is not a sufficient argument to make conclusions regarding the quantitative composition of fatty acids in sunflower oil, since the integrated intensities of allylic and bis-allylic protons can be significantly decreased

due to oxidation processes in the triacylglycerol molecules proceeding as a result of long-term storage of the oil. (Stephenson, 2005)

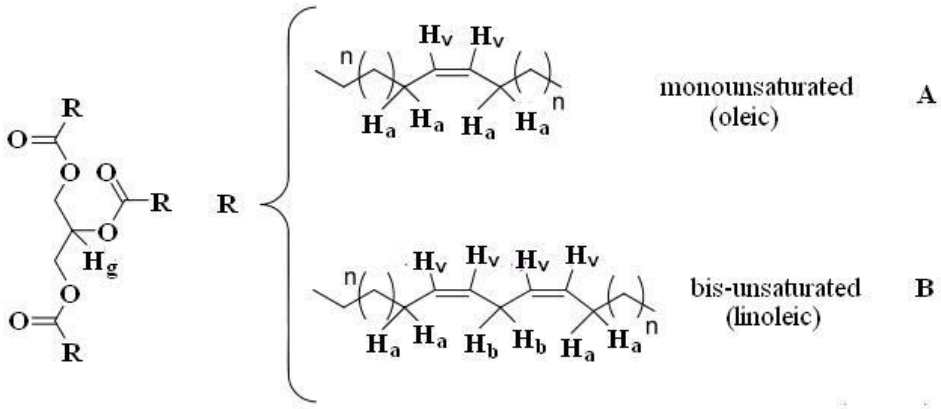

Fig. 3. General structure of triacylglycerides of sunflower oil.

Besides, in practice, it is often impossible to separate the signal of the tertiary hydrogen atom Hg of the glycerol residue from the signals of vinyl hydrogen Hv in the obtained spectra, that is, in the spectra the integrated signal intensity at 5.2 - 5.5 ppm depends on the total number of H_v and H_g atoms.

The results of the monitoring fatty acid composition by NMR ^{1}H spectroscopy confirm a progressive decrease in the contribution of linoleic acid throughout the store period. As result of the oxidative processes, at first, the ratios of relative intensities between bis-allylic protons (H_b) to allylic protons (H_a) decreased. Finally, peaks of bis-allylmethylene protons (H_b) almost disappeared.

With increasing storage time, the process becomes more and more complicated since primary oxidative products undergo further reactions forming volatile and non-volatile secondary products. Thus, the very small peak of aldehyde group is observed at δ9.74 ppm that is in according to literature (Wong, 2017).

Therefore, to determine the quantitative ratio of oleic and linoleic acids, it is necessary to compare the integrated intensities of the signals H_a at 1.95-2.07 ppm and H_b at about 2.75-2.85 ppm between themselves, relative to the secondary hydrogen atoms of the glycerol fragment at 4.1-4.3 ppm and the signals at 5.2 - 5.5 ppm, corresponding to the Hv and Hg atoms, taking into account the contribution of hydrogen atoms of each acyl residue (Vlahov, 1999).

It should be taken into account that the integral intensities of the hydrogen atoms of the CH_2 groups of the glycerol fragment and allylic group must be a multiple of four and the bis-allylic group must be multiple of two.

The NMR ^{1}H spectrum of the high-oleic oil obtained from sunflower seeds provided by Institute of Oilseed Crops of the Ukrainian Academy of Agricultural Sciences (IOC UAAS) is shown on Fig. 4. The spectrum of this sample demonstrates the presence of signals mainly oleates, and percentage of them is almost 95%. The content of linoleic acid detected by very weak typical signals at about 2.75-2.85 ppm does not exceed 1%.

In the spectrum of the sunflower oil sample № 2 (Fig.5), beside signals of oleic acid, peaks of polyunsaturated linoleic acid in the region of 2.75-2.85 ppm are identified exactly. The ratio of oleic and linoleic acids in this oil sample is 9:1, that is, the mass fraction of oleates reaches almost 89%, and of the polyunsaturated acids do not exceed 11%.

The ^{1}H NMR spectrum of the sunflower oil sample № 3 (see Fig. 6), obtained from sunflower seeds purchased in local markets, shows that the ratio of oleic and linoleic acids is 1: 2, that is, the oleic acid content in that sample is about 32%.

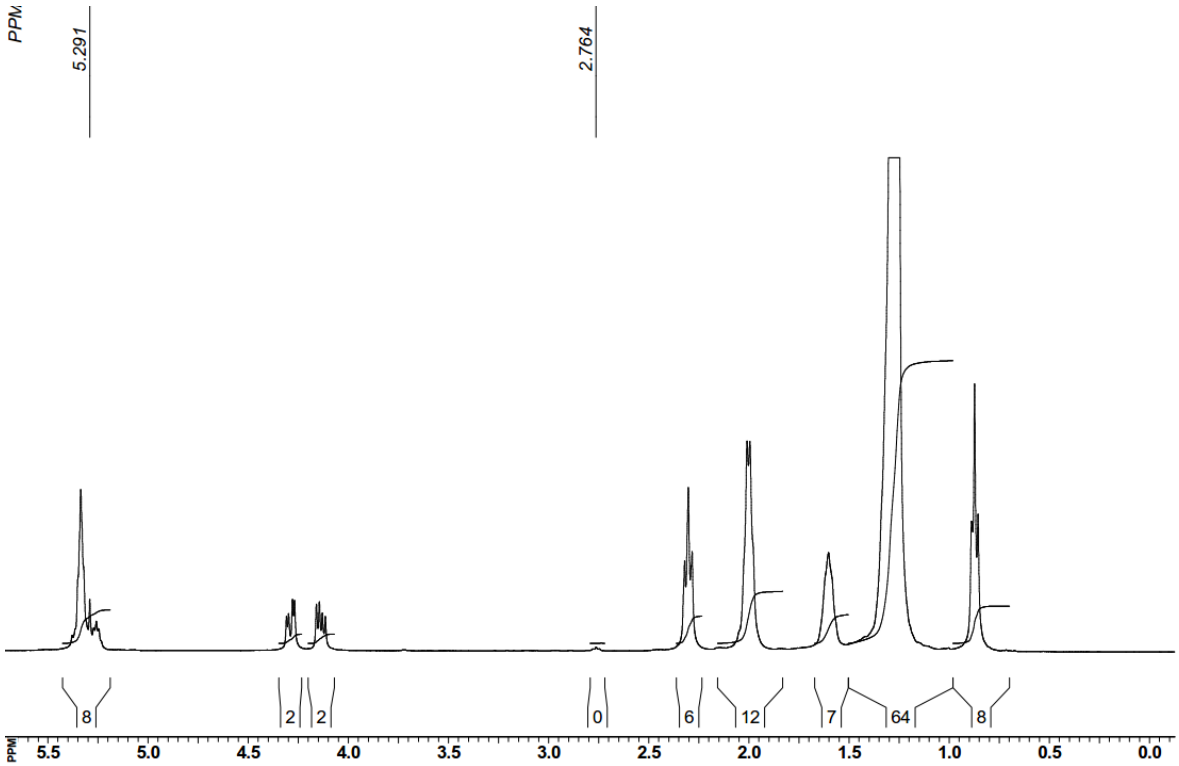

Fig. 4. NMR ^{1}H spectrum of high-oleic sunflower oil (sample №1)

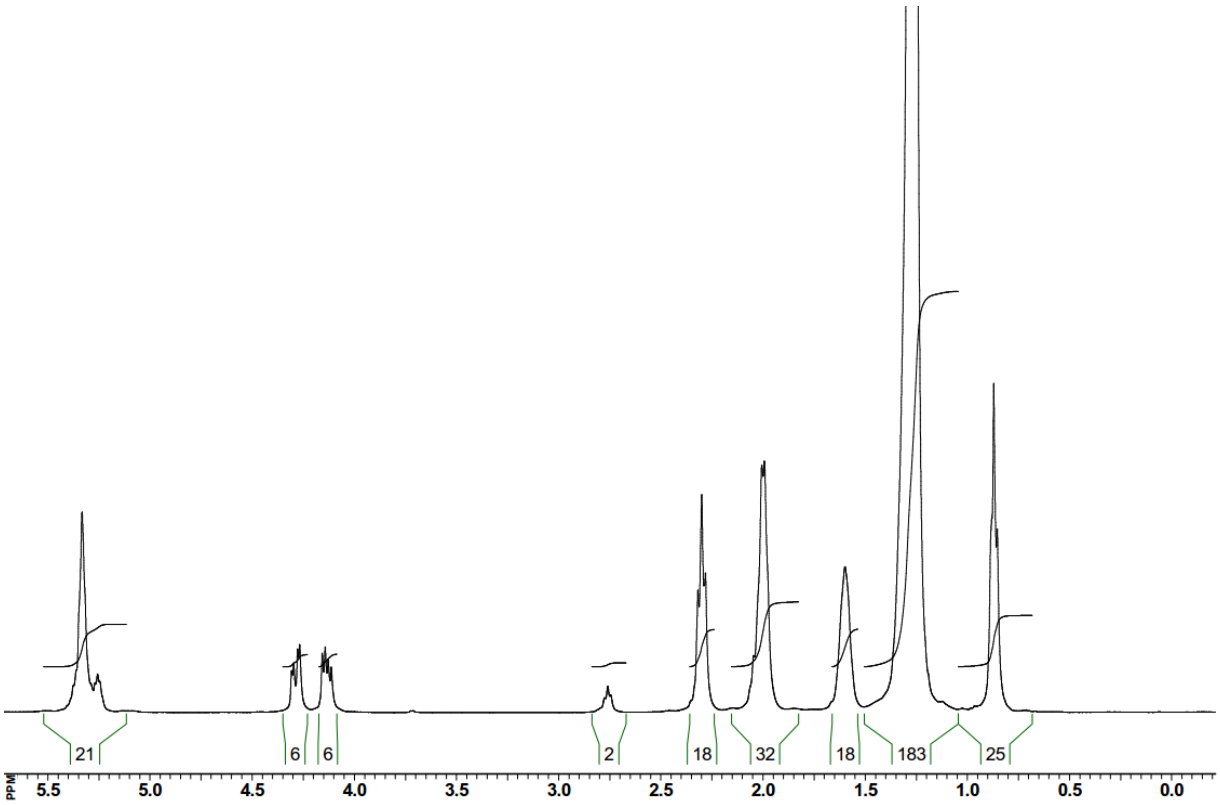

Fig. 5. NMR ^{1}H spectrum of the sunflower oil (sample № 2)

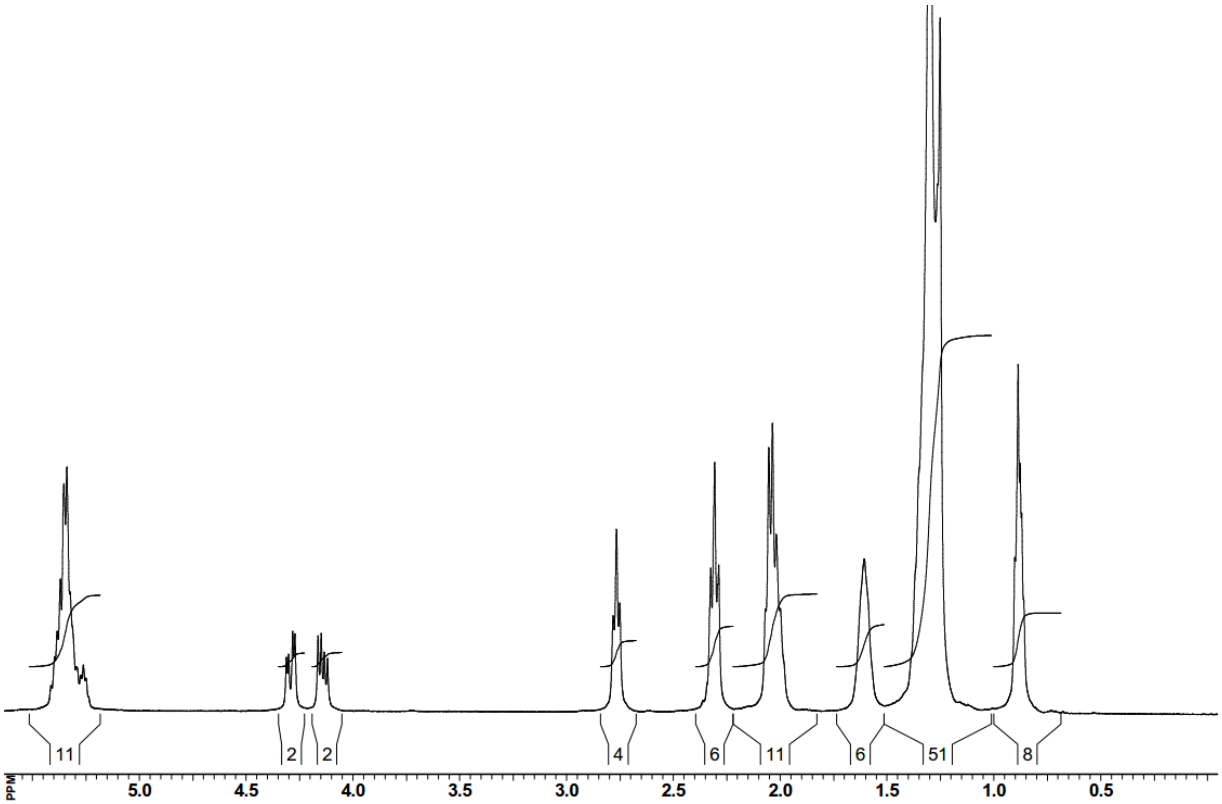

Fig. 6. NMR ^{1}H spectrum of the sunflower oil obtained from seeds bought in local markets (sample № 3)

CONCLUSION

The non-destructive and express method of NMR ^{1}H spectroscopy is a perspective tool for the quantitative determination of oleic and linoleic acids content in sunflower oil, that, under modern conditions of agricultural cultivation is characterized by a non-permanent fatty-acid composition, to aim of determination of the most optimal course for use of seed raw materials. NMR ^{1}H spectroscopy may be used as express and non-destructive method of estimation of sunflower oil quality.

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