

<sup>1</sup>Maryna LUTSENKO, <sup>2</sup>Viktoriia KALYNA, <sup>2</sup>Mykola KHARYTONOV,  
<sup>2</sup>Svitlana LEMISHKO, <sup>3</sup>Ana–Maria TĂBĂRAȘU

## ESTIMATION OF THE THERMAL EFFECT OF SUNFLOWER OIL PHOSPHATIDE CONCENTRATE DEGREASING PROCESS WITH ISOPROPYL ALCOHOL ON THE INACTIVATION OF PHOSPHOLIPID

<sup>1</sup>Luhansk Taras Shevchenko National University, Kovalia str. 3, Poltava, UKRAINE;

<sup>2</sup>Dnipro State Agrarian and Economic University, Serhiya Yefremova str. 25, Dnipro, UKRAINE;

<sup>3</sup>National Institute of Research – Development for Machines and Installations Designed to Agriculture and Food Industry – INMA Bucharest, ROMANIA

**Abstract:** The characteristic absorption bands in the IR spectra of sunflower oil phospholipids were studied under various temperature regimes of degreasing. The most active chemical centers in the phospholipid molecule are ethylene bonds (isolated and conjugated), amino groups, phosphate groups, methylene groups, especially in the  $\alpha$ -position to the double bond, and hydroxyl groups. The activity of P–O–groups and NH–groups increases at temperatures close to 80°C. The above information confirmed that the degreasing of the phosphatide concentrate in the temperature range of 50–70°C does not inactivate phospholipids. However, the degreasing rate of the phosphatide concentrate in this range already become high.

**Keywords:** phosphatide, phospholipide, thermal effect, degreasing, infrared spectra, absorption bands

### INTRODUCTION

Vegetable oils are complex mixtures of physiologically active substances depending on the species to which the oil belongs (Sukkasem *et al.*, 2015; Haque, Bubli, & Khan, 2021, Liang *et al.*, 2012). The main sources of phospholipids are vegetable oils. Phospholipids (PLs) are important biomolecules that play a vital role in cell biochemistry and physiology, constitute structural building blocks and organelle membranes (Parchem *et al.*, 2019). Phospholipids turn out to be nonionic surfactants, good emulsifiers (lecithin), antioxidants, liquid viscosity reducers, etc (Sagdullaeva *et al.*, 2021). The solvent usually used when the raw materials are oilseeds is hexane.

Due to its toxicity and flammability (Brevedan *et al.*, 2000). Alcohol extraction can be used as an alternative extraction solvent with less handling risks than hexane (Baümler *et al.*, 2016). It is known, that the solubility of lipids in ethanol is changed by the extraction temperature (Sineiro *et al.*, 1998). There several case studies focusing on the analysis of the total phospholipid content in vegetable oil samples by FTIR (Abramson *et al.*, 1971; Mendelsohn *et al.*, 1998; Illijas *et al.*, 2009).

The concentration range in which lecithin is present in dietary supplements make Fourier transform infrared (FTIR) spectrometry an appropriate approach for the quantification of these analytes in this type of samples (Kuligowski *et al.*, 2008).

During last four decades the FTIR (Fourier transform infrared spectroscopy) use as

analytical tool for simple and rapid quantitative determination of phospholipids in vegetable oil (Arrondo *et al.*, 1989; Nzai and Proctor, 1998). The optimal phospholipid absorption bands between 1200–970  $\text{cm}^{-1}$  were identified and used for quantitative determination. Analysis of 44 kinds of the vegetable oils and their two–component blends was carried out by refractometry and infrared spectrometry (Mukhametov *et al.*, 2022). The expressiveness of complex mixtures of physiologically active substances was connected with certain spectral bands responsible for vibrations of double C–bonds (at 1653  $\text{cm}^{-1}$ ), for valence and deformation–type vibrations in methyl C–H groups (at 3008  $\text{cm}^{-1}$  and 722 bands), for symmetric and asymmetric vibrations in  $\text{CH}_2$  groups (at 2923  $\text{cm}^{-1}$  as well as 2853).

The band from 1200–970  $\text{cm}^{-1}$  (due to P–O–C +  $\text{PO}_2$ ) was found to be extremely useful in the quantitative determination of phospholipids. It was established later, that different specific spectral regions can be distinguished (Brubach *et al.*, 2005; Garczarek and Gerwert, 2006). IR continua have been described involving hydrogen bonding features (Hielscher *et al.*, 2011).

This continuum position and broadness is seen at about 400 to 0  $\text{cm}^{-1}$  depending on the polarizability of the hydrogen bonding features. The three domains included the significant signals (600 – 480  $\text{cm}^{-1}$ , 460 –180  $\text{cm}^{-1}$  and below 300  $\text{cm}^{-1}$ ) which originates the head group domain, the vibrations of the torsion motions of

the hydrocarbon chains, the skeleton vibration of lipids and the broad hydrogen bonding signature (Hielscher and Hellwig, 2012).

The main objective of our case study was to study the thermal effects of degreasing the phosphatide concentrate with isopropyl alcohol on inactivation of phospholipids in the temperature range of 50–80°C.

### MATERIALS AND METHODS

Structural features of defatted phospholipids and their qualitative composition were studied by IR spectroscopy (Kuligowski et al, 2008). The absorption bands of =CH bonds in the cis-configuration to the double bond (3030 cm<sup>-1</sup>), C=C non-conjugated double bonds (1600 cm<sup>-1</sup>), OH- groups in alcohols, acids ( $\nu = 3400 \text{ cm}^{-1}$ ) were chosen as analytical bands.

Semi-quantitative evaluation of measurements in the spectra of food lecithin samples was carried out by the optical density intensity of the indicated bands, related to the absorption of the most intense band ( $\nu = 1170 \text{ cm}^{-1}$ ) of the triplet, characteristic of the glyceride structure in the spectra of food lecithin. The process of degreasing the phosphatide concentrate with isopropyl alcohol was carried out in the temperature range of 50–80°C.

This means that phospholipids are exposed to temperature and can be partially inactivated with some loss of their biological activity and emulsifying properties. The method of IR spectroscopy of phospholipid samples obtained under various temperature conditions of degreasing was used to study the thermal effect on their content. The most active chemical centers in the phospholipid molecule are ethylene bonds (isolated and conjugated), amino groups, phosphate groups, methylene (in the  $\alpha$ -position to the double bond) and hydroxyl groups.

The Fourier transform infrared analysis indicated the characteristic peak at 1,734/cm<sup>-1</sup> to see lecithin extrudates (Yang et al, 2016). The absorption bands corresponding to stretching vibrations of C=C non-conjugated in the cis configuration to the double bond ( $\nu = 3040\text{--}3010 \text{ cm}^{-1}$  and  $\nu = 1680\text{--}1620 \text{ cm}^{-1}$ ) were chosen as analytical bands. Stretching vibrations of methylene (CH<sub>2</sub>) groups are fixed at  $\nu = 2853 \text{ cm}^{-1}$ , bending vibrations of CH<sub>2</sub> groups – at  $\nu = 1465 \pm 10 \text{ cm}^{-1}$ , absorption bands due to bending and stretching vibrations of NH groups are sought in the ranges  $\nu = 1650\text{--}1590 \text{ cm}^{-1}$  and ( $\nu = 3500\text{--}3300 \text{ cm}^{-1}$ ).

Absorption bands due to stretching vibrations of free P=O groups (1300–1200 cm<sup>-1</sup>) were selected for the phosphate group. Stretching vibrations P–O–C are evaluated at  $\nu = 1070\text{--}1050 \text{ cm}^{-1}$  groups, P–O groups ( $\nu = 1040\text{--}1000 \text{ cm}^{-1}$ ) and groups –ROOH at  $\nu = 1240\text{--}1130 \text{ cm}^{-1}$ ) in R – O – RON=O.

### RESULTS

The characteristic absorption bands in the IR spectra of sunflower oil phospholipids were studied under various temperature regimes of degreasing. The most active chemical centers in the phospholipid molecule are ethylene bonds (isolated and conjugated), amino groups, phosphate groups, methylene groups, especially in the  $\alpha$ -position to the double bond, and hydroxyl groups.

A semi-quantitative assessment of changes in the spectra of phospholipids was carried out by changing the intensity of the indicated absorption bands related to the intensity (optical density) of the band ( $\nu = 1170 \text{ cm}^{-1}$ ) of the triplet characteristic of the glyceride structure in the spectra of each of the phospholipids. Significant changes during heating of phospholipids occur primarily in the position of the C=C absorption bands – the ethylene bond. Ethylene centers can be detected by absorption in four regions of the infrared spectrum:

- the region 3000–3100 cm<sup>-1</sup> refers to stretching vibrations of CH-groups in –CH=CH–;
- region 1585–1680 cm<sup>-1</sup> – absorption caused by stretching vibrations of the double bond itself;
- region 680–1000 cm<sup>-1</sup> – frequencies of non-planar deformation vibrations C–H;
- part of the spectrum near 1435 cm<sup>-1</sup> – absorption of the neighboring methylene group.

The change in the number of non-conjugated and conjugated ethylene bonds in the temperature range of 50–80°C is shown in fig. 1 and 2.

The decrease in the number of isolated ethylene bonds and the growth of conjugated dienes occurs with an increase in the temperature of degreasing the phosphatide concentrate with isopropyl alcohol. This process is already noticeable at temperatures above 70°C.

The fact of the formation of conjugated dienes should be explained by the positional isomerization of isolated double bonds in the fragment of acylalinoic acid (–C<sub>13</sub>H=C<sub>12</sub>H–C<sub>11</sub>H<sub>2</sub>–C<sub>10</sub>H=C<sub>9</sub>H–). Hydrogen of the  $\alpha$ -methylene group, located between two double bonds, is chemically mobile and under certain conditions

is easily cleaved off, forming a radical, leading to the appearance of several resonance forms.

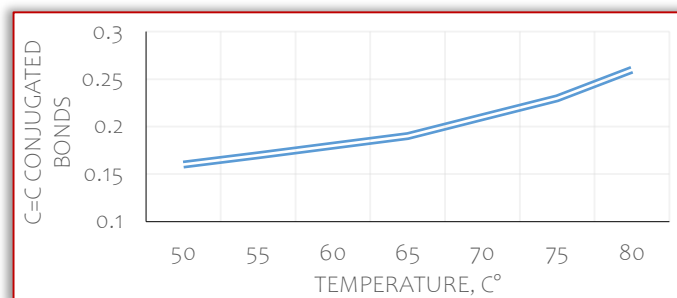
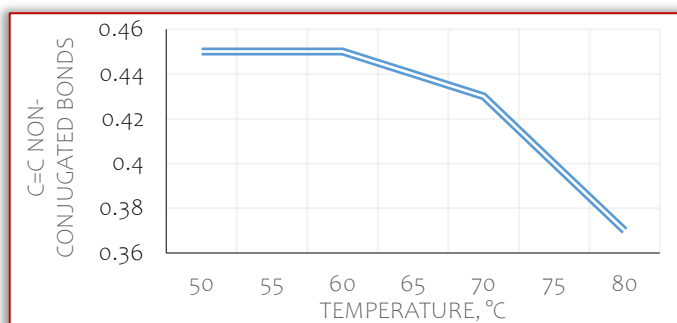


Figure 1 – Change in the number of non-conjugated and conjugated C=C bonds, (conventional units, c.u.)

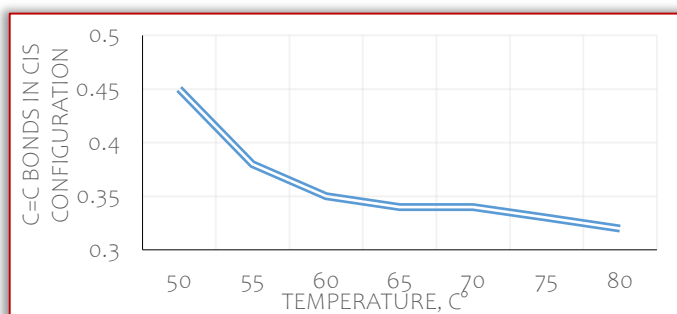


Figure 2 – Change in the number of C=C bonds in the cis-configuration (c.u.)

The resonant forms of radicals IV turned out to be the most thermodynamically favorable and require energy expenditure for their formation of only 78.5 kJ/mol compared to the resonant form II (126 kJ / mol), which, in turn, leads to the preferential formation of conjugated dienes according to the type of resonance form IV. The process of positional isomerization is also confirmed by the analysis of the chemical parameters of phospholipid samples obtained at different temperatures (Fig. 3 and 4).

The presented experimental data show that significant changes in the accumulation of conjugated dienes occur at temperatures close to 80°C. At the same time, iodine numbers, which characterize the presence of unsaturated bonds, practically do not change over the studied temperature range.

The number of  $\alpha$ -methylene groups decreases significantly at temperatures close to 80°C. This can be explained as follows: the number of  $\alpha$ -methylene groups in the acylalinoic acid

moiety  $-C^1H_2-CH=CH-C^2H_2-CH=CH-C^3H_2-$  three, and after positional isomerization, only two remain  $-C^1H_2-CH=CH-CH=CH-C^2H_2-$ . Trans-isomers absorbing in the region  $\nu = 3040\text{ cm}^{-1}$  (CH-valence),  $\nu = 970-960\text{ cm}^{-1}$  (CH-out-of-plane) and  $\nu=1310-1295\text{ cm}^{-1}$  (CH-plane deformation), in all samples of phospholipids obtained in the temperature range of 50–80°C were not detected.

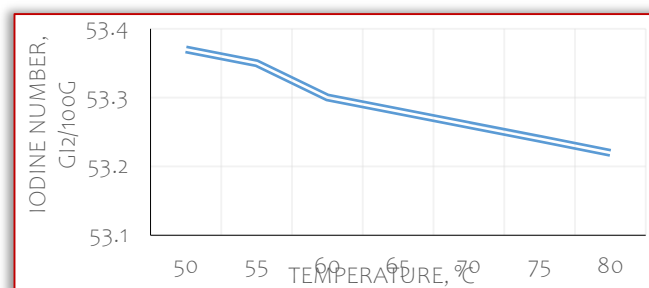
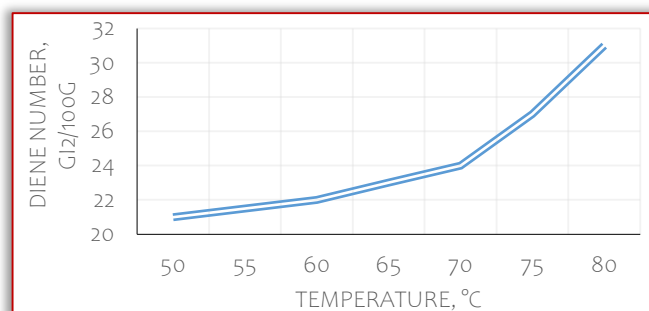


Figure 3 – Change in diene and iodine numbers and the number of  $\alpha$ -methylene groups at different decreasing temperatures

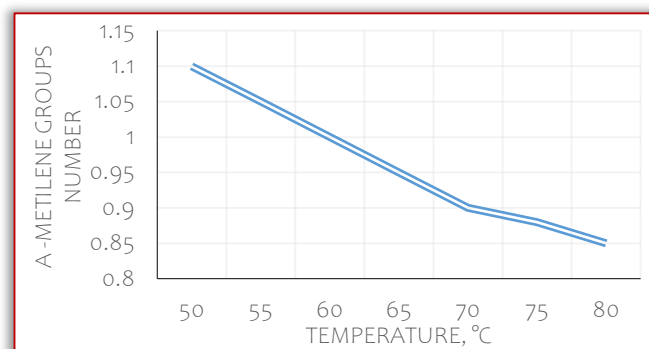


Figure 4 – Change in the number of  $\alpha$ -methylene groups at different decreasing temperatures

An increase in the intensity of the absorption band of the NH group ( $\nu=1640\text{ cm}^{-1}$ ) is observed in phospholipids isolated during degreasing at a temperature close to 80°C together with a decrease in the intensity of this band at a wavelength of  $\nu = 3350\text{ cm}^{-1}$  (Fig.5).

Consequently, the number of amino groups involved in the hydrogen bond somewhat decreases, and the number of free NH-groups increases, especially at the degreasing temperature of the phosphatide concentrate of 80°C.

The amounts of the indicated groups of the phosphate group (c.u.) during thermal action on phospholipids are shown in Fig. 6 – 8.

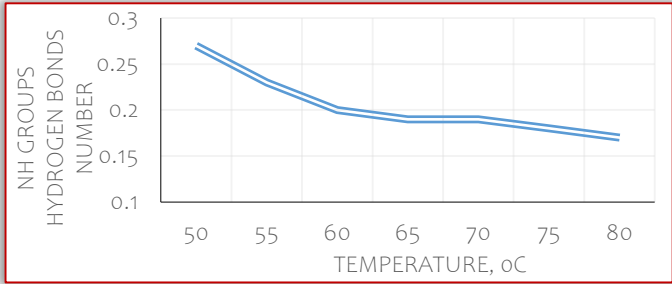
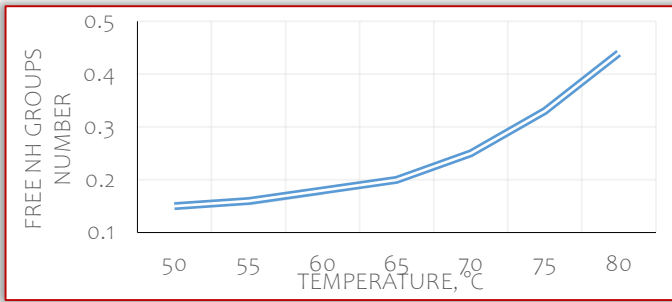


Figure 5 – Change in the intensity of the absorption bands of the NH group.

1 – the number of free NH groups (c.u.); 2 – is the number of NH groups involved in the hydrogen bond (c.u.).

Thus, the number of P–O– groups increases with an increase in the extraction temperature. This indicates an increase in polarization in phospholipid molecules.

The number of P–OH and P–O–C groups increases slightly with an increase in the extraction temperature, while the number of free and bound P=O decreases. It has been established that the thermal effect on phospholipids is directed primarily to the double bonds of acylalinoic acid.

The process of positional isomerization of isolated dienes into conjugated dienes becomes noticeable at temperatures close to 80°C. This process takes place at temperatures of 50 – 70°C. Meanwhile, the transition of isolated ethylene bonds into conjugated bonds is small.

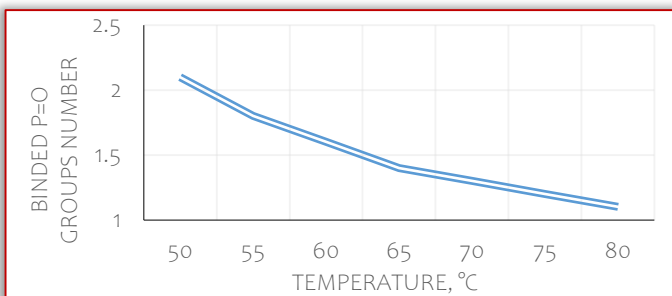
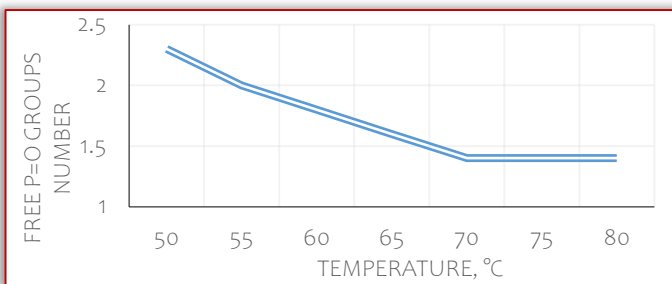


Figure 6 – Change in the number of groups of phospholipids in free P=O and bined P=O groups

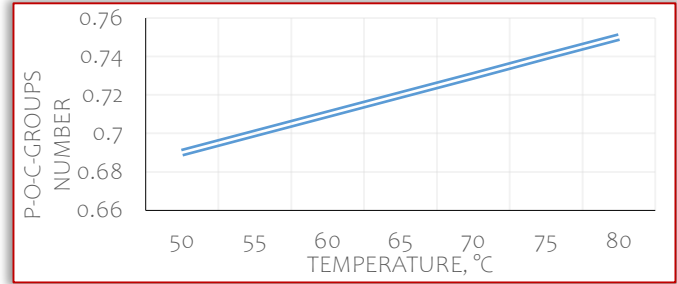
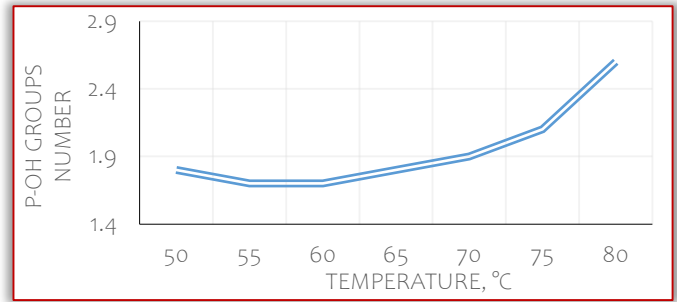


Figure 7 – Change in the number of groups in the phosphate group of phospholipids: – P–OH (c.u.) and – P–O–C

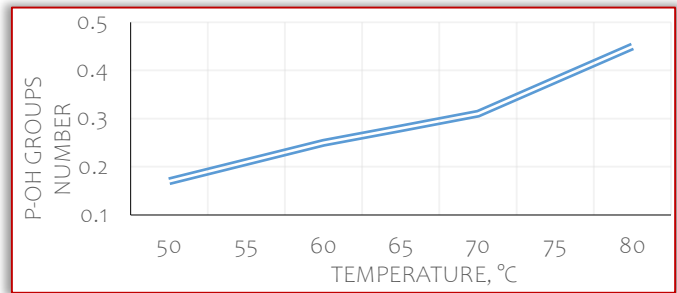


Figure 8 – Change in the number of groups in the phosphate group of phospholipids. P–O<sup>-</sup> (c.u.)

## CONCLUSIONS

The characteristic absorption bands in the IR spectra of sunflower oil phospholipids were studied under various temperature regimes of degreasing.

The most active chemical centers in the phospholipid molecule are ethylene bonds (isolated and conjugated), amino groups, phosphate groups, methylene groups, especially in the  $\alpha$ -position to the double bond, and hydroxyl groups.

The activity of P–O–groups and NH–groups increases at temperatures close to 80°C. This is due to ionization and an increase in charges on the nitrogen of the amino group and phosphorus in the phosphate group.

The above information allows concluding that the degreasing of the phosphatide concentrate in the temperature range of 50–70°C does not inactivate phospholipids. However, the degreasing rate of the phosphatide concentrate in this range already becomes high.

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## References

- [1] Brevedan, M.I., Carelli, A.A., & Crapiste, G.H. (2000). Changes in composition and quality of sunflower oils during extraction and degumming. *Grasas y Aceites*. 51(6), 417–423.  
<https://grasasyaceites.revistas.csic.es/index.php/grasasyaceites/article/view/460>
- [2] Bäumler, E.R., Carrín, M.E., & Carelli, A.A. (2017) Diffusion of tocopherols, phospholipids and sugars during oil extraction from sunflower collets using ethanol as solvent. *Journal of Food Engineering*, 194, 1–8
- [3] Brubach, J. B., Mermet, A., Filabozzi, A., Gerschel, A., & Roy, P. (2005). Signatures of the hydrogen bonding in the infrared bands of water, *Journal of Chemical Physics*, 122 (18), Article ID 184509, 1–7
- [4] Garczarek, F. and Gerwert, K. (2006). Functional waters in intraprotein proton transfer monitored by FTIR difference spectroscopy, *Nature*, 439 (7072), 109–112
- [5] Haque, F., Bubli, S. Y., & Khan, M. S. (2021). UV–Vis Spectroscopy for Food Analysis. In book: *Techniques to measure food safety and quality* edited by M. S. Khan & R. M. Shafiur, 169–193
- [6] Hielscher R., Friedrich T., & Hellwig P. (2011). Far- and mid-infrared spectroscopic analysis of the substrate-induced structural dynamics of respiratory complex I, *ChemPhysChem*, 12(1), 217–224
- [7] Hielscher, R., & Hellwig, P. (2012). Specific Far Infrared Spectroscopic Properties of Phospholipids. Hindawi Publishing Corporation *Spectroscopy. An International Journal*, 27(5–6), 525–532
- [8] Illijas, M.I., Indy, J.R., Yasui, H., & Itabashi, Y. (2009) Lipid Class and Fatty Acid Composition of a Little-known and Rarely Collected *Alga Exophyllum wentii* Weber–van Bosse from Bali Island, Indonesia. *Journal of Oleo Science*, 58(3), 103–110
- [9] Kuligowski, J., Quintás, G., Garrigues, S., & De la Guardia M. (2008). Determination of lecithin and soybean oil in dietary supplements using partial least squares–Fourier transform infrared spectroscopy. *Talanta*, 77, 229–234.
- [10] Liang, P., Wang, H., Chen, C., Ge, F., Liu, D., Li, S., Han, B., Xiong, X., & Zhao, S. (2012). The Use of Fourier Transform Infrared Spectroscopy for quantification of adulteration in Virgin walnut oil. *Journal of Spectroscopy*, 2013, Article ID 305604
- [11] Mukhametov, A., Mamayeva, L., Kazhymurat, A., Akhlan, T., & Yerbulekova, M. (2022) Study of vegetable oils and their blends using infrared reflectance spectroscopy and refractometry. *Food Chem X.*, 17(30)
- [12] Mendelsohn, R., Davies, M.A., Brauner, J.W., Schuster, H.F., & Dluhy, R.A. (1989) Quantitative determination of conformational disorder in the acyl chains of phospholipid bilayers by infrared spectroscopy. *Biochemistry*, 28(22), 8934–8939.
- [13] Mendelsohn, R., Davies, M.A., Schuster, H.F., Xu Z., & Bittman R. (1991). CD<sub>2</sub> rocking modes as quantitative infrared probes of one-, two-, and three-bond conformational disorder in dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylcholine/cholesterol mixtures. *Biochemistry*, 30(35), 8558–8563
- [14] Nzai, J.M., & Proctor, A. (1998). Determination of phospholipids in vegetable oil by fourier transform infrared spectroscopy. *J Amer Oil Chem Soc* 75, 1281–1289
- [15] Parchem, K., Sasson, S., Ferreri, C., & Bartoszek, A. (2019). Qualitative analysis of phospholipids and their oxidised derivatives – used techniques and examples of their applications related to lipidomic research and food analysis. *Free Radic Res.*, 53(1), 1068–1100
- [16] Saqullaeva, D., Turaev, A.S., Salikhanova, D., Abdurakhimov, S., Jumaeva, D., & Ruzmetova, D. (2021) Research of ecological safety and chemical compositions of phospholipids obtained from local vegetable oils and their miscelles. *E3S Web of Conferences* 288, 01084.7p
- [17] Sineiro, J., Domínguez, H., Núñez, M. J., & Lema, J.M. (1998). Ethanolic extraction of sunflower oil in a pulsing extractor. *Journal of the American Oil Chemists' Society*, 75(6), 753–754
- [18] Sukkasem, C., Machikowa, T., Tanthanuch W., & Wonprasaid, S. (2015). Rapid chemometric method for the determination of oleic and linoleic acid in sunflower seeds by ATR–FTIR spectroscopy. *Chiang Mai J. Sci.*, 42(4), 930–938. <http://epg.science.cmu.ac.th/ejournal/>
- [19] Yang, Q., Yang, Y., Luo, Z., Xiao, Z., Ren, H., Li, D., & Yu, J. (2016). Effects of lecithin addition on the properties of extruded maize starch. *J. Food Process. Preserv.*, 40, 20–28

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