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

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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 α–position to the double bond, and hydroxyl groups. The activity of P–O–groups and NH–groups increases at temperatures close to 80^{0}C. The above information confirmed that the degreasing of the phosphatide concentrate in the temperature range of 50–70^{0}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 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 cm^{-1}), for valence and deformation–type vibrations in methyl C–H groups (at 3008 cm^{-1} and 722 bands), for symmetric and asymmetric vibrations in CH_2 groups (at 2923 cm^{-1} as well as 2853).

The band from 1200–970 cm^{-1} (due to P–O–C + 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 cm^{-1} depending on the polarizability of the hydrogen bonding features. The three domains included the significant signals (600 – 480 cm^{-1} , 460 –180 cm^{-1} and below 300 cm^{-1})which originates the head group domain, the vibrations of the torsion motions of

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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^{0}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^{-1}), C=C non–conjugated double bonds (1600 cm^{-1}), OH– groups in alcohols, acids (υ = 3400 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 (υ = 1170 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^{0}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 a–position to the double bond) and hydroxyl groups.

The Fourier transform infrared analysis indicated the characteristic peak at 1,734/cm^{-1} 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 (υ = 3040–3010 cm^{-1} and υ = 1680–1620 cm^{-1}) were chosen as analytical bands. Stretching vibrations of methylene (CH_2) groups are fixed at υ = 2853 cm^{-1}, bending vibrations of CH_2 groups – at υ = 1465±10 cm^{-1}), absorption bands due to bending and stretching vibrations of NH groups are sought in the ranges υ = 1650–1590 cm^{-1} and (υ = 3500–3300 cm–1).

Absorption bands due to stretching vibrations of free P=O groups (1300–1200 cm^{-1}) were selected for the phosphate group. Stretching vibrations P–O–C are evaluated at υ = 1070–1050 cm^{-1} groups, P–O groups (υ = 1040–1000 cm–1) and groups – ROOH at υ = 1240–1130 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 a–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 (υ = 1170 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^{-1} refers to stretching vibrations of CH–groups in –CH=CH–;
- region 1585–1680 cm^{-1} – absorption caused by stretching vibrations of the double bond itself;
- region 680–1000 cm^{-1} – frequencies of non–planar deformation vibrations C–H;
- part of the spectrum near 1435 cm^{-1} – absorption of the neighboring methylene group.

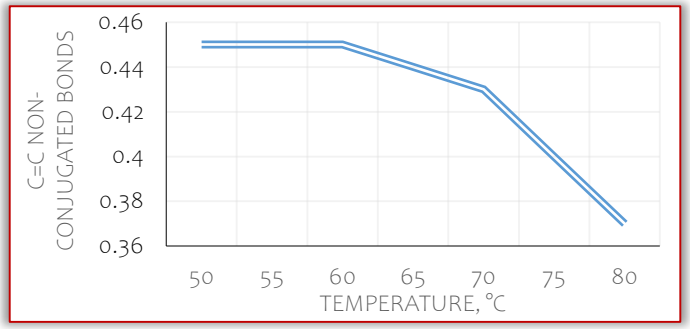
The change in the number of non–conjugated and conjugated ethylene bonds in the temperature range of 50–80^{0}C is shown in fig. 1 and 2.

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^{0}C.

The fact of the formation of conjugated dienes should be explained by the positional isomerization of isolated double bonds in the fragment of acylalinolic acid (–C_{13}H=C_{12}H–$C_{11}H_2$–C_{10}H=C_9H–). Hydrogen of the a–methylene group, located between two double bonds, is chemically mobile and under certain conditions

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



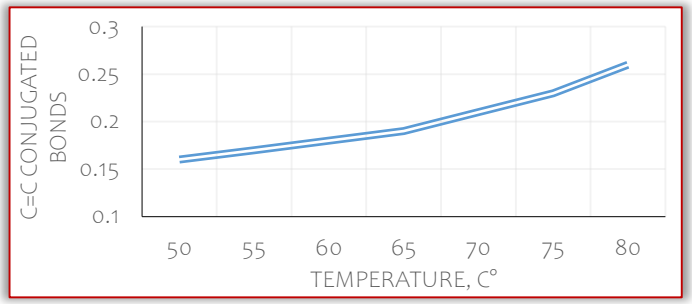


Figure 1 – Change in the number of non–conjugated and conjugated C=C bonds, (conventional units, c.u.)

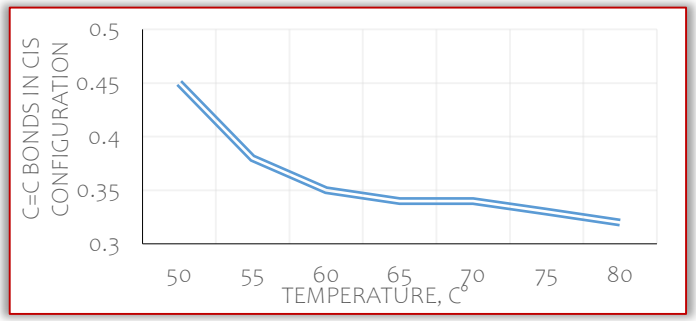
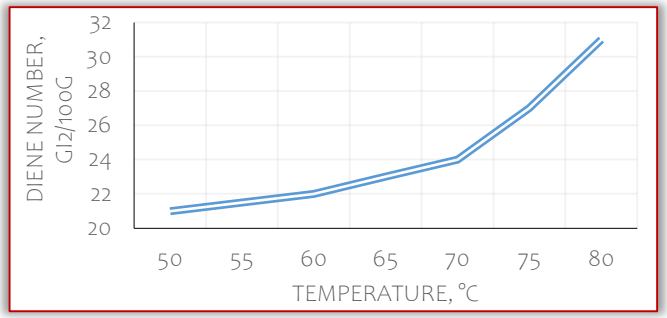


Figure 2 – Change in the number of of C=C bonds in the cis–configuration (c.u.)

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 80⁰C. 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 α–methylene groups decreases significantly at temperatures close to 80⁰C. This can be explained as follows: the number of α–methylene groups in the acylalinoleic 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 υ = 3040 cm^{-1} (CH–valence), υ = 970–960 cm^{-1} (CH–out–of–plane) and υ=1310–1295 cm^{-1} (CH–plane deformation), in all samples of phospholipids obtained in the temperature range of 50–80⁰C were not detected.



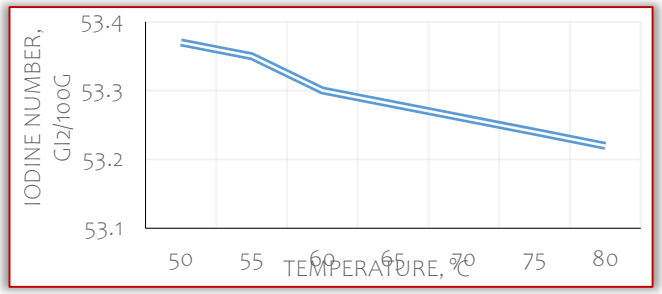


Figure 3 – Change in diene and iodine numbers and the number of α–methylene groups at different degreasing temperatures

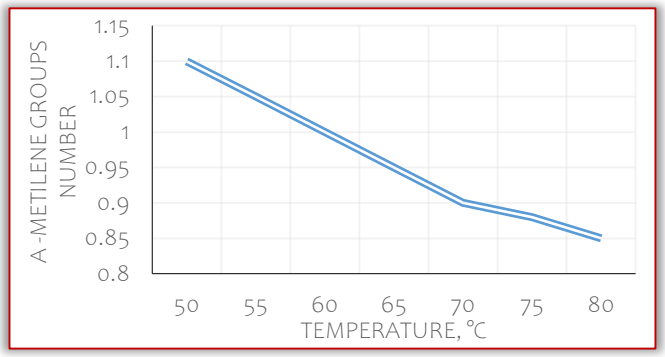


Figure 4 – Change in the number of α–methylene groups at different degreasing temperatures

An increase in the intensity of the absorption band of the NH group (υ=1640 cm^{-1}) is observed in phospholipids isolated during degreasing at a temperature close to 80⁰C together with a decrease in the intensity of this band at a wavelength of υ = 3350 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 80⁰C.

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

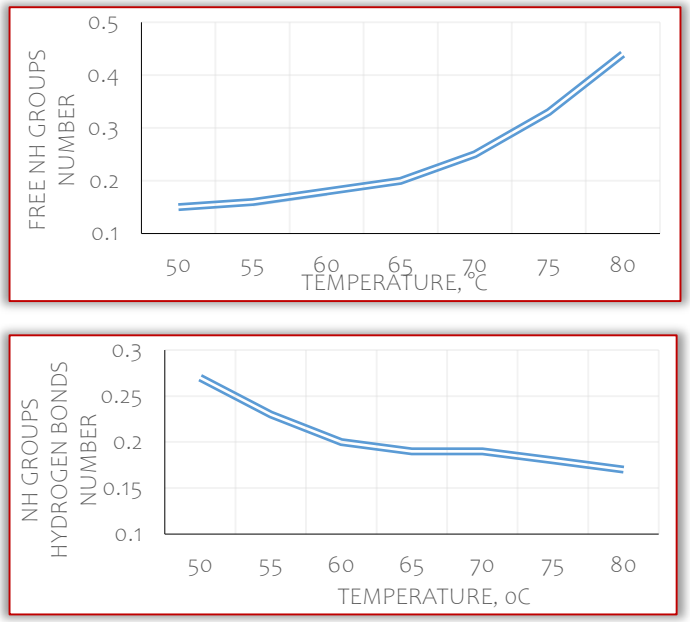


Figure 5 – Change in the intensity of the absorption bands of the NH group.
1 – the number of free NH groups (c.u.); 2 – is the number of NH groups involved in the hydrogen bond (c.u.).

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 acylalinoleic 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.

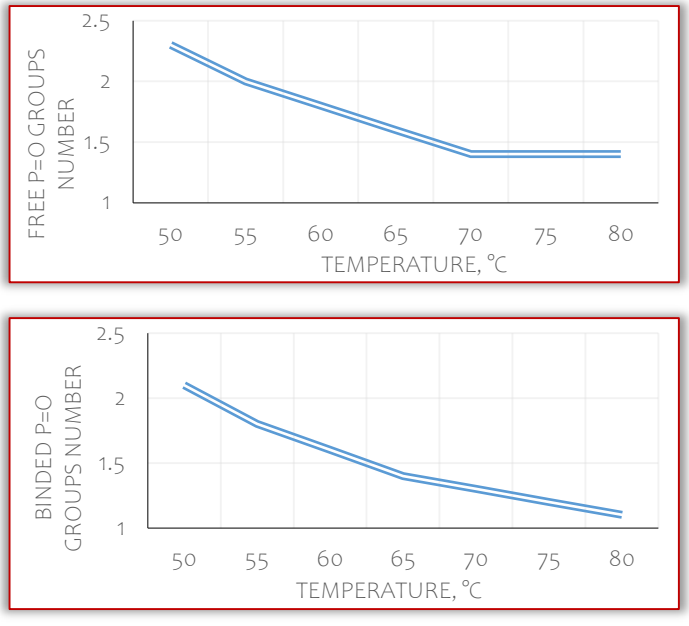


Figure 6 – Change in the number of groups of phospholipids in free P=O and binded P=O groups

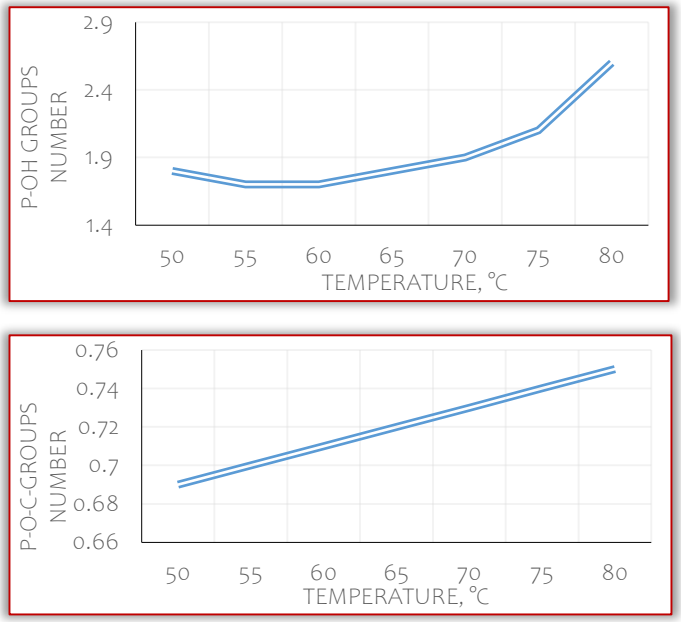


Figure 7 – Change in the number of groups in the phosphate group of phospholipids:
– P–OH (c.u.) and – P–O–C

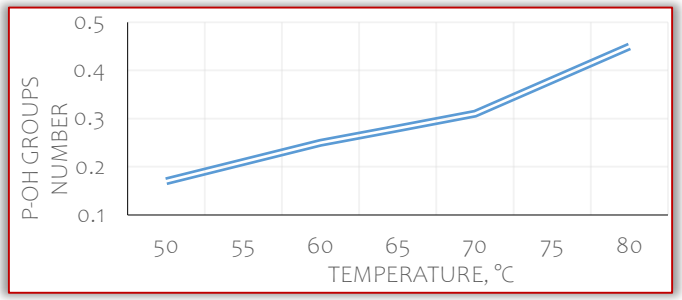


Figure 8 – Change in the number of groups in the phosphate group of phospholipids. $P–O^-$ (c.u.)

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 a–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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