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Production of edible lecithin from sunflower-oil refining waste

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ABSTRACT

The spent meal from the production of sunflower oil is a phosphatide concentrate that contains 50–60% of phospholipids (lecithin). We have developed a methodology for the defatting of phosphatide concentrate with isopropyl alcohol at an elevated temperature and low ratios of the phosphatide concentrate to the extractant, and subsequent identification of the components of food lecithin. Extraction at 60°C attains a relatively high yield of phospholipids; phosphatidylcholine, phosphatidylinositol, and phosphatidylethanolamine predominate in the fractional composition of phospholipids; at the same time, the carbohydrate content is reduced.

KEYWORDS

Defatting; fatty acids; phospholipids; food lecithin

Introduction

Sunflower (*Helianthus annuus*) is a highly prized oil crop, widely cultivated in Ukraine. Following the extraction of sunflower oil, the residual sunflower meal contains 50–60% of phospholipids (*lecithin*, which is used in the food industry as a natural emulsifier and as dietary supplement). In recent years, as much as 35 million tonnes of sunflower meal has been exported to the European Union as stockfeed [1,2]. The valuable phospholipids can be recovered, and refined products from solvents to antioxidants have a range of high-value nutraceutical and biomaterial applications [3,4].

Crude sunflower oil is obtained from partly dehulled seeds by mechanical pressing followed by hexane extraction and water defatting with petroleum ether and hexane [5]. Removal of phospholipids by hydration from a solution of a phosphatide concentrate in hexane is effective when the water content of the phosphatide concentrate is 0.4–0.5%. Most phosphatide concentrates produced at Ukrainian enterprises contain up to 0.9–1.6% water; the solubility of phospholipids in hexane decreases with increasing water content; separation of hydrated phospholipids and neutral oils dissolved in hexane is unsatisfactory; and it is hard to dehydrate the isolated phospholipids. Chemical modifications, fractionation and, in particular, extracting phospholipids using aliphatic alcohol at a controlled temperature promise effective separation of phospholipids in the aqueous-liquid fraction [6–8].

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National and EU regulations permit the use of only a few solvents (water and highly volatile solvents like ethyl acetate, ethanol, acetone) and the residues are strictly regulated [9,10]. Fractionation of lecithin is usually carried out with absolute ethanol to extract fats; insoluble residues are washed with acetone [11]; and the phospholipid fraction stored at 0°C [12]. The quality and stability of the products depend on seed quality, seed treatment prior to extraction, extraction method and processing conditions [13–16]. Solvent-extracted oils have higher total phospholipid content, being higher in phosphatidylcholine and phosphatidylethanolamine [17,18] which have valuable hypocholesterolemic, hypolipidemic and hepatoprotective properties [19].

Phospholipids are a by-product in the vegetable oil industry but most of them are simply used as stockfeed. Their extraction from crude vegetable oil typically requires heating the oil to an appropriate temperature, treatment with a peroxide solution, separating the lecithin precipitate from the oil, and drying the lecithin [20]. Defatting, or degumming of the oil [21] commences with heating the crude oil [22] and treating with water, or citric and phosphoric acid [23], decreasing the phosphorus content (<20 mg/kg [24]), fatty acid content, peroxide value and wax content, and yielding considerably less-stable oils [16]. The content of phosphatidylcholines in sunflower lecithin is somewhat greater than in soy lecithin [25]. Fatty acids are categorised as saturated (SFAs), mono (MUFAs), and polyunsaturated (PUFAs) on the basis of presence or absence of double bonds in side chains [26]. Sunflower oleic type lecithins have a high content of MUFAs (81.3%) compared to rapeseed and soybean lecithins [27].

Our goal, here, was to develop a methodology for defatting phosphatide concentrate with the subsequent identification of the components of food lecithin.

Materials and methods

Phosphatide concentrate samples were taken at the Dnipro and Pology oil-extraction plants (DOEP and POEP) situated in Dnipropetrovsk and Zaporozhka provinces.

Extraction procedure

The kinetics of extraction of phosphatide concentrate with isopropyl alcohol were carried out in a laboratory batch unit, consisting of a round-bottomed 1 L three-necked flask in a heated water bath. The extractor was equipped with a stirrer; other components were a thermometer and a reflux condenser. The process temperature was varied from 50°C to 80°C; the weight ratio of phosphatide concentrate to isopropyl alcohol was from 1:1 to 1:3; the stirring speed varied from 60 to 140 revolutions per minute. Extraction time was 5 minutes.

A 100 g sample of phosphatide concentrate was loaded into the extractor; the appropriate amount of isopropyl alcohol was added and heated to the required temperature. Heating and extraction were stopped after stirring the phosphatide concentrate with isopropyl alcohol for 5 minutes. This mixture was poured into a settling flask and cooled to room temperature. After separating the extractives from the resin-like extractable phospholipids, the liquid phase was decanted on a suction filter. The residue in the flask was washed with a small amount of isopropyl alcohol and transferred to a suction filter in the same way. Partially defatted phospholipids from the settling flask and suction filter were transferred back to the extractor.

This operation was repeated twice, until the fat was completely removed from the phosphatide concentrate. The extract obtained after filtering was placed in a pre-weighed flask of the distillation unit and the weight of the extracted substances was determined after distillation of the extractant. Purified phospholipids were dried under vacuum at 30–35°C in a nitrogen stream and weighed: powdered food lecithin, light yellow in colour, was obtained.

Isolation of phospholipids

The indicators of the fat-free phospholipid content (iodine, peroxide and diene numbers, *etc.*) were determined at the first stage [16]. Individual groups of phospholipids were isolated from defatted phosphatide concentrate by high-performance micro-thin layer chromatography on glass plates (6 × 9 cm) with silica gel L 5/40 (Lachema, Czech Republic), using a mixture of hexane:diethyl ether: glacial acetic acid (85:15:1) as solvent system. Individual phospholipids were identified by known R_f values using qualitative tests for functional groups.

Nonspecific manifestation of plates by thin layer chromatography was carried out by spraying with a 10% solution of sulphuric acid in methanol with at a temperature of 180°C. Specific determination of lipids was carried out using rhodamine 6G [26], ninhydrin [27], anthrone reagent [28], malachite green and Dragendorff's reagent.

Analytical procedures

Fatty acid methyl esters were prepared according to Christie [29]. The fatty acid composition of phospholipids was studied by chromatography as described above; likewise, for the separation of phospholipids [30,31]. Isolated groups of phospholipids were then heated at 100°C for 1 hour with 2N HCl in absolute methyl alcohol to obtain methyl esters of fatty acids.

Macro- and microelements in phospholipids were determined by atomic absorption spectrophotometry. The separation and identification of individual carbohydrates soluble in isopropyl alcohol was carried out using their separation by thin-layer chromatography on fast paper based on R_f of the simplest carbohydrates [32]. The total content of carbohydrates in the form of reducing substances was determined by the ebullioscopy method by titrating copper-alkaline solutions with the obtained samples [33].

The data obtained were processed by statistical methods using StatGraphics Plus at significance level of 0.95% ($p < 0.05$).

Results

Phosphatide concentrate obtained by hydrating vegetable oil with water was defatted using isopropyl alcohol at elevated temperatures and low weight ratios of phosphatide concentrate to alcohol. In essence, the phosphatide concentrate is treated with isopropyl alcohol in a weight ratio of 1:2 (at the initial stage) and then, to reduce the solubility of lecithin in isopropyl alcohol, at 1:1 at a temperature of 50–80°C. In these conditions, hydrocarbon part of the phospholipids is partially melted, which promotes greater contact of the solvent with the components of the phosphatide concentrate.

Table 1 shows the results of extraction of phosphatide concentrate with isopropyl alcohol. The greatest influence on the rate of defatting of phosphatide concentrate is exerted by two factors: extraction temperature and the ratio of the weight parts of

Table 1. Effect of technological factors on the rate of extraction of phosphatide concentrate with isopropyl alcohol.

Extraction temperature, °C	Phosphatide concentrate/isopropyl alcohol ratio	Extraction time, min	% extracted substances
50	1:2 at the initial stage, then 1:1	5	38.00
		10	43.80
		15	45.50
		20	45.70
60	1:2 at the initial stage, then 1:1	5	38.75
		10	44.85
		15	45.80
		20	46.00
70	1:2 at the initial stage, then 1:1	5	38.30
		10	44.53
		15	46.20
		20	46.40
80	1:2 at the initial stage, then 1:1	5	37.90
		10	41.21
		15	43.92
		20	44.90
		25	45.71
		30	46.00
60	1:1 in all extractions	5	38.90
		10	45.00
		15	45.82
		20	46.40
60	1:2 in all extractions	5	39.00
		10	45.71
		15	46.00

phosphatide concentrate to isopropyl alcohol. The use of elevated temperatures is advisable considering that: a) after the first treatment of the phosphatide concentrate with an extractant, a resinous mass is formed into which the solvent does not penetrate well, which leads to a significant decrease in the extraction rate; b) at elevated temperatures, the hydrocarbon part of the phospholipids partially transforms into a molten state, and the rest becomes permeable to the solvent.

A significant part of phospholipids melts already at 50°C so, to increase the rate of defatting with isopropyl alcohol, it helps to carry out the extraction at an elevated temperature close to the boiling point of isopropyl alcohol. On the one hand, the transfer of the non-polar hydrocarbon part of the phospholipids into a molten state facilitates the access of the extractant to the remaining polar part of the phospholipid molecule. On the other hand, it is possible to reduce the ratio of phosphatide concentrate to isopropyl alcohol.

Figure 1 indicates that 37–38% of the substances making up the phosphatide concentrate are extracted at the initial stage of the process which means that, during the first defatting, 84–88% of lipids are extracted.

The total consumption of isopropyl alcohol per part by weight (pbw) of phosphatide concentrate during extraction at elevated temperatures was 4–5pbw, and extraction at room temperature (20–25°C) increases this ratio to 20–25pbw of isopropyl alcohol per 1 pbw of phosphatide concentrate (Figure 2).

Figure 3 shows the data on dependence of five indicators of the fat-free phospholipids on extraction temperature. Acid, peroxide and diene numbers increase with an increase in the defatting temperature, and the saponification number decreases; iodine numbers in the temperature range of 60–80°C remain practically constant; it is clear that 60°C is the optimum

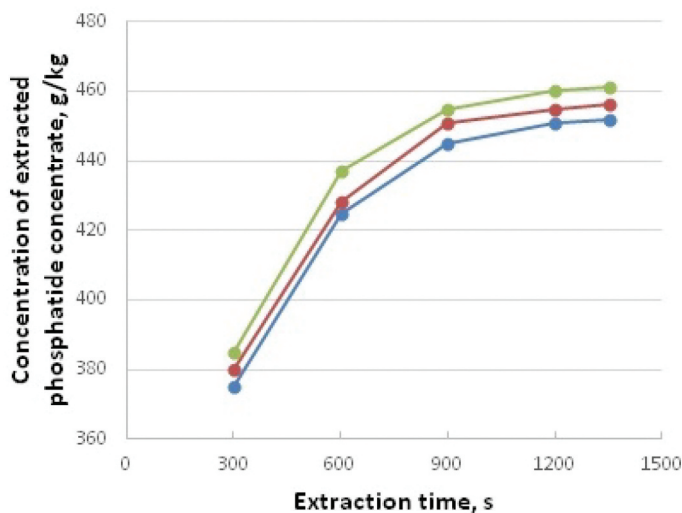


Figure 1. Kinetic curves for extraction of phosphatide concentrate with isopropyl alcohol at: 1: 50°C, 2: 60°C, 3: 70°C. The ratio of phosphatide concentrate to extractant is 1:2 at the initial stage, and then 1:1.

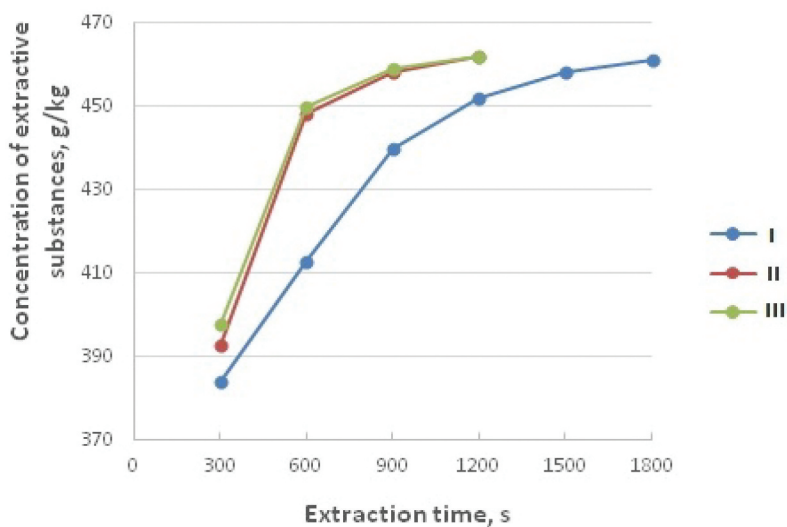


Figure 2. Kinetic curves for defatting phosphatide concentrate with isopropyl alcohol depending on the ratio of phosphatide concentrate to isopropyl alcohol: I) 1:1 at all stages of defatting; II) 1:2 at all stages of defatting; III) 1:2 at the initial stage of degreasing, and then 1:1.

temperature for extraction of the phosphatide concentrate with isopropyl alcohol. These trends coincide with the data on phospholipids heat capacity of unrefined sunflower oil [34,35].

Figure 4 shows the dependence of the main chemical parameters of phospholipids on the weight ratio of the phosphatide concentrate to isopropyl alcohol. An increase in the ratio of phosphatide concentrate to isopropyl alcohol leads to only a slight increase in acid and peroxide numbers. Other indicators remain stable.

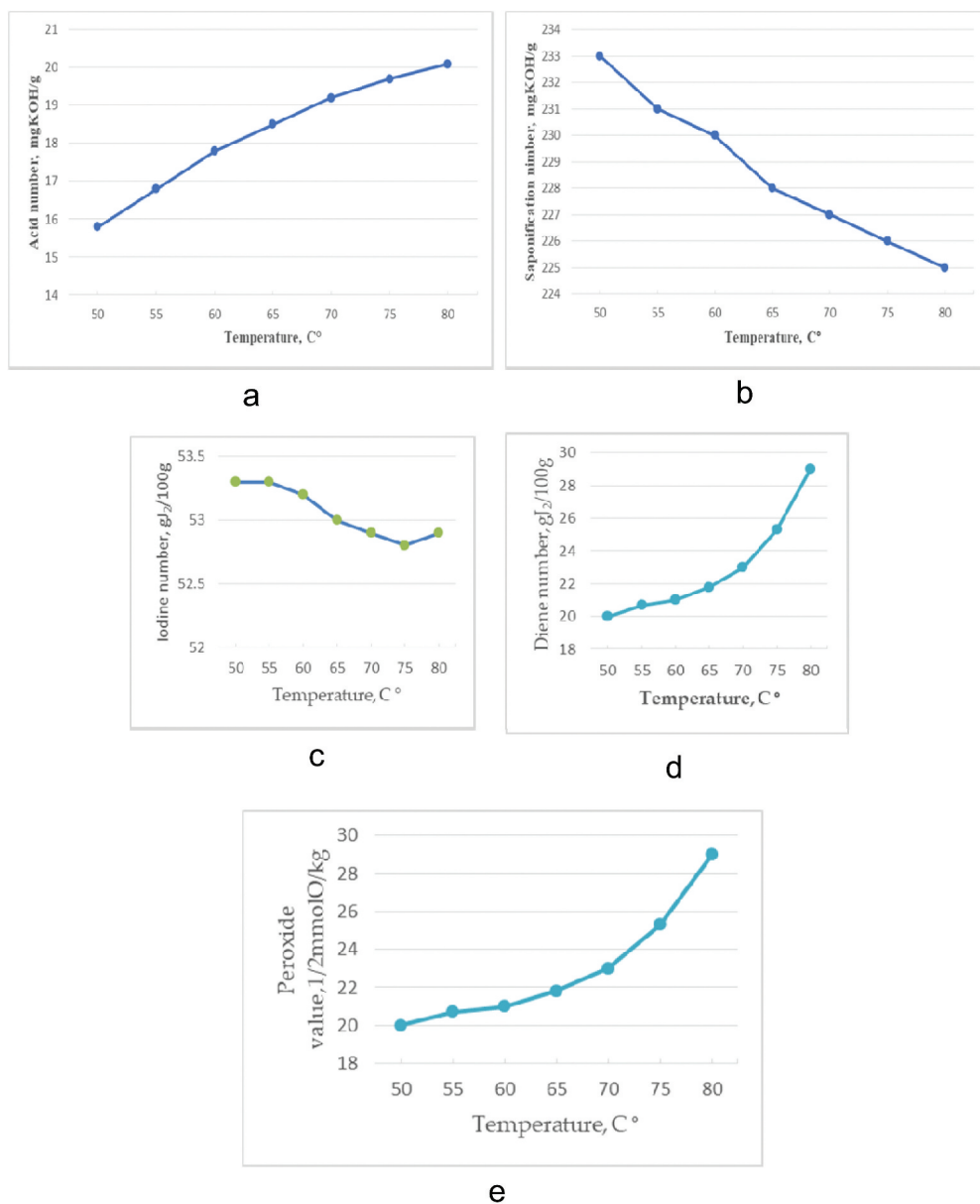


Figure 3. a, b - dependence of acid and saponification nos. of phospholipids on extraction temperature, mg KOH/g; c, d - dependence of iodine and diene nos. of phospholipids on the extraction temperature, gI₂/100g; E - dependence of the peroxide value of phospholipids on the extraction temperature, ½mmolO/kg.

A comprehensive assessment of the essential phospholipids that comprise sunflower oil lecithin was carried out. Table 2 shows the general characteristics of phospholipids isolated at different extraction temperatures. Free and bound carbohydrates, and unsaponifiable lipids, were found in the composition of the isolated phospholipids. The presence of free carbohydrates in hydrated phospholipids is explained by the fact that certain groups of

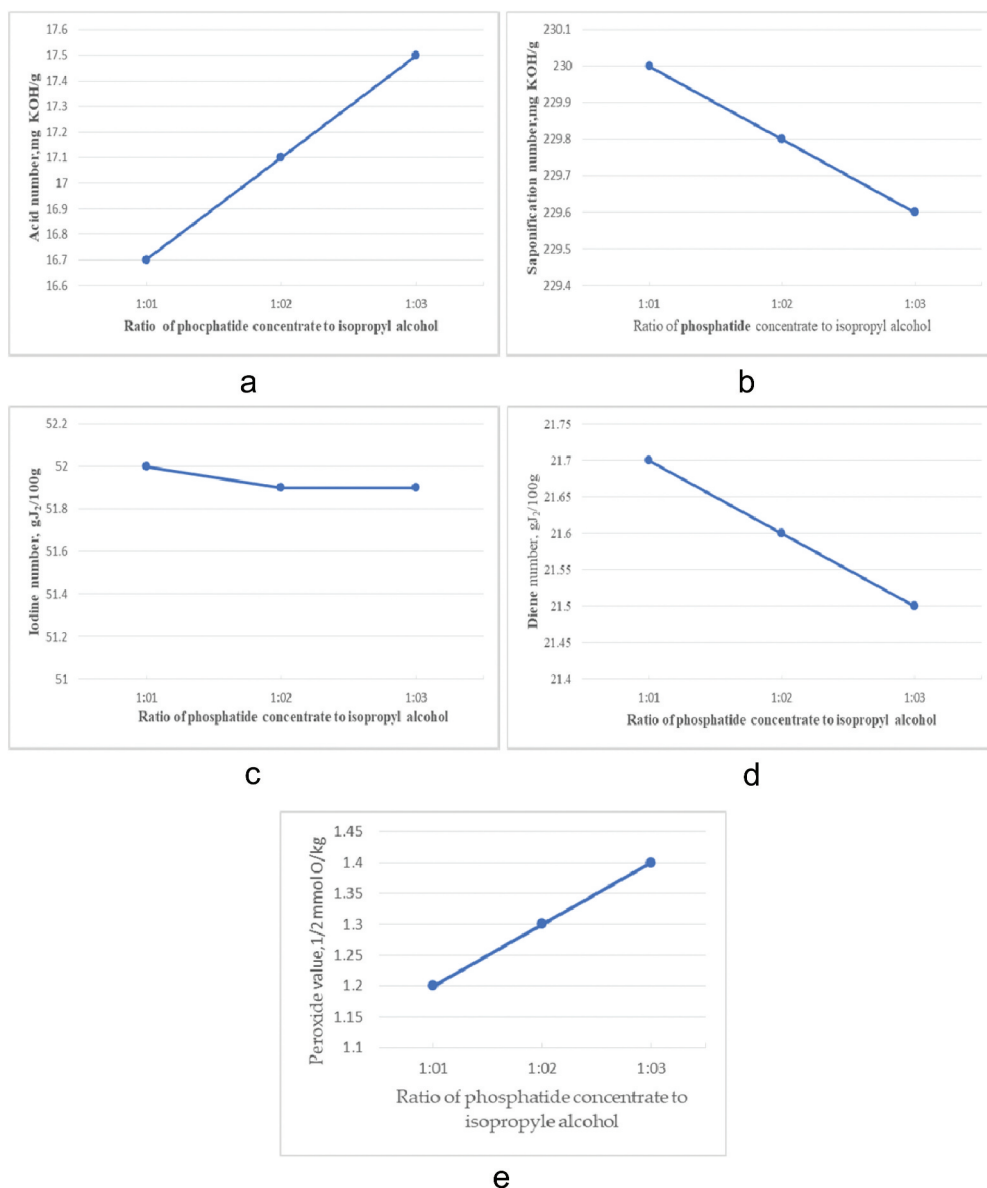


Figure 4. a, b - dependence of acid and saponification nos. of phospholipids on the weight ratio of phosphatide concentrate to isopropyl alcohol, mgKOH/g; c, d - iodine and diene nos. of phospholipids v. that weight ratio, gJ₂/100 g; e - peroxide value of phospholipids v. that weight ratio, 1/2mmolO/kg.

phospholipids are not only chemically bound with carbohydrates but, also, associated by intermolecular hydrogen bonds between the P=O, P-OH, NH₂ – groups of phospholipid molecules and C=O, C-OH of carbohydrate molecules [36]. During defatting of the phosphatide concentrate, isopropyl alcohol extracts 2.9–3.2% of the carbohydrate.

Figure 5 shows the content of individual monosaccharides extracted with isopropyl alcohol from phosphatide concentrate from the Pology and Dnipro oil extraction plants (OEP).

Table 2. General characteristics of defatted phospholipids.

Content, %	Defatting stage			
	I	II	III	IV
Ash	4.20	4.31	4.42	4.44
Nitrogen	1.23	1.30	1.03	1.03
Phosphorus	3.59	3.96	4.38	4.84
Carbohydrates	3.03	3.13	3.18	3.33
Unsaponifiable lipids	1.90	2.00	2.00	1.88

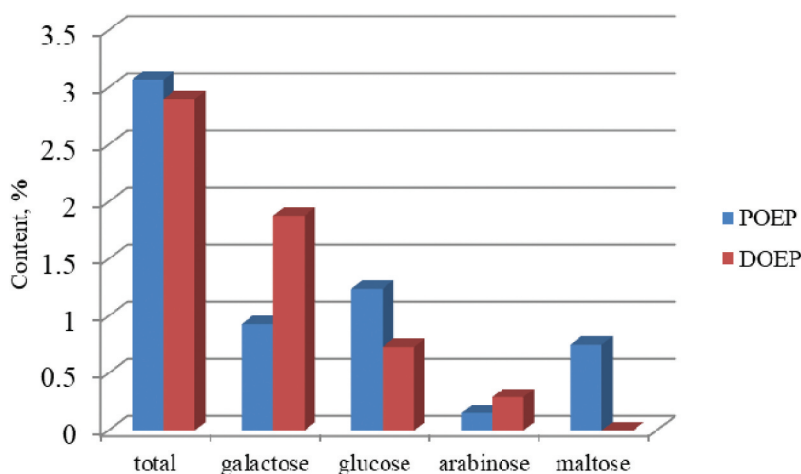
**Figure 5.** Content of carbohydrates in the isopropyl extract of phosphatide concentrates.

Figure 6 shows the content of bound carbohydrates in sunflower oil phospholipids from the two OEPs

Isopropyl alcohol extracted half of the carbohydrates from the phosphatide concentrate; the remainder are chemically bound to the phospholipids, so acid hydrolysis is an indispensable part of their isolation.

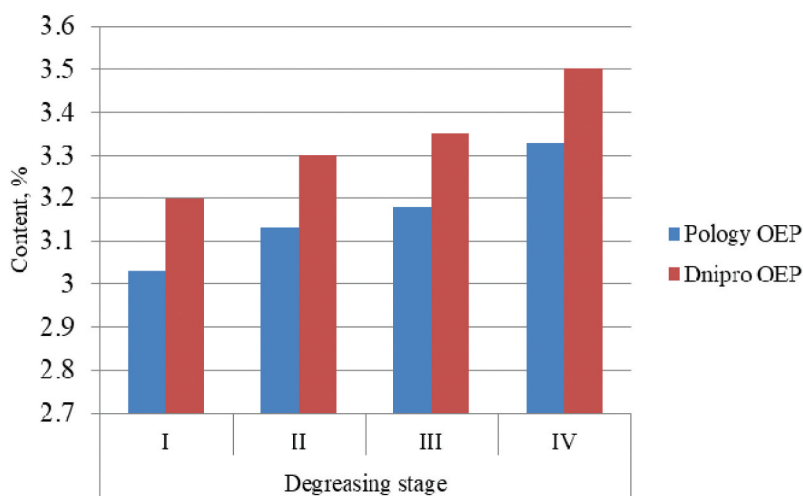
**Figure 6.** Content of bound carbohydrates in defatted phospholipids.

Table 3 shows the content of macro and microelements in phospholipids. Sunflower lecithin contains a high level of potassium and magnesium, beneficial for people with cardiovascular disease [22]. Metals with variable valence are also a component of phospholipids [37]. There are traces of Pb, Cd and As. Table 4 shows chemical indicators of fat-free phospholipids.

Ten individual phospholipids were identified. Figure 7 shows the kinetic diagram of the five principal compounds: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA). The content of phospholipids hardly changes with an increase in the temperature of extraction of the phosphatide concentrate with isopropyl alcohol. The other five defatted phospholipids are present in smaller amounts from 0.2% to 6.5% (Table 5); two glycolipids (diphosphatidylglycerol and phosphatidylglycerol) comprise 83% of this profile.

Figure 8 shows the fatty acid composition of phospholipids isolated from sunflower oil phosphatide concentrate at various temperatures. Saini and Keum [38] classify naturally

Table 3. The content of macro and microelements in phospholipids.

Plant	Macroelements, %				Microelements, mg/kg					
	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Pb	Cd	As	Cu	Zn	Fe
POEP	0.514	0.15	0.1	0.06	0.05	0.001	0.001	3.3	4.2	19.6
DOEP	0.49	0.14	0.07	0.02	0.06	0.001	0.001	3.2	4.2	19.5

Table 4. Chemical indicators of phospholipids.

Indicator	Defatting stage				Phospholipids isolated with acetone at 30°C
	I	II	III	IV	
Acid number (AN), mgKOH/g	16.26	17.58	19.48	20.11	15.3
Saponification number (SN), mgKOH/g	232.8	231.4	228.1	226.9	233.0
Iodine number (IN), gJ ₂ /100 g	53.90	53.40	53.10	53.10	53.9
Peroxide number (PN), mmol ¹ / ₂ O/kg	1.30	1.42	1.81	2.30	1.28
Diene number (DN), gJ ₂ /100 g	20.36	21.34	23.26	30.12	19.82
Hydroxyl number (HN), mgKOH/g	21.85	22.73	24.44	24.44	21.84

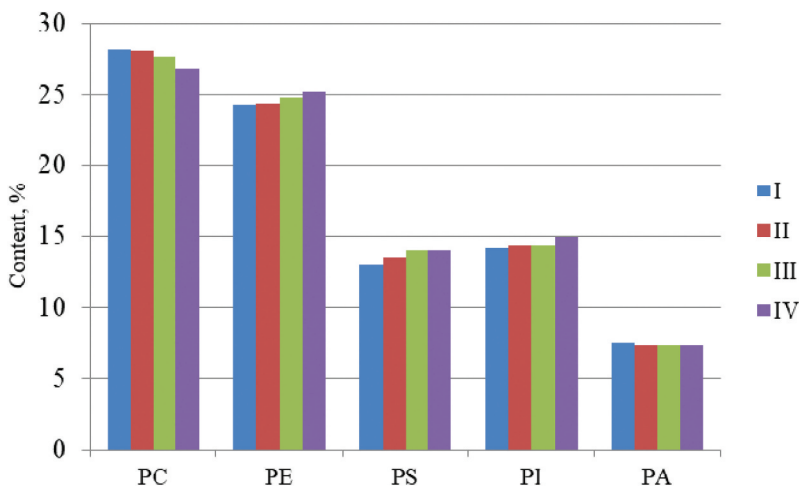
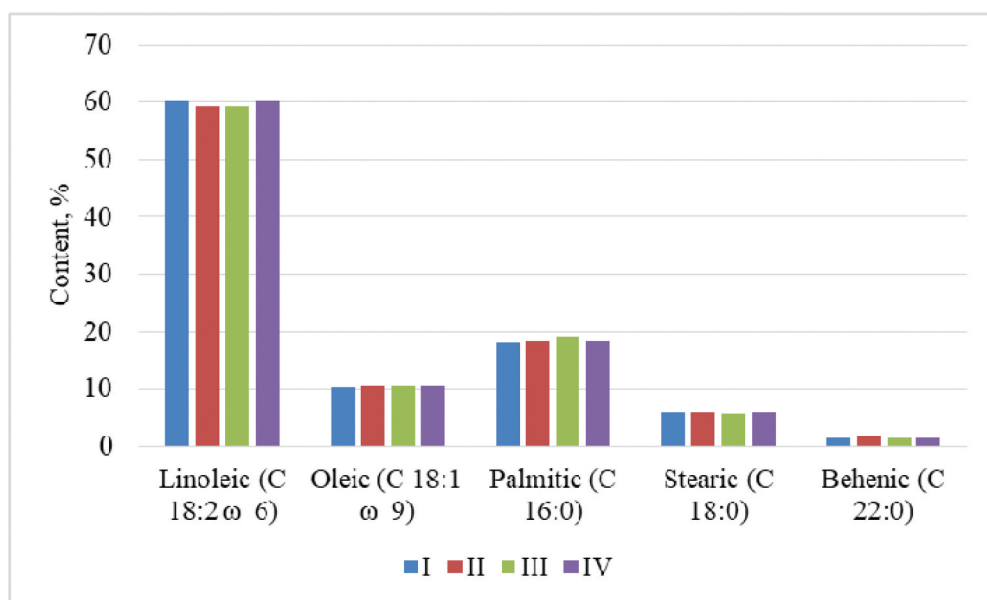


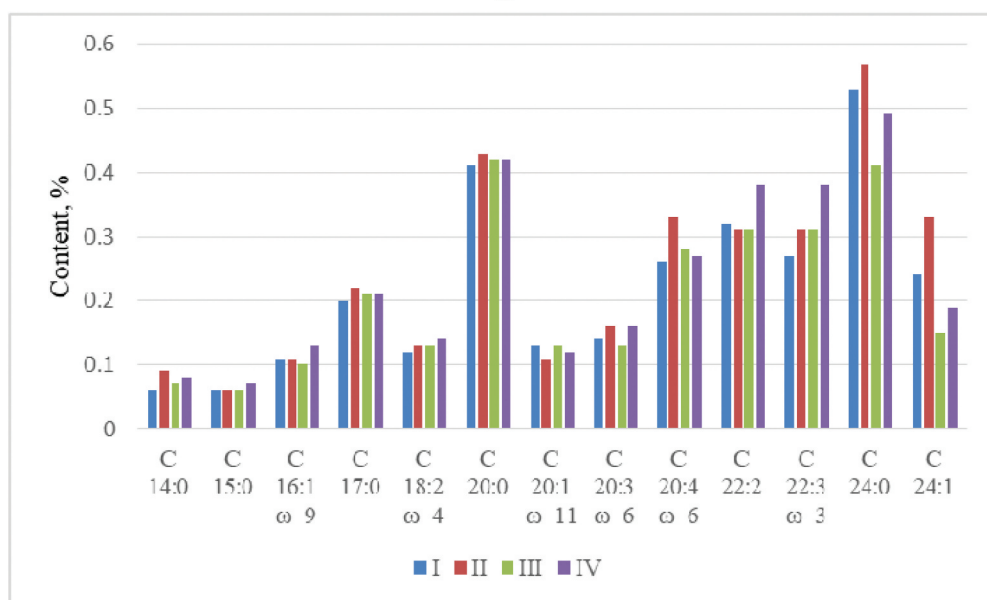
Figure 7. Main phospholipids composition depending on the defatting temperature of the phosphatide concentrate.

Table 5. Minor phospholipids composition depending on the defatting temperature, %.

Phospholipid	Degreasing conditions			
	I	II	III	IV
Diphosphatidylglycerol	6.3	6.6	6.9	6.5
Phosphatidylglycerol	4.3	4.2	3.9	3.9
N-acylphosphatidylethanolamine	1.6	1.1	0.6	0.7
Lysophosphatidylcholine	0.3	0.3	0.4	0.4
Lysophosphatidylethanolamine	0.3	0.3	0.2	0.2



a



b

Figure 8. Fatty acid composition of lecithins: a - main components; b - minor components.

Table 6. Group fatty acid composition of lecithins.

Fatty acid	%
Myristic (C 14:0)	0.08
Palmitic (C 16:0)	18.58
Stearic (C 18:0)	5.94
Arachinoic (C 20:0)	0.42
Behenic (C 22:0)	1.51
Lignoceric (C 24:0)	0.5
Total saturated	27.25
Palmitoleic (C 16:1 ω 9)	0.1
Oleic (C 18:1 ω 9)	10.53
Linoleic (C 18:2 ω 6)	59.67
Linoleic (C 18:2 ω 4)	0.13
Eicosenoic (C 20:1 ω 11)	0.12
Arachidonic (C 20:4 ω 6)	0.39
Others	1.3
Total unsaturated	72.25

occurring fatty acids (FAs) by their carbon chain length and the position of the first double bond on methyl terminal (omega; ω ; or n-FAs) into three categories: saturated FAs (SFAs, no double bonds), monounsaturated FAs (MUFAs, a single double bond), and polyunsaturated FAs (PUFAs, ≥ 2 double bonds). In our case, main SFAs are C 14:0, C 16:0, C 18:0, C 20:0, C 22:0 and C 24:0. Main MUFAs and PUFAs are represented by C 16:1 ω 9, C 18:1 ω 9, C 18:2 ω 6, C 18:2 ω 4, C 20:1 ω 11 and C 20:4 ω 6.

Table 6 shows the average group fatty acid composition:

Thus, defatting phosphatide concentrate at 50–70°C does not inactivate the phospholipids and achieves a high rate of defatting.

Discussion

Considering that the extraction of lecithin from oilseed by-products has not been adequately explored, it was important to investigate methods for the extraction of phospholipids from plant materials and agro-food by-products. We find that the shortcomings of conventional defatting of the phosphatide concentrate can be avoided if the extraction is carried out with isopropyl alcohol at elevated temperatures (50–80°C) and low weight ratios of the phosphatide concentrate to isopropyl alcohol.

In terms of abundance, the extracted phospholipids can be ranked: PC>PE>PI>PS>PA.

Sunflower lecithin has a high proportion of oleic and linoleic acids [22]: linoleic 59.17–60.15%; oleic acid 10.34–0.58%. The high content of unsaturated palmitic acid in phospholipids (up to 19.0%) is noteworthy, but its content in the feedstock (unrefined sunflower oil), does not exceed 7.2% [39].

Conclusions

The new procedure allows an increase in the extraction rate, reduces the consumption of the extractant by 5–6 times, reduces energy costs for the regeneration of the extractant, and improves the quality of defatted phospholipids (food lecithin).

Lecithin should be extracted from phosphatide concentrate at elevated temperatures, close to the boiling point of isopropyl alcohol, to increase the rate of defatting. At 60°C, the effect of temperature on lecithin is insignificant and the extraction rate becomes relatively high.

An increase in the ratio of phosphatide concentrate to isopropyl alcohol leads to a slight increase in acid and peroxide numbers. Other indicators remain stable.

The composition of phospholipids changes with a change in the technological parameters of defatting: the percentage of ash, phosphorus and carbohydrates increases with an increase in the temperature of extraction but the content of nitrogen and unsaponifiables is reduced.

Up to 50% of the carbohydrates in the phosphatide concentrate are extracted with isopropyl alcohol. These carbohydrates may be classified as free and they are represented by glucose and galactose.

The chemical parameters of phospholipids change depending on the defatting temperature. Phospholipids obtained at higher extraction temperatures have higher acid, peroxide, diene and hydroxyl numbers and a lower saponification number. The iodine number practically does not change. The component composition of phospholipids is represented by phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and others. Linoleic, oleic and palmitic acids predominate in the fatty acid composition.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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