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Molecular Characterization of Phospholipids by High-Performance Liquid Chromatography Combined with an Evaporative Light Scattering Detector, High-Performance Liquid Chromatography Combined with Mass Spectrometry, and Gas Chromatography Combined with a Flame Ionization Detector in Different Oat Varieties

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ABSTRACT: Oat (*Avena sativa* L.) is an important crop produced in various regions of Europe and North America. Oat lipids are a heterogeneous mixture of acyl lipids and unsaponifiable components. The neutral lipids are mainly triacylglycerols and account for 50−60% of total oat lipids. Oat oil is also rich in polar lipids, that is, phospholipids and glycolipids. Characterization of oat polar lipids has largely been performed by thin-layer chromatography (TLC), but the composition of phospholipid classes has been poorly studied. The aim of our work was the determination of different phospholipids in Romanian oat samples. For that purpose, one commercial sample (Comun) and four pure varieties (Jeremy, Lovrin 1, Lovrin 27-T, and Mures) were used. High-performance liquid chromatography combined with an evaporative light scattering detector results allowed us to establish that phosphatidylethanolamine was the most representative phospholipid in all of the oat samples. In addition, high-performance liquid chromatography combined with electrospray ionization mass spectrometry analysis showed that C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, and C20:1 were the fatty acids bound to the glycerol backbone. Using first-preparative TLC and later gas chromatography, it was demonstrated that linoleic acid (C18:2) was the main fatty acid of the phospholipid fraction in all of the samples.

KEYWORDS: *Oat, phospholipids, HPLC−ELSD, phospholipid fatty acids, HPLC−ESI−MS, GC−FID*

INTRODUCTION

Oat has been traditionally used as a feed source for animal diets. However, its human consumption has increased in recent years because of some studied health benefits, above all, in the United States, Mexico, and Japan, with Canada being the world's largest oat exporter. Oat grains have been established as a good source of B complex vitamins, protein, fat, minerals, and β-glucans.[1] The soluble fiber from oat has been studied to be very effective at decreasing blood cholesterol. Particularly, β-glucans, which are present in oat in relatively high proportions, can act in control of blood glucose in diabetes and cardiovascular diseases.[1] Moreover, the inclusion of moderate amounts of this cereal in a gluten-free diet has been demonstrated to be safe.[2] In fact, the concept of oat as preventive medicine in some particular diseases has been reviewed,[2,3] as well as the implementation of oat with β-glucans in functional foods.[4]

Apart from the importance of its soluble fiber content, oat is also characterized by high protein and lipid contents.[3] Among other cereal grains, oat presents the highest oil content, between 2 and 12% according to Zhou et al.[5] This lipid fraction has been studied on several occasions because it determines some flavor properties and the energy contribution to food and feed.[5] However, the phospholipid composition of oat has been scarcely studied despite the important role of phospholipids in the growth, maturing, and functioning of all body cells, their character as antioxidants,[6−8] and their increasing uses in the supplement industry as the main constituents of lecithin.

The content of the phospholipid fraction in oat has been estimated to be 5−26% of the total lipids.[9−11] Price and Parsons[12] used a first step of separation by silicic acid column chromatography and thin-layer chromatography (TLC) analysis and found that

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L-α-phosphatidylcholine (PC), L-α-phosphatidylethanolamine (PE), and -L-α-lysophosphatidylcholine (lyso-PC) were the most abundant phospholipids of all of the cereal grains analyzed. Sahasrabudhe et al.,[11] using the same experimental procedure as Price and Parsons,[12] determined L-α-lysophosphatidylethanolamine (lyso-PE) (20.4%), PE (14.8%), L-α-phosphatidylglycerol (PG) (9.5%), L-α-phosphatidylinositol (PI) (3.9%), and L-α-phosphatidylserine (PS) (3.2%) in six oat cultivars, with PC (29.9%) being the most abundant. The same result for the PC content was obtained by Aro et al.[13] using high-performance liquid chromatography combined with an evaporative light scattering detector (HPLC−ELSD). Finally, the most recent work about phospholipids in oat reported a solid-phase extraction (SPE) to separate polar and neutral lipids and HPLC−ELSD to quantitate the polar fraction.[14] However, the obtained HPLC−ELSD profile was only related to one oat variety.

In the past few decades, HPLC has become the preferred method for determining phospholipids, because quantitative and qualitative analyses can readily be obtained at a relatively low cost. Phospholipid analyses by means of liquid chromatography can be carried out in different ways, ultraviolet (UV), mass spectrometers, and refractive index detectors. However, in recent years, HPLC−ELSD has become an useful alternative to perform these kinds of analyses in different matrices, such as milk and dairy products, meat, fish, eggs, cereals, and oils.[15]

The aim of this work was to study the phospholipid composition of different oat cultivars using a HPLC−ELSD method. In addition, the determination of the molecular species of each individual phospholipid class was performed by high-performance liquid chromatography combined with mass spectrometry (HPLC−MS), and the specific fatty acid composition of phospholipids in the different oat varieties was determined using gas chromatography (GC) analysis with a previous preparative TLC.

MATERIALS AND METHODS

Samples. Oat samples analyzed in this work belonged to five Romanian varieties corresponding to one commercial sample (Comun) and four pure varieties (Jeremy, Lovrin 1, Lovrin 27-T, and Mures). "Comun" is a commercial common oat, and it is not a pure cultivar. Jeremy, Lovrin 1, and Lovrin 27-T were registered as varieties of oat in 2005, 2002, and 2005, respectively, at the research center of Lovrin (Timisoara, Romania), and Mures was registered as a variety of oat in 1991 at the research center of Turda (Cluj-Napoca, Romania). The samples were cultivated in an experimental field under the same agronomic conditions in Timisoara (Romania) in 2009. Oat grains were briefly milled before extraction for 3 min at 15 °C to a particle size of <0.6 mm using a laboratory mill IKA A10 (IKA-Werke GmbH and Co., Staufen, Germany).

Chemicals. All of the solvents and reagents were purchased from Merck (Darmstadt, Germany). The following phospholipid standards were supplied by Sigma-Aldrich (St. Louis, MO): PE, PC, PS, sphingomyelin (SM), PI, and lyso-PC. GLC-463 mix was from Nu-Check (Elysian, MN), and FAME 189-19 was from Sigma-Aldrich (St. Louis, MO). Both GLC-463 and FAME 189-19 are fatty acid methyl ester mixtures.

Lipid Extraction. Lipids were extracted with the Folch procedure according to Boselli et al.[16] Briefly, 100 g of milled sample was added to 300 mL of 1:1 (v/v) chloroform/methanol and then homogenized for 3 min using a Ultra-Turrax T25 homogenizer and a S18N dispersing tool both from Ika-Werke (IKA-Werke GmbH and Co., Staufen, Germany). The mixture was successively maintained at 60 °C for 20 min, added to 150 mL of chloroform, homogenized again for 2 min, and filtered through filter paper Albet 400 purchased from Barloworld Scientific (Stone, Staffordshire, U.K.). The organic phase was mixed with 75 mL of potassium chloride (1 M), shaken for about 1 min, and left overnight at 4 °C to permit a better separation between the organic matter and the aqueous phase. The separation of organic and aqueous phases was performed using a separatory funnel, after which the collected organic phase was filtered over anhydrous sodium sulfate. After the organic solvent was removed using a vacuum evaporator (bath temperature of 40 °C), the lipid fraction was dried under nitrogen, weighed, and stored in 25 mL of 4:1 (v/v) *n*-hexane/isopropanol at −18 °C until further analyses. To determine the phospholipids in oat extract, 100 mg of fat were weighed and dissolved in 1 mL of 88:12 (v/v) chloroform/methanol and transferred to capped test tubes for HPLC analysis.

Each oat sample was extracted 3 times, and each extract was injected twice.

Phospholipid Determination by HPLC−ELSD. The quantitation of the phospholipid classes was performed using HPLC−ELSD. The chromatographic method used for the separation of the polar lipids extracted from cream by Lopez et al.[17] was carried out with some modifications. Phospholipid separation was performed on an Agilent liquid chromatography HP 1200 Series (Agilent Technologies, Palo Alto, CA). The detector was an ELSD (PL-ELS1000, Polymer Laboratories, Church Stretton, Shropshire, U.K.). The control of the HPLC system was accomplished by the software Agilent ChemStation (Agilent Technologies, Santa Clara, CA), while chromatogram registration and data processing were assessed by ClarityLite (version 2.4.0.190, DataApex, Praha, The Czech Republic). The separation was achieved using a silica column, 150 × 3 mm with a 3 μm particle diameter (Phenomenex, Torrance, CA). Dried and filtered compressed nitrogen was used as the nebulizer gas at a flow rate of 1.0 L/min and temperature of 50 °C. The evaporating temperature was 85 °C. The elution program comprised isocratic conditions with 87.5:12:0.5 (v/v/v) chloroform/methanol/buffer (1 M formic acid, neutralized to pH 3 with triethylamine) from 0 to 7 min, followed by a linear gradient from 87.5:12:0.5 (v/v/v) to 28:60:12 (v/v/v) chloroform/methanol/buffer from 7 to 27 min. The mobile phase was brought back to the initial conditions, from 27 to 29 min, and the column was equilibrated until the next injection for 7 min. The flow rate was maintained at 0.25 mL/min. The column was maintained at room temperature (25 °C), and the injection volume was 15 μL per sample.

Determination of Phospholipid Molecular Species by HPLC−MS. HPLC−MS analyses were carried out on the same extract analyzed by HPLC−ELSD. A liquid chromatography apparatus HP 1100 Series from Agilent Technologies, including a degasser, a binary pump delivery system, and an automatic liquid sampler, was used and coupled with a mass spectrometer (model G1946A) from Agilent Technologies that operated in negative and positive modes using an electrospray ionization (ESI) source. The chromatographic conditions were the same as those used for the HPLC−ELSD protocol. MS settings were in accordance with the study by Pelillo et al.,[18] with some modification as follows: drying gas flow (nitrogen), 9 L/min; nebulizer pressure, 50 psi; gas drying temperature, 350 °C; capillary voltage, 5000 V; fragmentor voltage, 100 mV; mass scan range, m/z 400−1000.

Isolation of Phospholipids by TLC and Gas Chromatographic Determination of Phospholipid Fatty Acids. About 20 mg of the extracted lipids in chloroform/methanol were dried under nitrogen, dissolved in 0.2 mL of chloroform, and loaded on a silica gel 60 TLC plate, 20 × 20 cm (Merck KGaA, Darmstadt, Germany). The mobile phase was 100 mL of a 3:2 (v/v) *n*-hexane/diethyl ether mixture. The phospholipid band was visualized under UV light (254 nm) by spraying the TLC plate with a 0.02% (m/v) ethanolic solution of 2,7-dichlorofluorescein (sodium salt) and then scraped off and collected. Phospholipids were extracted 3 times with chloroform (3 × 1 mL). Organic extracts were pooled and dried under nitrogen, and to convert fatty acids to the corresponding methyl esters (FAMEs), the method by Christie[19] was carried out. The FAMEs were analyzed by GC using a fused silica capillary column BPX70 (10 m × 0.1 mm inner diameter, 0.2 μm footing thickness) from SGE Analytical Science (Ringwood, Victoria, Australia). The column was fitted on a GC-2010 Plus gas chromatograph from Shimadzu (Shimadzu Corp., Tokyo, Japan). The injector and flame ionization detector (FID) temperatures were set at 240 °C. Hydrogen was used as a carrier gas at the flow of 0.80 mL/min. The oven temperature was held at 50 °C for 0.2 min, increased from 50 to 175 °C at 120.0 °C/min, held at 175 °C for 2 min, increased from 175 to 220 °C at 20.0 °C/min, and finally increased from 220 to 250 °C at

50.0 °C/min. The samples were injected in split mode (0.4 μL) with a split ratio set at 1:10. Peak identification was accomplished by comparing peak retention times to those of two FAME standard mixtures: reference standard GLC-463 from Nu-Check (Elysian, MN) and FAME 189-19 from Sigma-Aldrich (St. Louis, MO). The two solution were used for their different fatty acid compositions and furnished complementary information.

Statistical Analysis. Tukey's honest significant difference multiple comparison [one-way analysis of variation (ANOVA)], $p < 0.05$ level, was evaluated using Statistica 6.0 software (StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Identification of Phospholipids and Their Relative Proportion in Different Oat Samples by HPLC−ELSD. The first aim of this work was to carry out a simple and sensitive method enabling the determination of oat phospholipids directly from fat extract, without a previous purification. Basically, the different methods reported in the literature centered on the phospholipid fraction of different foods using HPLC−ELSD and changed as a function of the mobile phases used. Pelillo et al.[18] determined the phospholipids of wheat using 80:19.5:0.5 (v/v/v) chloroform/methanol/30% ammonia solution as mobile phase A and 60:34:5.5:0.5 (v/v/v/v) chloroform/methanol/water/30% ammonia solution as mobile phase B. Recently, a separation of the different classes of phospholipids was achieved by Lopez et al.[17] using a gradient elution ending with a mobile phase of increased polarity. This method used a gradient with 87.5:12:0.5 (v/v/v) chloroform/methanol/buffer (1 M formic acid, neutralized to pH 3 with triethylamine) as phase A, and different proportions of the same solvents, 28:60:12 (v/v/v) chloroform/methanol/buffer, as phase B, in a flow rate of 0.5 mL/min.

Initially, chromatographic conditions developed by Pelillo et al.[18] were applied. Although the fat extract without purification steps was injected, the elution of high amounts of nonpolar (triglycerides) and more polar (diglycerides, monoglycerides, and free fatty acids) lipids did not affect the correct identification and quantitation of the main phospholipid classes. However, as reported by Pelillo et al.,[18] in the case of PG, its quantitation was difficult because PG co-eluted with some free fatty acids (triglycerides and diglycerides eluted before). A standard mix solution was spiked with oleic acid to verify the co-elution, and the results confirmed that the PG peak was overlapped with free fatty acids. For this reason, the method developed by Lopez et al.[17] was tested to evaluate the phospholipid content in the oat lipid extract. It was noted, however, that to obtain an adequate separation of phospholipids from other interfering compounds and a satisfactory signal-to-noise ratio required optimizing some chromatographic conditions, such as the flow rate in HPLC and the gas flow in the ELSD. The best results were obtained using a flow rate of 0.25 L/min in HPLC and a gas flow of 1.0 mL/min in ELSD. With this method, contrary to the results obtained with alkaline mobile phases by Pelillo et al.,[18] the PG peak was not overlapped with free fatty acids. In fact, this method permitted the separation and quantitation of PG easily.

The repeatability of the method was assessed for an extract of Comun oat. The extract was injected 4 times on the same day (intraday precision) and for 3 consecutive days (interday precision; $n = 12$). The percent relative standard deviations (% RSDs) of the peak areas and retention times were determined for each peak detected. The intraday repeatability (expressed as % RSDs) of the retention times was 0.3−3.0%, whereas the interday repeatability was 1.3−3.1%. The intraday repeatability (expressed as % RSDs) of the total peak area was 0.8%, whereas the interday repeatability was 1.9%.

Figure 1 shows the phospholipid profile obtained for the Comun variety. Phospholipids identified in all oat samples

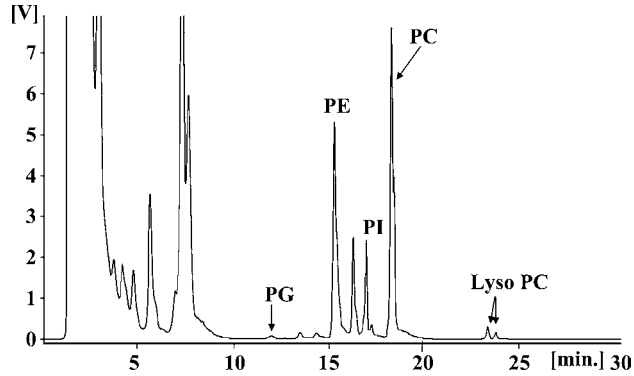

Figure 1. Chromatogram of oat phospholipids using the optimized HPLC−ELSD conditions: 87.5:12:0.5 (v/v/v) chloroform/methanol/buffer (1 M formic acid, neutralized to pH 3 with triethylamine) as mobile phase A and 28:60:12 (v/v/v) chloroform/methanol/buffer as mobile phase B. The chromatogram corresponds to the Comun oat variety analyzed.

analyzed corresponded to PG, PE, PC, PI, and lyso-PC. Calibration curves were obtained using diluted concentrations of the different phospholipid standards as PG, PE, PC, PI, and lyso-PC at a concentration range from 1 to 500 μg/mL, injected in triplicate. A satisfactory limit of detection (LOD), ranging from 0.002 to 0.010 mg/mL, was obtained. The relative proportion of each class of phospholipid is presented in Table 1. These data were calculated using three different lipid extracts of the oat samples injected in duplicate. The phospholipid percentage of the five varieties Comun, Lovrin 1, Lovrin T-27, Jeremy, and Mures was 4.6, 3.8, 4.1, 4.0, and 10.0% of the total lipids, respectively. These results were near the values reported by Moazzami and co-workers,[20] who showed that phospholipid concentrations in oats were in the range of 6−26%, considering that some factors, such as cultivar and agronomic conditions, can influence the phospholipid content. In fact, Jood et al.[21] demonstrated that phospholipids in wheat were more adversely affected than other classes of lipids because of the attack of insect species. The average total phospholipid content was 53.2 mg/100 g of fat (coefficient of variation of 49.8%), and phospholipids ranged from 38.0 to 100.3 mg/100 g of fat. The phospholipid content for each variety was similar, with the exception of Mures oat, which presented a high content for PE, PI, PC, and lyso-PC compared to other cultivars.

The individual content of oat phospholipids was also determined. Comun, Lovrin 1, Lovrin 27-T, and Mures samples were characterized by PE as the main component. The PE concentration in the Mures sample was high (35%) compared to the values for the other varieties analyzed (22−29%). In the case of PI, PC, and lyso-PC, their contents were similar in all samples analyzed. The percentage of PG ranged from 2% (in the Mures variety) to 6% (in the Jeremy variety) of total phospholipids.

Currently, information about the phospholipid content in cereals is very poor.[20] Our results were compared to previous data reported in the literature. Although Doehlert and co-workers[14] reported high concentrations of PC, PG, PE, and lyso-PC, results obtained in this work differ, possibly because of differences in the oat varieties analyzed and also the extraction procedure used. With regard to PI, our results are higher than those reported using TLC,[11] possibly because of an incomplete recovery from TLC. The high content of PI (about 20% of total

Table 1. Phospholipid Content (mg/g of Total Lipids) and Percentage of Each Phospholipid (on Total Phospholipid Content) in Different Oat Varieties[a]

	Comun		Lovrin 1		Lovrin 27-T		Jeremy		Mures	
	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%
PG	2.4 ± 0.2 a	5	1.7 ± 0.3 a,b	5	2.1 ± 0.4 a	5	2.2 ± 0.2 a	6	1.8 ± 0.3 a,b	2
PE	13.3 ± 0.4 b	29	10.3 ± 0.2 c	27	11.5 ± 0.4 c	28	8.9 ± 0.2 d	22	35.6 ± 0.3 a	35
PI	9.3 ± 0.5 b	20	8.2 ± 0.4 b	22	8.7 ± 0.9 b	21	9.0 ± 0.7 b	22	16.9 ± 0.2 a	17
PC	10.4 ± 0.6 b	22	8.4 ± 0.1 b	22	9.2 ± 0.3 b	22	8.9 ± 0.4 b	22	23.4 ± 0.4 a	23
lyso-PC	10.9 ± 0.5 b	24	9.4 ± 0.7 b	25	9.7 ± 0.9 b	24	11.3 ± 0.7 b	28	22.6 ± 0.3 a	23
sum	46.3 ± 1.0 b		38.0 ± 0.9 c		41.2 ± 1.2 b,c		40.3 ± 0.9 b,c		100.3 ± 0.7 a	

[a]Different letters in the same line indicate significant differences ($p < 0.05$).

Table 2. Molecular Species of Phospholipids Extracted from Oats Determined by Negative and Positive Ions by HPLC−ESI−MS Analysis[a]

phospholipid class	retention time (min)	observed ion	*m/z*	combination of molecular species
PG	11−12	$[M - H]^-$	743	C16:0/C18:3
		$[M - H]^-$	745	C16:0/C18:2
		$[M - H]^-$	747	C16:0/C18:1
		$[M - H]^-$	767	C18:2/C18:3
		$[M - H]^-$	769	C18:2/C18:2; C18:1/C18:3
		$[M - H]^-$	771	C18:1/C18:2; C18:0/C18:3
PE	14.2−15.3	$[M - H]^-$	688	C14:0/C18:1
		$[M - H]^-$	712	C16:0/C18:3
		$[M - H]^-$	714	C16:0/C18:2
		$[M - H]^-$	716	C16:0/C18:1
		$[M - H]^-$	736	C18:2/C18:3
		$[M - H]^-$	738	C18:2/C18:2; C18:1/C18:3
		$[M - H]^-$	740	C18:1/C18:2; C18:0/C18:3
		$[M - H]^-$	742	C18:1/C18:1
PI	16.5−17.4	$[M - H]^-$	831	C16:0/C18:3
		$[M - H]^-$	833	C16:0/C18:2
		$[M - H]^-$	835	C16:0/C18:1
		$[M - H]^-$	837	C16:0/C18:0
		$[M - H]^-$	857	C18:2/C18:2; C18:1/C18:3
		$[M - H]^-$	859	C18:1/C18:2; C18:0/C18:3
		$[M - H]^-$	861	C18:0/C18:2; C18:1/C18:1
		$[M - H]^-$	863	C18:0/C18:1; C16:0/C20:1
PC	17.8−18.7	$[M + H]^+$	756	C16:0/C18:3
		$[M + H]^+$	758	C16:0/C18:2
		$[M + H]^+$	760	C16:0/C18:1
		$[M + H]^+$	762	C16:0/C18:0
		$[M + H]^+$	782	C18:2/C18:2
		$[M + H]^+$	784	C18:1/C18:2
		$[M + H]^+$	786	C18:1/C18:1; C18:0/C18:2
		$[M + H]^+$	788	C18:1/C18:0
		$[M + HCOO]^-$	802	C16:0/C18:2
		$[M + HCOO]^-$	804	C16:0/C18:1
		$[M + HCOO]^-$	806	C16:0/C18:0
		$[M - CH_3]^-$	768	C18:1/C18:2
		$[M - CH_3]^-$	770	C18:1/C18:1; C18:0/C18:2
lyso-PC	22.6−24.0	$[M + HCOO]^-$	540	C16:0
		$[M + HCOO]^-$	564	C18:2
		$[M + HCOO]^-$	566	C18:1
		$[M - CH_3]^-$	480	C16:0
		$[M - CH_3]^-$	504	C18:2
		$[M - CH_3]^-$	506	C18:1
		$[M + H]^+$	496	C16:0
		$[M + H]^+$	520	C18:2
		$[M + H]^+$	522	C18:1
		$[M + H]^+$	550	C20:1

[a]Retention times, observed ions, and nominal masses obtained for each molecular species are also included.

phospholipid composition) is very interesting because several studies have shown that PI affects lipoprotein metabolism by controlling interactions and regulating signaling pathways.[22−24] In fact, it has been demonstrated that PI can act with increasing cholesterol excretion.[24] Burgess et al.[25] determined the safety and therapeutic value, after oral administration, of PI in normolipidemic (normal levels of lipids) human subjects. The administration of PI increased the high-density lipoprotein cholesterol (HDL-C), which has an inverse association with coronary artery disease, in the plasma of subjects receiving PI with food, while also reducing the plasma triglycerides.

Identification of Phospholipids and Their Relative Proportions in Different Oat Samples by HPLC−MS. HPLC coupled to ESI−MS has been successfully used in the analysis of phospholipids.[26,27] This analysis confirmed the presence of five principal phospholipid classes in oats: PG, PE, PI, PC, and lyso-PC, all of which eluted at different retention times, as reported in Table 2. The mass spectra of PG, PE, and PI species showed ions corresponding to the deprotonated molecules $[M - H]^-$. In contrast, $[M - CH_3]^-$, $[M + H]^+$, and $[M + HCOO]^-$ ions were observed for the charged PC and lyso-PC (Table 2).

As Table 2 shows, ESI−MS spectra of PG in negative-ion mode revealed six major molecular species, all represented by deprotonated ion $[M - H]^-$. According to other authors,[17,28] the main molecular species identified in PG were C16:0, C18:0, C18:1, C18:2, and C18:3. As reported in Table 3, from the different molecular species of PG, PG (C18:2/C18:3) and PG (C18:1/C18:2 or C18:0/C18:3) were the main species.

Analysis in negative-ion mode of the PE class revealed eight major molecular species. As reported in Table 2 and according to Pelillo et al.[18] and Herchi et al.,[28] these values confirmed the presence of C14:0, C16:0, C18:0, C18:1, C18:2, and C18:3 on the glycerol backbones of phospholipids. For PE, the pre-

Table 3. Distribution of the Molecular Species (%) of Phospholipids in Oat Samples[a]

phospholipids	Comun	Lovrin 1	Lovrin 27-T	Jeremy	Mures
		PG			
C16:0/C18:3	14.9 ± 0.8	14.6 ± 0.5	14.7 ± 0.7	14.9 ± 0.5	15.1 ± 0.8
C16:0/C18:2	9.9 ± 0.3	10.1 ± 0.6	9.8 ± 0.5	9.7 ± 0.4	9.9 ± 0.7
C16:0/C18:1	5.9 ± 0.2	6.0 ± 0.3	6.2 ± 0.3	6.2 ± 0.2	5.8 ± 0.1
C18:2/C18:3	25.7 ± 0.6	26.0 ± 0.9	25.8 ± 0.8	25.5 ± 0.5	25.4 ± 0.7
C18:2/C18:2; C18:1/C18:3	19.8 ± 0.4	19.6 ± 0.2	19.5 ± 0.5	19.6 ± 0.3	20.1 ± 0.5
C18:1/C18:2; C18:0/C18:3	23.8 ± 0.7	23.7 ± 0.8	24.0 ± 0.6	24.1 ± 0.6	23.8 ± 0.5
		PE			
C14:0/C18:1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C16:0/C18:3	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
C16:0/C18:2	21.4 ± 0.6	21.5 ± 0.4	21.3 ± 0.6	20.9 ± 0.5	21.3 ± 0.4
C16:0/C18:1	9.8 ± 0.5	10.0 ± 0.6	10.2 ± 0.6	10.1 ± 0.8	10.5 ± 0.5
C18:2/C18:3	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
C18:2/C18:2; C18:1/C18:3	32.6 ± 0.9	32.4 ± 0.6	32.5 ± 0.7	32.8 ± 0.8	31.8 ± 0.5
C18:1/C18:2; C18:0/C18:3	27.3 ± 0.6	27.1 ± 0.3	27.2 ± 0.5	26.8 ± 0.4	27.5 ± 0.5
C18:1/C18:1	7.4 ± 0.3	7.7 ± 0.4	7.5 ± 0.4	7.8 ± 0.5	7.2 ± 0.4
		PI			
C16:0/C18:3	5.2 ± 0.3	5.0 ± 0.1	5.1 ± 0.4	4.9 ± 0.3	5.0 ± 0.4
C16:0/C18:2	2.4 ± 0.3	2.5 ± 0.2	2.3 ± 0.4	2.6 ± 0.3	2.5 ± 0.4
C16:0/C18:1	43.6 ± 0.6	43.5 ± 0.5	43.2 ± 0.5	43.6 ± 0.7	43.0 ± 0.6
C16:0/C18:0	16.3 ± 0.3	16.5 ± 0.4	16.7 ± 0.4	16.2 ± 0.3	16.5 ± 0.5
C18:2/C18:2; C18:1/C18:3	8.2 ± 0.3	8.0 ± 0.3	8.2 ± 0.2	8.4 ± 0.4	8.1 ± 0.2
C18:1/C18:2; C18:0/C18:3	10.4 ± 0.3	10.3 ± 0.4	10.0 ± 0.3	9.7 ± 0.2	10.2 ± 0.4
C18:0/C18:2; C18:1/C18:1	8.7 ± 0.2	9.1 ± 0.4	9.2 ± 0.3	9.7 ± 0.4	9.5 ± 0.3
C18:0/C18:1; C16:0/C20:1	5.2 ± 0.1	5.1 ± 0.3	5.3 ± 0.2	4.9 ± 0.2	5.2 ± 0.4
		PC			
C16:0/C18:3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
C16:0/C18:2	2.1 ± 0.1	1.9 ± 0.1	2.1 ± 0.2	2.2 ± 0.2	1.8 ± 0.1
C16:0/C18:1	21.8 ± 0.4	22.0 ± 0.3	22.3 ± 0.4	22.1 ± 0.2	22.5 ± 0.4
C16:0/C18:0	15.9 ± 0.2	15.7 ± 0.2	15.4 ± 0.3	15.6 ± 0.2	15.5 ± 0.3
C18:2/C18:2	2.3 ± 0.2	2.4 ± 0.1	2.2 ± 0.1	2.4 ± 0.2	2.3 ± 0.2
C18:1/C18:2	21.5 ± 0.5	21.3 ± 0.5	21.0 ± 0.4	21.5 ± 0.5	21.4 ± 0.3
C18:1/C18:1; C18:0/C18:2	25.3 ± 0.4	25.5 ± 0.3	25.6 ± 0.5	25.0 ± 0.4	24.9 ± 0.5
C18:1/C18:0	11.0 ± 0.3	11.1 ± 0.2	11.3 ± 0.3	11.0 ± 0.2	11.5 ± 0.4
		Lyso-PC			
C16:0	43.0 ± 0.7	43.2 ± 0.5	43.5 ± 0.8	43.0 ± 0.3	42.6 ± 0.4
C18:2	36.1 ± 0.5	36.0 ± 0.4	36.2 ± 0.4	36.1 ± 0.5	36.3 ± 0.6
C18:1	20.0 ± 0.3	19.8 ± 0.4	19.5 ± 0.3	19.5 ± 0.2	20.0 ± 0.3
C20:1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.4 ± 0.2	1.2 ± 0.2

[a]PG, PE, and PI were quantified as $[M - H]^-$, and PC and lyso-PC were quantified as $[M + H]^+$.

Table 4. Fatty Acids (% Area of Total Methyl Esters) of Phospholipids Isolated with TLC from Oats Determined by GC−FID

	Comun	Lovrin 1	Lovrin 27-T	Jeremy	Mures
C14:0	2.5 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	1.8 ± 0.1	2.9 ± 0.2
C16:0	24.2 ± 0.6	23.3 ± 0.5	24.4 ± 0.7	23.3 ± 0.4	24.3 ± 0.5
C18:0	13.7 ± 0.2	14.5 ± 0.2	13.2 ± 0.1	10.9 ± 0.2	13.0 ± 0.2
C18:1c9	17.7 ± 0.1	17.8 ± 0.1	17.0 ± 0.1	22.1 ± 0.3	17.1 ± 0.1
C18:1c	1.2 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.1 ± 0.0
C18:2n-6	26.4 ± 0.7	25.9 ± 0.8	27.0 ± 0.7	31.0 ± 1.0	27.7 ± 0.8
C18:3	1.7 ± 0.1	2.7 ± 0.3	2.6 ± 0.2	1.6 ± 0.1	2.6 ± 0.2
C20:1	6.8 ± 0.3	5.7 ± 0.2	6.4 ± 0.3	3.9 ± 0.1	5.4 ± 0.2
C20:2	5.8 ± 0.2	6.3 ± 0.2	5.8 ± 0.3	4.2 ± 0.2	5.8 ± 0.2
SFA	40.4	40.5	40.0	36.1	40.2
MUFA	25.7	24.6	24.6	27.1	23.6
PUFA	33.9	34.9	35.4	36.8	36.2
SFA/UFA	0.7	0.7	0.7	0.6	0.7

dominant species were PE (C18:2/C18:2 or C18:1/C18:3) and PE (C18:1/C18:2 or C18:0/C18:3) (Table 3).

In the case of PI, analysis in negative-ion mode revealed eight principal molecular species corresponding to PI with a combination

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of C16:0, C18:0, C18:1, C18:2, C18:3, and C20:1 fatty acids. Similar data were reported in the literature.[18,28] PI reported PI (C16:0/C18:1) as the main molecular species (Table 3).

For PC, analysis in positive- and negative-ion mode furnished more and/or complementary information to identify main molecular species. Effectively, according to Pulfer and Murphy,[26] eight major molecular species were reported when positive ionization was applied; in addition, five molecular species were described in negative-ion mode according to Pelillo et al.[18] and Herchi and coworkers.[28] The identification of the molecular species for PC was possible in both ionization modes, as $[M + H]^+$ in the positive-ion mode and $[M + HCOO]^-$ or $[M - CH_3]^-$ in the negative-ion mode. PC (C18:1/C18:1 or C18:0/C18:2), PC (C16:0/C18:1), and PC (C18:1/C18:2) were the most abundant molecular species (Table 3).

As reported for the PC class, lyso-PC fatty acid constituent was analyzed in negative- and positive-ion mode. Except for lyso-PC (C20:1) that showed only a protonated molecule ($[M + H]^+$) with a nominal mass of m/z 550, all of the identified fatty acids C16:0, C18:1, and C18:2 present in lyso-PC were described as $[M + HCOO]^-$, $[M - CH_3]^-$, and $[M + H]^+$. Lyso-PC (C16:0), lyso-PC (C18:2), and lyso-PC (C18:1) were the first, second, and third molecular species, respectively. C20:1 was the least abundant in lyso-PC (Table 3).

Total Fatty Acid Composition of Phospholipids. To obtain the phospholipid fraction from the total lipid extract, a TLC separation was carried out. This technique permits the isolation of the different lipid classes.[29] To establish the retention factor (R_f) of the phospholipid class and to verify the absence of co-elution with other lipid compounds, a solution containing triglycerides, free fatty acids, tocopherols, sterols, esterified sterols, and phospholipids was deposited on a TLC plate. After elution, the R_f value of each lipid class was established. As expected, phospholipids reported a R_f value equal to 0 because they did not migrate with the solvent mixture used. R_f values of 0.15, 0.26, 0.53, and 0.94 were reported for sterols + diglycerides, free fatty acids, triglycerides, and esterified sterols, respectively.

The phospholipid class of oat samples was obtained after TLC elution. The band relative to phospholipids was collected and extracted, as reported in the Materials and Methods. The phospholipid extract was methylated, and the fatty acids were analyzed by GC−FID. The fatty acid content of total phospholipids from oat samples is shown in Table 4. In all samples, the unsaturated fatty acids (UFAs) ranged from 60 to 63% of total phospholipid fatty acids. More specifically, polyunsaturated fatty acids (PUFAs) were the most representative UFAs, ranging between 33.9 and 36.8%, while the monounsaturated fatty acids (MUFAs) were 23.6−27.1% of the total phospholipid fatty acids. Saturated fatty acids (SFAs) were in the range of 36.1−40.5%. The SFA/UFA ratio was 0.7 in all samples, except in the Jeremy sample that reported a SFA/UFA ratio equal to 0.6.

The fatty acid content reported significant differences in several fatty acids, including major (C16:0, C18:0, C18:1, and C18:2) and minor (C14:0, C18:3, C20:1, and C20:2) species. The linoleic acid (C18:2) was the major fatty acid of the phospholipid fraction in the range of 25.9−31.0%, according to previous results obtained by Sahasrabudhe et al.[11] Palmitic (C16:0), oleic (C18:1), and stearic (C18:0) acids were the second, third, and fourth phospholipid fatty acids in the range of 23.3−24.4, 17.0−22.1, and 10.9−14.5%, respectively. Other fatty acids were gondoic acid (C20:1), eicosadienoic acid (C20:2), myristic acid (C14:0), and linolenic acid.

Using a combination of techniques, such as HPLC−ELSD, HPLC−ESI−MS, and GC, some differences about polar lipid composition of oats were provided. The variety of oat showed a consistent effect on the phospholipid content and their fatty acid profile. The total phospholipid content of the Mures cultivar was the highest compared to the other oat lines.

The HPLC−ESI−MS and GC analyses showed complementary qualitative and quantitative information about the phospholipid fatty acid composition. UFAs represented about 60% of total phospholipid fatty acids. Because of the fact that the information available about the phospholipid fraction in oats is very limited, all data obtained here allow for the collection of more information about these polar lipids. The results obtained in this work corroborate oat as an important cereal for its proven benefits in human health and nutrition.

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Notes

The authors declare no competing financial interest.

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