

Triacylglycerols and Phospholipids Composition of Caper Seeds (*Capparis spinosa*)

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Abstract The goal of this study is to evaluate for the first time the composition of triacylglycerols (TAG) using ESI-TOF-MS and phospholipids species using HPLC-ESI-TOF-MS of two *Capparis spinosa* seed oil populations. Results show that LOO, LOP, LLO, OOO, PLL and POO were the major molecular species of triacylglycerol detected in caper seeds; where L represents linoleic acid; O, oleic acid; and P, palmitic acid. The TAG composition was significantly different among the two *C. spinosa* populations. In Ghar el Melh population, LOO (15.7%) was detected as the dominant TAG molecular species, followed by LOP (13.2%), LLO (12.0%) and OOO (11.4%); while, the dominant fraction was LLO (14.2%) followed by LOO (14.1%), LOP (11.5%) and PLL (10.5%) in Chouigui samples. The major component in the phospholipids fraction was

phosphatidylinositol (ca. 54–91%), followed by phosphatidylglycerol, phosphatidylethanolamine and phosphatidic acid. A variety of molecular species within each class were identified. The major component in all phospholipids species contains a C-18:1 lipid chain. C16:0/C18:2-PI (ca. 28–31%) was the most abundant PI. PG species were mainly C18:2/C18:1-PG (25–32%). The major PE was C18:1/C18:1-PE (44–75%). The major PA species was C18:1/C18:1-PA (22–24%).

Keywords Caper (*Capparis spinosa*) · LC/ESI-MS · Triacylglycerols · Phospholipids · Composition

Introduction

The biosynthesis and accumulation of storage lipids by plants has been the subject of intense study for several decades [1]. Food chemists have given considerable attention to the structures and ratios of the different fractions of vegetable oil acylglycerols [2]. Four classes of lipids are habitually found in vegetable oils: triacylglycerols (TAG), diacylglycerols, polar lipids and free fatty acids. In most plant seeds, lipids are predominantly of TAG. TAG composition has been established as a measurement of the quality and purity of vegetable oils [3].

The stability and quality of vegetable oils are influenced by the presence of minor constituents, such as phosphatides. Phospholipid components can be used to evaluate the quality of crude oil from oilseeds [4]. Indeed, phospholipids are essential components of cell membranes and play many roles such as maintaining homeostasis of cells, mediation of signal transduction and as ligands for receptors [5]. In addition, they can act as antioxidants due to their synergistic action, their metal scavenging activity, and

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their catalytic activity to decompose hydroperoxides [6, 7]. Phospholipids have positive effects on human health and have a wide industrial application in food and nutraceuticals, cosmetics, agricultural products, and pharmaceuticals [8, 9].

Caper is a perennial shrub and is the common name of the genus *Capparis*, family Capparidaceae. *Capparis spinosa* has a large natural distribution in the Mediterranean Sea basin. Different parts of this plant can be used as drugs or in cosmetics. Before its commercialization, flower buds are pickled in vinegar or preserved in salt. Furthermore, the fruit with small soft seeds is preferred for the production of pickles. Previous studies reported the tocopherols, carotenoids, sterols, aliphatic and triterpenic alcohols contents of caper (*Capparis spinosa*) seeds [10–13]. Seeds of *C. spinosa* are rich in oil (ca. 30%) mainly unsaturated (ca. 70%). Oleic and linoleic acid are the major fatty acids. High quantities of sterols have been detected in this plant (more than 2,200 mg kg⁻¹) and β -sitosterol was the major form. *C. spinosa* seed oil also contains high levels of tocopherols (between ca. 250 and 1,980 mg 100 g⁻¹); γ -tocopherol was the major homologue followed by α -tocopherol. Total carotenoids were also quantified in a good amount (ca. 457 mg 100 g⁻¹) and β -carotene was the major form. Triterpenic and aliphatic alcohol contents were ca. 396 and 45 mg kg⁻¹, respectively. All these compounds have a wide range of nutritional and medicinal uses. For these reasons, seeds of *C. spinosa* are especially attractive for producing oil for both food and pharmaceutical applications.

However, molecular species of the TAG and PL have not yet been reported. Therefore, the objective of this study was to characterize TAG and PL molecular species of *Capparis spinosa* seeds.

Materials and Methods

Chemicals

Methanol, and *n*-hexane, solvents of LC grade, were purchased from Panreac Quimica SA. (Barcelona, Spain). Isopropanol and acetic acid were from Fisher Scientific SA (Loughborough, Spain).

Sample Preparation and Oil Extraction

Seeds were collected from 8 to 13 plants from each sampling location, i.e., Ghar el Melh (GM) and Chouigui (CH). Seeds collected from a specific location were mixed and then a representative sample used for oil extraction. The oil from the seeds was extracted as reported previously [13] following ISO method 659:1998 [14].

Phospholipids Determination

The phospholipid composition was determined according to the method described by Harrabi et al. [15]. Liquid chromatography was performed on an HP 1050 Ti series gradient pump having a 20- μ l sample loop. A Lichrospher 100 diol column (250 mm \times 4.6 mm, 5 μ m particle size; Merck, Germany) was used to separate the phospholipids by class. The binary solvent gradient consisted of solvent mixture A: hexane–isopropanol–acetic acid–triethylamine (82:17:1:0.08 v/v/v/v) and mixture B: isopropanol–water–acetic acid–triethylamine (85:14:1:0.08 v/v/v/v). The gradient started at 5% mixture B and its percentage was increased to 80% over 25 min. This composition was maintained for 1 min before being returned to 5% B over 10 min and maintained at 5% for another 4 min (40 min total run time). The flow rate of 150 μ l/min through the column was introduced directly to the electrospray ionization source of the mass spectrometer. Glycerophospholipids were detected with a QTOF2 (Waters Micromass, Manchester, UK) electrospray ionization quadrupole time-of-flight mass spectrometer (operating in negative ion mode) employing the MassLynx (v.4.0) control and processing software. The 150 μ l/min flow of eluent was passed through the electrospray capillary, which was held at –3.11 kV. The source block temperature was 100 °C and the cone voltage was set at –68 V. N₂ drying gas was passed coaxially to the capillary with a flow rate of approximately 440 l/h and N₂ cone gas was passed around the entrance to the mass spectrometer at a rate of 200 l/h. Relative quantification between the different glycerophospholipids classes was achieved by the summation of the chromatogram ion peak areas of all the species of one class divided by the total chromatogram ion peak areas of all classes. The two fatty acid chains (C1, C2) of the glycerophospholipids were identified using tandem mass spectrometry with collision-induced dissociation [16].

Triacylglycerol Determination

The TAG composition was determined according to the method described by Harrabi et al. [17]. Triacylglycerol containing sodium adducts (+23 *m/z*) were detected with the same QTOF2 electrospray ionization quadrupole time-of-flight mass spectrometer (operating in positive ion mode). The TAG-containing solution was injected into a 20- μ l sample loop and carried by a methanol phase with a flow of 60 μ l/min to the electrospray capillary, which was held at 3.3 kV. The source block temperature was 100 °C and the cone voltage was set at 50 V. N₂ drying gas was passed coaxially to the capillary with a flow rate of approximately 440 l/h and N₂ cone gas was passed around the mass spectrometer entrance at a rate of 200 l/h. The

total mass spectral intensities were used to quantify the different TAG species. Quantification was then achieved by comparison with the total ion count of the internal standard (Tripalmitin) with intensity corrections made for isotopic peak overlap using the following equation:

$$[\text{TAG}] = \text{TAG}_{\text{intensity}} \frac{[\text{STD}]}{\text{STD}_{\text{intensity}}}$$

Statistical Analysis

The experimental data were analyzed using the analysis of variance (ANOVA) and the Statistical Analysis System (XLSTAT 2008). Differences at $P < 0.05$ were considered statistically significant by Duncan's new multiple range test. Analyses were performed in triplicate.

Results and Discussion

Triacylglycerols Composition

As we have previously reported [11, 13], the mean content of total lipids was $32.2 \pm 1.95\%$ dry weight basis. The values were between 30.8% (GM) and 33.6% (CH).

Figure 1 shows a typical TAG *C. spinosa* chromatogram. The distribution of TAG detected in caper seed oil is presented in Table 1. Seventeen molecular species of TAG were detected.

The total mass spectral intensities (Fig. 2) were used to quantify the different TAG species; as one TAG that contains one more or one fewer double bonds than a different TAG will only differ by 2 mass units, isotopic effects had to be considered. For example, the sodium adducted PPL and POP will have an m/z of 853.7 and 855.7, respectively. The theoretical percentage of PPL containing two C_{13} isotopes (855.7 m/z) is 18.4% (relative to PPL containing no C_{13} isotopes). This directly coincides with the m/z of POP. The experimental intensities arising from PPL (853.7) and PPL- $1C_{13}$ (854.7 m/z) were summed with the theoretical intensities of PPL- $2C_{13}$ (855.7 m/z) and PPL- $3C_{13}$ (856.7 m/z) to give the total ion intensity.

A corrected intensity of POP (855.7 m/z) was calculated by subtracting the theoretical intensity of PPL- $2C_{13}$ (855.7 m/z) from the experimental intensity at that m/z . The same was done for POP- $1C_{13}$; experimental intensity 856.7 less the theoretical intensity of PPL- $3C_{13}$ to give corrected POP- $1C_{13}$ intensity. These corrected intensities were then themselves used to calculate the theoretical intensities of their $2C_{13}$ and $3C_{13}$ counterparts. Quantification was then achieved by comparison with the total ion count of the internal standard (Tripalmitin). No distinction is made between triacylglycerols which are positional isomers.

Results show that the species LOO, LOP, LLO, OOO, PLL and POO were the major forms. To identify the TAG, we did not use the MS/MS. Indeed, the OOO can be designated by OLS, except that OOO is more abundant in plants.

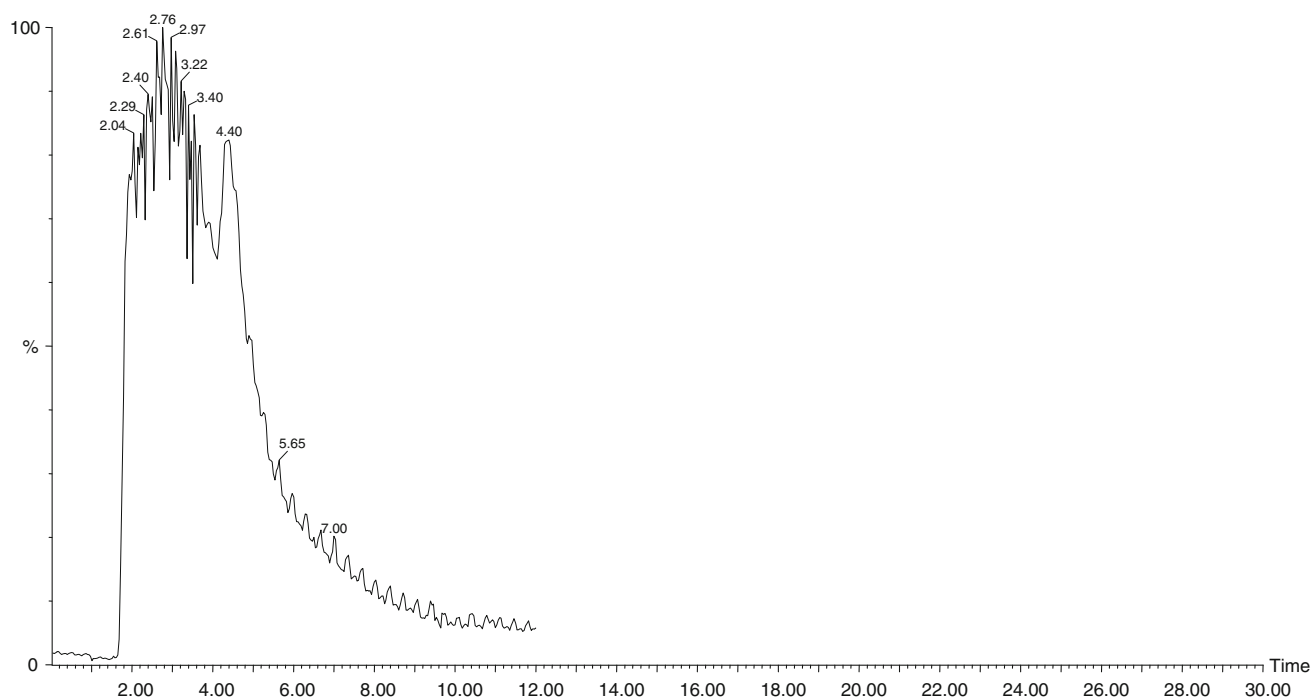


Fig. 1 Total ion chromatogram (TIC) of TAG (ESI-TOF-MS) of caper seeds in the positive mode of ionization

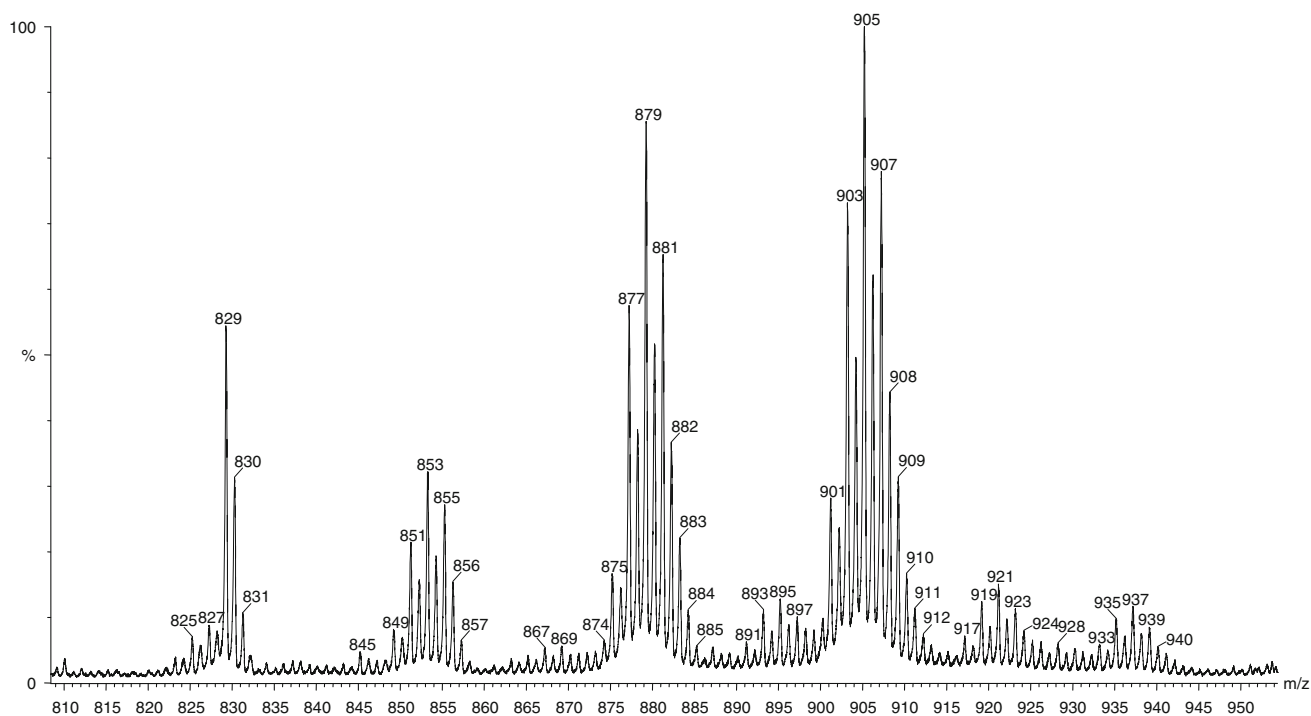
Table 1 Triacylglycerol content (mg/g) and composition (%) of *C. spinosa* seed oil

	GM		CH		<i>P</i> value*
	mg/g	%	mg/g	%	
PPL	141.1 ± 11	5.1	124.7 ± 25.3	4.9	0.0388
POP	96.3 ± 9.2	3.5	66.2 ± 15.3	2.6	0.0009
PLL _n	84.1 ± 5.7	3.0	113.4 ± 16.8	4.5	0.0020
PLL	251.0 ± 21	9.2	263.8 ± 28.7	10.5	NS
LOP	360.6 ± 32	13.2	288.9 ± 52.1	11.5	0.0058
POO	250.5 ± 22	9.2	172.8 ± 31.2	6.8	0.0009
SOP	64.2 ± 12	2.3	35.7 ± 5.6	1.4	0.0002
LnLnLn	59.8 ± 11	2.2	121.7 ± 12	4.8	<0.0001
LLnLn	ND	ND	76.5 ± 3.6	3.0	–
LLL _n	1.6 ± 0.9	0.1	ND	ND	–
LLL	143.6 ± 11	5.2	198.0 ± 31.2	7.9	0.0015
LLO	327.6 ± 26.3	12.0	357.8 ± 36.5	14.2	NS
LOO	427.1 ± 29	15.7	354.8 ± 34.8	14.1	0.0108
OOO	311.2 ± 24	11.4	198.3 ± 22.1	7.9	0.0004
OOS	104.6 ± 15	3.8	64.3 ± 12.3	2.5	0.0003
ALO	59.5 ± 5.8	2.1	44.5 ± 8.9	1.7	0.0022
BOP	32.4 ± 5.8	1.1	24.2 ± 4.8	0.9	0.0022

Each value in the table is represented as the mean ± SE (*n* = 3)

A arachidic acid; B behenic acid; P palmitic; S stearic; O oleic; L linoleic; Ln linolenic; NS not significant; ND not detected; GM Ghar el Melh; CH Chouigui

* *P* values were determined by the Duncan test, significantly different at (*P* < 0.05)

**Fig. 2** Spectrum of the different molecular species of TAG

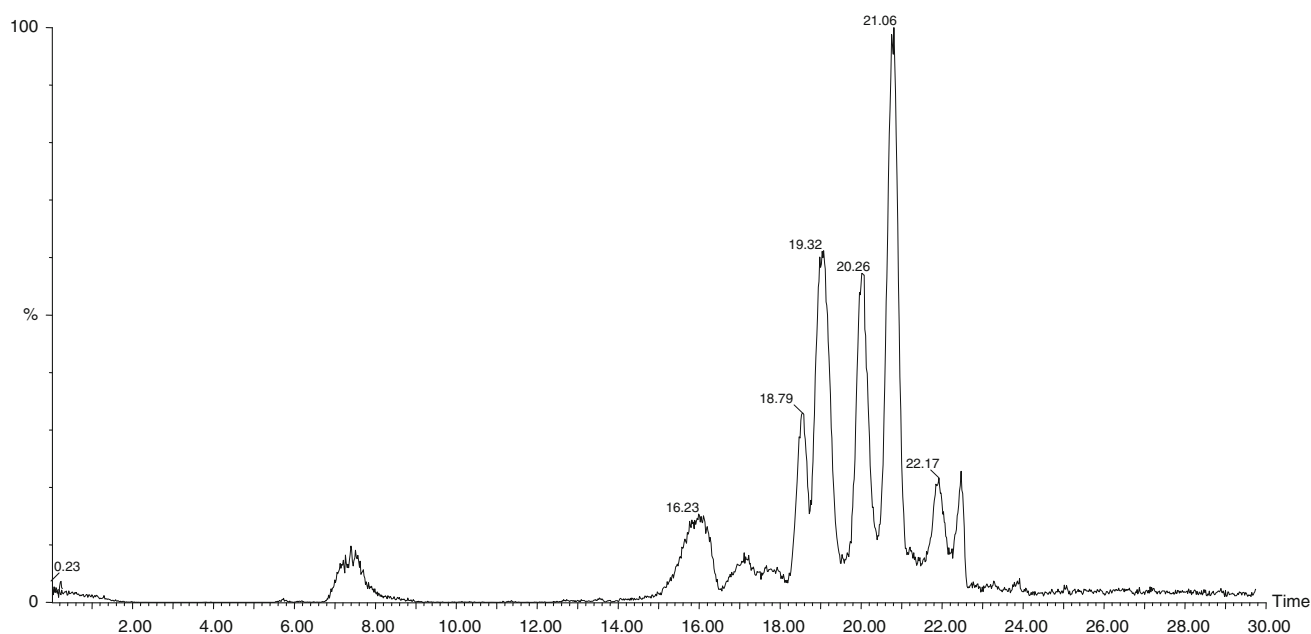


Fig. 3 Total ion chromatogram (TIC) of the different classes of phospholipids (LC/ESI-TOF-MS) of caper seeds in the negative mode: PA (16.23 min), PE (19.32 min), PG (20.26 min), PI (21.06 min)

Table 2 Glycerophospholipid classes identified by HPLC–MS in *C. spinosa* seed oil

Class	Odd/even mass	<i>m/z</i> values for major species	<i>m/z</i> values for major fragments	GM (%)	CH (%)
Phosphatidic acid (PA)	Odd	671, 673, 697, 699	255, 279, 281	2.0 ± 0.58	2.5 ± 0.9
Phosphatidylethanolamine (PE)	Even	742, 740, 716	281, 279, 255	7.1 ± 1.25	1.7 ± 0.62
Phosphatidylglycerol (PG)	Odd	771, 769, 745, 743	279, 281, 255, 277	36.3 ± 2.65	4.2 ± 1.26
Phosphatidylinositol (PI)	Odd	835, 833, 863, 861	255, 279, 281, 283	54.5 ± 3.25	91.5 ± 3.65

255 *m/z*: C16:0; 277 *m/z*: C18:3; 279 *m/z*: C18:2; 281 *m/z*: C18:1; 283 *m/z*: C18:0

GM Ghar el Melh; CH Chouigui

The TAG composition was significantly different between the two populations (Table 1). In GM population, the major fraction was LOO (15.7%) followed by LOP (13.2%), LLO (12.0%) and OOO (11.4%). While, the dominant fraction was LLO (14.2%) followed by LOO (14.1%), LOP (11.5%) and PLL (10.5%) in CH samples. Moreover, significant difference was detected between the minor fractions; such as SOP (ca. 64 and 35 mg/g, in GM and CH, respectively) and LnLnLn (ca. 59 and 121 mg/g, in GM and CH, respectively). In addition, LLnLn was detected in GM (76.5 mg/g) while CH did not contain this species.

These results agree with other researchers who reported that the TAG content of *Pinus pinea* can vary among populations [18]. Moreover, TAG composition can vary among species [19, 20].

Phospholipids Composition

Figure 3 shows a typical LC–MS chromatogram which displayed numerous peaks. As shown in Table 2, four

glycerophospholipid classes were detected in caper seed oil: phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidylinositol (PI). Generally, the most common phospholipids of eukaryotes are PC, PE, PI and PS [21].

Results show a significant difference between the two populations. PI is the major form (ca. 54 and 91% for GM and CH, respectively), followed by PG (ca. 36 and 4% for GM and CH, respectively). Moreover, PE occupy the third position for GM (ca. 7%) followed by PA (ca. 2%); while for CH it is the inverse (ca 2 and 1.5% for PA and PE, respectively). Indeed, the relative amounts of individual phospholipid species can vary greatly between different sources due to the influence of environmental stresses, such as temperature, nutrient supply, light and age [22, 23]. The differences can be also due to the species effect. Indeed, the major phospholipids in sunflower seed oil are PC followed by PE, PI and PA [22]. While, in corn seed oil the major phospholipids are PC followed by PI, and PE [15]. Moreover, Harrabi et al. [15] suggested that the quantitative and

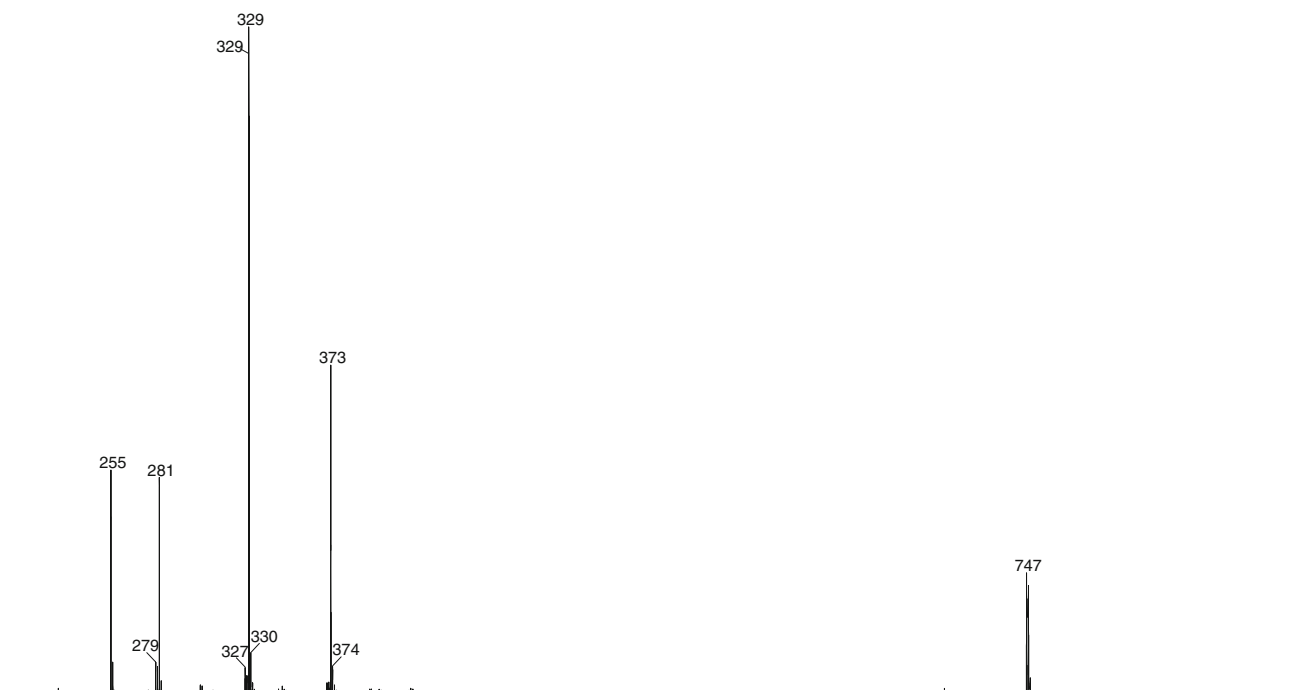


Fig. 4 Mass spectrum of the PG-C16:0/18:1 (m/z 747) (ESI-TOF-MS/MS) of caper seeds in the negative mode. 255 m/z : C16:0; 281 m/z : C18:1

Table 3 Distribution of the major molecular species of glycerophospholipids found in *C. spinosa* seed oil

	GM (%)	CH (%)	<i>P</i> value*
PA			
PA-C16:0/18:3 (m/z 669)	6.3 ± 0.8	6.9 ± 0.9	NS
PA-C16:0/18:2 (m/z 671)	16.7 ± 1.5	15.1 ± 1	0.0715
PA-C16:0/18:1 (m/z 673)	18.7 ± 1.8	14.3 ± 0.8	0.0029
PA-C18:3/18:2 (m/z 693)	6.0 ± 1	ND	–
PA-C18:2/18:2 (m/z 695)	7.3 ± 0.9	7.3 ± 1	NS
PA-C18:1/18:2 (m/z 697)	17.1 ± 2.3	24.1 ± 3.2	0.0012
PA-C18:1/18:1 (m/z 699)	22.1 ± 1.8	24.1 ± 2.3	NS
PA-C18:0/18:1 (m/z 701)	5.3 ± 0.8	7.9 ± 2.4	0.0007
PE			
PE-C16:0/18:2 (m/z 714)	5.0 ± 0.7	12.2 ± 1.4	<0.0001
PE-C16:0/18:1 (m/z 716)	8.8 ± 1.4	18.0 ± 2.5	<0.0001
PE-C18:2/18:2 (m/z 738)	2.0 ± 0.1	7.6 ± 1.5	<0.0001
PE-C18:2/18:1 (m/z 740)	8.5 ± 0.8	17.9 ± 3.1	<0.0002
PE-C18:1/18:1 (m/z 742)	75.5 ± 3.1	44.1 ± 5	0.0002
PG			
PG-C16:0/18:3 (m/z 743)	14.7 ± 1.2	ND	–
PG-C16:0/18:2 (m/z 745)	19.0 ± 1.3	20.3 ± 1.8	NS
PG-C16:0/18:1 (m/z 747)	2.1 ± 0.8	4.2 ± 1.2	0.0001
PG-C18:3/18:2 (m/z 767)	8.1 ± 1.7	9.3 ± 1.6	0.0254
PG-C18:2/18:2 (m/z 769)	23.0 ± 2.1	28.8 ± 2.3	0.0056

Table 3 continued

	GM (%)	CH (%)	<i>P</i> value*
PG-C18:2/18:1 (m/z 771)	25.1 ± 3.1	32.0 ± 1.5	0.0041
PG-C18:1/18:1 (m/z 773)	7.7 ± 0.9	5.2 ± 2.4	0.0007
PI			
PI-C16:0/18:3 (m/z 831)	2.8 ± 0.7	1.5 ± 0.5	0.0002
PI-C16:0/18:2 (m/z 833)	31.3 ± 0.6	28.7 ± 0.9	NS
PI-C16:0/18:1 (m/z 835)	29.9 ± 3.5	28.7 ± 1.8	NS
PI-C18:2/18:1 (m/z 859)	2.8 ± 0.7	3.7 ± 3.1	0.0033
PI-C18:1/18:1 (m/z 861)	16.3 ± 1.2	16.8 ± 1.5	NS
PI-C18:1/18:0 (m/z 863)	16.6 ± 1.6	20.3 ± 2.1	0.0078

Each value in the table is represented as the mean ± SE ($n = 3$)

GM Ghar el Melh; CH Chouigui; NS not significant; ND not detected

* *P* values were determined by the Duncan test, significantly different at ($P < 0.05$)

qualitative glycerophospholipid class compositions could be used for the detection of oil adulteration.

We do not know the relative position of the two fatty acyl substituents. However, the mass spectrum (Fig. 4) gives us the identify of the two fatty acyl substituents as outlined in Table 2. The content of the phospholipids in *C. spinosa* seed oils is given in Table 3. Mixtures of molecular species were identified in each class of glycerophospholipid. A significant difference was also detected.

The major components in all phospholipids species contain monounsaturated fatty acids (18:1) and polyunsaturated fatty acids (C18:2). C16:0/C18:2-PI (ca. 28–31%) was the most abundant PI followed by the C18:1/C18:0-PI (ca. 16–20%) and the C18:1/C18:1-PI (ca. 16–16%). PG species were mainly C18:2/C18:1-PG (25–32%) and C18:2/C18:2-PG (23–28%). The major PE was C18:1/C18:1-PE (44–75%). The major PA species was C18:1/C18:1-PA (22–24%) followed by C18:1/C18:2-PA (17–24%).

Conclusion

Different molecular species of TAG and PL were detected in *C. spinosa* seed oil for the first time. Therefore, our analysis and previous studies confirm that caper seeds can be considered a healthy oil source from their lipid composition point of view.

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