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Phospholipid composition of *Cucurbitaceae* seed oils

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Dedicated to Acad. Dimiter Ivanov on the occasion of his 120th birth anniversary

Phospholipid composition of three species of *Cucurbitaceae* family (*Cucurbita moschata, Cucurbita pepo* and *Cucurbita maxima*) grown in the southern Bulgaria, was investigated. Oil content of *Cucurbita moschata, Cucurbita pepo* and *Cucurbita maxima* seeds was 45.1%, 46.8% and 51.5%, respectively. Phospholipids were determined spectrophotometrically after separation by two dimensional thin layer chromatography. Total phospholipid content in *Cucurbita moschata, Cucurbita pepo* and *Cucurbita maxima* seed oils was 0.5%, 1.1% and 1.0%, respectively. Phosphatidylinositol (19.7-29.4%), phosphatidylcholine (34.8-48.6%) and phosphatidylethanolamine (16.0-23.7%) predominated in phospholipid fraction. The quantity of phosphatidic acids was found to be from 2.1% to 5.5%. Fatty acid composition of individual phospholipids was determined by gas chromatography. The predominant fatty acids of phospholipids were palmitic, stearic, oleic and linoleic acids.

Key words: phospholipids, pumpkin seed oil, fatty acids

INTRODUCTION

Phospholipids are the major structural constituents of all biological membranes. They are also present in plants, especially in seeds and kernels of different flora species. Phospholipids are complex lipids which are amphipathic in nature i.e. each molecule consists of a hydrophilic and a hydrophobic parts, thus tending to form lipid bilayers. They are present in many natural sources like human and animal tissues, plants and microbial organisms. Vegetable materials usually contain only small amounts of phospholipids, ranging from 0.3 to 2.5 wt% [1]. The major phospholipids present in plant sources are phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine. Phospholipids are removed as by-products during the degumming process of vegetable oil refining, too. They are used in food and cosmetic industry [2,3], thence a more detailed study is necessary. Phospholipids are present in pumpkin seeds; therefore the latter could be used as a source of these biologically active components. Their content in pumpkin seeds range from 1.09 to 1.27% [1,4-6]. Nevertheless, the phospholipid content and quantities in pumpkin seed oils have not been investigated in depth.

The most valuable pumpkin species in Bulgaria are *Cucurbita moschata*, *Cucurbita pepo* and *Cucurbita maxima*. There is no information about their phospholipid content and composition, so the aim of the current study is to present the comparative results of the investigation of phospholipid content and their individual composition in some *Cucurbitaceae* seed oils (three species of pumpkin seed oils) grown in southern Bulgaria. Fatty acid composition of the major phospholipids was investigated, too, and the results were collated to the respective glyceride oils.

MATERIALS AND METHODS

The investigation was carried out with seeds of *Cucurbita moschata*, *Cucurbita pepo*, *Cucurbita maxima* grown in southern Bulgaria, crop 2012. All investigations were carried out with hulled and air-dried seeds. All solvents and reagents were of analytical grade from Merck (Darmstadt, Germany) and were used without additional purification.

Extraction of lipids

The seeds (50 g sample) were ground to powder and the oil was extracted with n-hexane in Soxhlet apparatus for 8 h. After rotation vacuum evaporation of the solvent the extracted oils were dried and weighed [7].

Analysis of phospholipids

Another part (50 g) of air-dried seeds was subjected to Folch extraction [8] and polar lipids were isolated from the total lipids by column

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chromatography. The column chromatography was performed according to the procedure reported by Angelova-Romova *et al.* [9]. The sample (about 100 mg oil) was applied into column (2 cm diameter and 40 cm long) filled with silica gel (100-200 mesh). The eluting solvents were chloroform and methanol, respectively. Neutral lipid classes were eluted in sequence of 50 ml chloroform and phospholipids were isolated with 75 ml methanol. The purity of the different classes was verificated with thin-layer chromatography. The solvents in the different fractions were evaporated by using N_2. The isolated phospholipids classes were separated by two-dimensional thin-layer chromatography (TLC) on 20 x 20 cm glass plates with 0.2 mm silica gel 60 G layer impregnated with aqueous $(NH_4)_2SO_4$ (1 g in 100 ml water). In the first direction the plate was developed with chloroform:methanol:ammonia, 65:25:5 (v/v/v) and in the second - with chloroform:acetone:methanol:acetic acid:water, 50:20:10:10:5 (v/v/v/v/v). The individual phospholipids were detected and identified by spraying with specific reagents: Dragendorff test (detection of choline-containing phospholipids), ninhydrin spray (for phospholipids with free amino groups) and Shiff's reagent (for inositol containing phosphorlipids). Additional identification was performed by comparing the respective R_f values with those of authentic commercial standards subjected to silica gel TLC under identical experimental conditions. The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm after scraping the respective phospholipid zone and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 (v/v) [10].

Determination of fatty acid composition of phospholipids

The fatty acids of phospholipids were determined by gas chromatography (GC). The method is based on isolation of phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidyl-ethanolamine (PE) from the phospholipid fraction by preparative TLC. The obtained free fatty acids after saponification of main phospholipids and acidification [11] were esterified with 2% H_2SO_4 in absolute CH_3OH at 50°C to methyl esters of the fatty acids (FAME) [12]. FAMEs were purified by TLC on 20 x 20 cm plates covered with 0.2 mm silica gel 60 G (Merck, Darmstadt, Germany) layer with mobile phase n-hexane:diethyl ether, 97:3 (v/v). GC was performed on a HP 5890 gas chromatograph (Hewlett Packard GmbH, Austria) equipped with a 60 m x 0.25 mm capillary DB-23 column and a flame ionization detector. The column temperature was programmed from 130°C (1 min), at 6.5°C/min to 170°C, at 3.0°C/min to 215°C (9 min), at 40.0°C/min to 230°C (1 min), injector and detector temperatures were kept at 270°C and 280°C. The carrier gas was hydrogen at a flow rate 0.8 ml/min; split was 1:50. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of methyl esters of fatty acids subjected to GC under identical experimental conditions [13]. The standard mixture of fatty acid methyl esters was provided by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

RESULTS AND DISCUSSION

The content of oil, phospholipids in oil and phospholipids in seeds are presented in Table 1.

Table 1. Oil content, phospholipids in oil and phospholipids in seeds*.

Source	Content, wt %		
	Oil in the seeds	Phospholipids in the oil	Phospholipids in the seeds
Cucurbita moschata	45.1±0.5	0.5±0.1	0.2±0.05
Cucurbita pepo	46.8±0.2	1.1±0.2	0.5±0.1
Cucurbita maxima	51.5±0.3	1.0±0.1	0.5±0.1

*Means of triplicate analysis ± SD.

Based on the data, it is obvious that *Cucurbita maxima* has the highest amount of oil in the seeds (51.5%), followed by *Cucurbita pepo* (46.8%) and *Cucurbita moschata* (45.1%) seeds. According to Kim *et al*. [14], lipid content in *Cucurbita moschata*, *Cucurbita pepo* and *Cucurbita maxima* seed oils was 45.7%, 43.9% and 52.4%, respectively. These results were similar to those obtained in our investigation; according to these authors, only the oil content of *Cucurbita pepo* seeds was a bit lower. On the other hand, according to Ihediohanma *et al*. [15] the lipid content of *Cucurbita pepo* seed oil was 50.3% which was higher than in the species grown in Bulgaria. Fokou *et al*. and Achu *et al*. [16,17] presented the oil content of *Cucurbita moschata* and *Cucurbita maxima* seeds from 48.56 to 52.02% and from 49.05 to 50.81%, respectively.

Cucurbita pepo and *Cucurbita maxima* seeds had the highest amount of phospholipids (0.5%) and phospholipid content in the oil of the same

varieties had also higher amount (1.1 and 1.0%, respectively) than in *Cucurbita moschata* seed oil (0.5% in the oil and 0.2% in the seeds). These values are lower than those in previous publications by Raharjo *et al*. and El-Adawy and Taha [4,6] who report that phospholipid content in *Cucurbita moschata* seeds are 1.27% and 1.09%, respectively. The reasons for the different values of the phospholipids are probably origin and geographic region of the growing [6]. Phospholipid content of pumpkin seed oils is similar to that of sunflower, flaxseed, corn germ, soybean and canola oil (0.7-1.0%) [18-20].

Individual phospholipid composition of pumpkin seed oils is shown in Fig. 1.

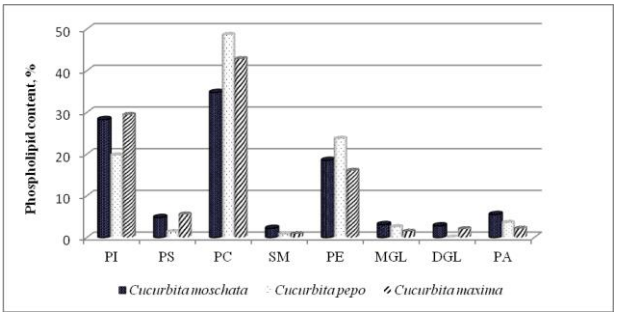

Fig. 1. Individual phospholipid composition of pumpkin seed oils. PI - Phosphatidylinositol; PS - Phosphatidylserine; PC - Phosphatidylcholine; SM - Sphingomyelin; PE - Phosphatidylethanolamine; MGL - Monophosphatidylglycerol; DGL - Diphosphatidylglycerol; PA - Phosphatidic acid.

All main classes of phospholipids were presented in pumpkin seed oils. Phosphatidylcholine was the predominant constituent in all pumpkin seed oils (from 34.8% to 48.6%) followed by phosphatidylinositol (from 19.7% to 29.4%) and phosphatidylethanolamine (from 16.0% to 23.7%). Phosphatidylcholine was detected in highest quantity in *Cucurbita pepo* - 48.6%, while in the other two species its amount was 42.8% in *Cucurbita maxima* and 34.8% in *Cucurbita moschata*. The amount of phosphatidylinositol in *Cucurbita moschata* and *Cucurbita maxima* seed oils was 28.3% and 29.4%, respectively, while the quantity of this class of phospholipids in *Cucurbita pepo* seed oil was 19.7%. The quantity of phosphatidylethanolamine in *Cucurbita pepo* species was 23.7%, while the other two varieties contained lower amounts of it - 18.5% in *Cucurbita moschata* and 16.0% in *Cucurbita maxima* seed oil. Phosphatidylserine (1.3-5.5%), monophosphatidylglycerol (1.4-3.1%) and phosphatidic acids (2.1-5.5%) were presented in small quantities. The amount of sphingomyelin (0.7-2.2%) and diphosphatidylglycerol (~2.8%) was minimal in all pumpkin seed oils.

Fatty acid composition of the main phospholipids (phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine) is shown in Fig. 2. The most predominant component in all species was palmitic acid, followed by oleic, stearic and linoleic acids.

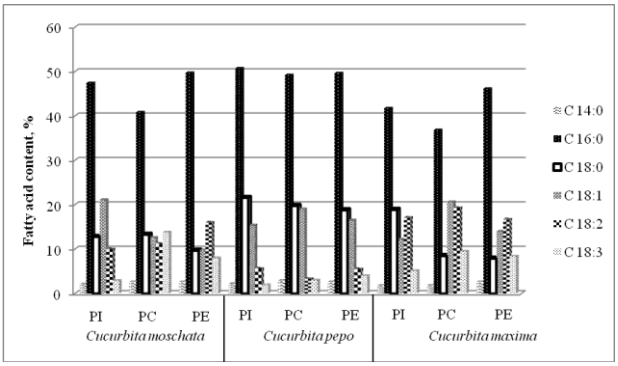

Fig. 2. Fatty acid composition of the main phospholipids classes. $C_{14:0}$- Myristic acid; $C_{16:0}$- Palmitic acid; $C_{18:0}$- Stearic acid; $C_{18:1}$- Oleic acid; $C_{18:2}$- Linoleic acid; $C_{18:3}$- Linolenic acid.

The quantity of palmitic acid in phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine in *Cucurbita moschata* was 47.4%, 40.9% and 49.7%, respectively. The amount of oleic acid in phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine was 21.1%, 12.6% and 9.8%, respectively, while the quantity of linoleic acid in the same phospholipids was 10.0%, 11.2% and 16.0%, respectively. The levels of stearic acid were close - 12.8%, 13.3% and 9.7%. Linolenic acid was presented in higher quantity in phosphatidylcholine (13.8%) and in phosphatidylethanolamine (8.0%), while in phosphatidylinositol it was significantly lower (2.9%). Myristic acid in these three phospholipid classes was detected in similar amounts (2.1-2.6%).

The quantity of palmitic acid in phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine in *Cucurbita pepo* was 50.7%, 49.2% and 49.5%, respectively followed by stearic acid - 21.6%, 19.8% and 18.8%. The levels of oleic acid in phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine were from 21.6% to 18.8%, respectively. The quantity of linoleic acid in the same phospholipids was a bit higher - 15.4%, 16.5% and 19.0% in phosphatidylinositol, phosphatidylethanolamine and phosphatidylcholine. Linolenic and myristic acids were presented in lower quantities (1.9-4.0% and 2.2-2.9%, respectively), in these phospholipids.

The quantity of palmitic acid in phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine in *Cucurbita maxima* was 41.7%, 36.8% and 46.1%, respectively, and that of stearic acid was 18.9%, 8.5% and 7.9%. The amount of oleic acid was higher in phosphatidylcholine (20.6%), while in phosphatidylinositol and phosphatidylethanolamine it was 12.2% and 14.0%, respectively. Linoleic acid was presented in a relatively higher amount (17.1% in phosphatidylinositol, 19.3% in phosphatidylcholine and 16.7% in phosphatidylethanolamine). The quantity of linolenic acid was lower - 5.1% in phosphatidylinositol, 9.5% in phosphatidylcholine and 8.4% in phosphatidylethanolamine. There was also a small amount of myristic acid (from 1.8% in phosphatidylinositol and phosphatidylcholine to 2.6% in phosphatidylethanolamine).

These results differ from the data obtained by Vidhute *et al.* [21] who reported that linoleic acid predominated in phospholipid fraction (37.1%), followed by oleic (27.4%) and palmitic acid (24.0%).

The ratio between saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of the main phospholipids was presented in Fig. 3 and these results were collated to the respective triacylglycerols (TAG) reported by Petkova and Antova [22]. Identical fatty acids have been detected in the TAG and phospholipids, but they are in different amounts.

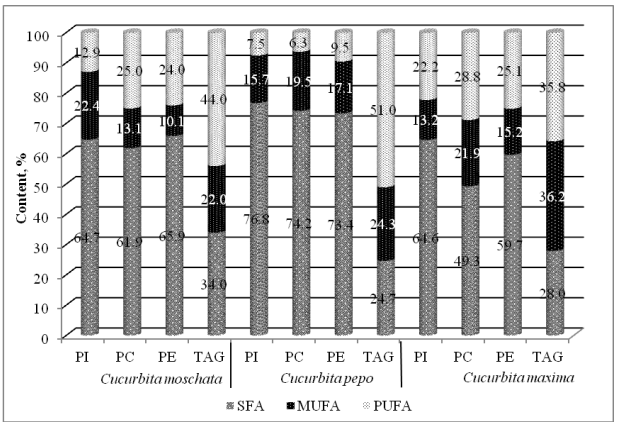

Fig. 3. The ratio between saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of the main phospholipids and triacylglycerols (TAG).

The highest content of SFAs was detected in *Cucurbita pepo* (73.4-76.8%), while the amount of PUFAs was very low (6.3-9.5%). The phospholipid fraction of *Cucurbita maxima* had a significantly higher content of PUFAs (25.1-28.8%) which was at the expense of the content of SFAs in the same species (49.3-59.7%). There are considerable differences in the fatty acid composition of phospholipid classes in all pumpkin species. The highest quantity of SFAs was detected in phosphatidylinositol (64.6-76.8%), followed by phosphatidylethanolamine (59.7-73.4%) and phosphatidylcholine (49.3-74.2%). On the other hand, phosphatidylcholine contained the highest level of PUFAs (6.3-28.8%). The differences in the fatty acid composition can be explained by the different individual stages of biosynthesis of the separated phospholipids. Saturated fatty acids were synthesized in the first stage of biosynthesis and were included in phospholipids when the last were synthesized, too [23]. On the other hand, phosphatidylinositol was synthesized first, thus saturated fatty acids were predominant in this phospholipid. Phosphatidylcholine was synthesized last, when the biosynthesis of polyunsaturated fatty acids began. Therefore, polyunsaturated fatty acids predominate in the phosphatidylcholine.

The fatty acid composition of the phospholipids and triacylglycerols was very different. The fatty acid profiles of the phospholipid fraction were characterized by a significant amount of saturated acids, while unsaturated acids predominated in the triacylglycerols [22]. The increase of unsaturated fatty acids was most significant in *Cucurbita pepo* seed oils (from 6.3-9.5% in phospholipids to 51.0% in triacylglycerols), while the changes of the same acids in *Cucurbita maxima* seed oils were only about 10% (from 22.2-28.8% in phospholipids to 35.8% in triacylglycerols).

CONCLUSION

This study shows that the phospholipid content and the fatty acid composition of the main phospholipids classes in pumpkin seed oils do not differ significantly from those in other seed oils as sunflower, canola, corn germ oil, etc. The pumpkin seed oils and seeds have a relatively high content of biologically active compounds, such as phospholipids (0.5-1.1% in the oils and 0.2-0.5% in the seeds). Phospholipids have a similar qualitative and quantitative composition in the studied pumpkin varieties and are similar to those of other vegetable oils, such as sunflower, soybean, flaxseed oils, etc. Palmitic acid is the major component in phospholipids (36.8-50.7%), followed by stearic acid (7.9-21.6%), oleic acid (9.8-21.1%) and linoleic acid (3.3-19.3%). Saturated fatty acids predominate

in phospholipids, while unsaturated fatty acids are predominant in triacylglycerols.

This leads to their complete and versatile examination. The studies about phospholipid content of pumpkin seed oils also contribute to a full assessment of their nutritional value.

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ФОСФОЛИПИДЕН СЪСТАВ НА МАСЛА ОТ СЕМ. *CUCURBITACEAE*

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(Резюме)

Изследван е фосфолипидният състав на масла от семена на три сорта тиква от сем. *Cucurbitaceae* (*Cucurbita moschata*, *Cucurbita pepo* и *Cucurbita maxima*). Маслено им съдържание е съответно 45.1%, 46.8% и 51.5%. Фосфолипидите са определени спектрофотометрично, след разделянето им чрез двупосочна тънкослойна хроматография. Общото съдържание на фосфолипиди в тиквените масла от семена на сортове *Cucurbita moschata*, *Cucurbita pepo* и *Cucurbita maxima* е съответно 0.5%, 1.1% и 1.0%. Във фосфолипидната фракция преобладават основно фосфатидилинозитол (19.7-29.4%), фосфатидилхолин (34.8-48.6%) и фосфатидилетаноламин (16.0-23.7%). Количеството на фосфатидните киселини е от 2.1% до 5.5%. Мастнокиселинният състав на индивидуалните фосфолипиди е определен чрез газова хроматография. Установено е, че преобладаващите мастни киселини във фосфолипидната фракция са палмитиновата, стеариновата, олеиновата и линоловата.