GRASAS Y ACEITES 66 (1)
January–March 2015, e058
ISSN-L: 0017-3495
doi: http://dx.doi.org/10.3989/gya.0706142

Changes in the composition of pumpkin seeds (*Cucurbita moschata*) during development and maturation

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Submitted: 26 June 2014; Accepted: 22 September 2014

SUMMARY: Changes in the chemical and lipid composition of *Cucurbita moschata* seeds and seed oils at different stages of development were investigated. The oil content of the seeds at 30, 60 and 90 days after flowering was 10.7, 41.1, and 47.1%, respectively. The amount of proteins was 26.0, 35.9, and 38.2%. The contents of carbohydrates soluble in ethanol were 9.5, 1.8 and 1.3%. The starch and fiber percentage contents were 16.3, 6.8, 2.3 and 4.0, 6.9 and 10.0, respectively and the ash contents were 7.2, 4.7, and 4.5%. The total sterol percentages were found to be 2.0, 0.8 and 0.6 in the oils and 0.2, 0.3 and 0.3 in the seeds. The tocopherol contents were 2010, 512 and 527 mg·kg^{-1} in the oil, and 215, 210 and 250 mg·kg^{-1} in the seeds. The total phospholipid percentages were 8.7, 0.8 and 0.4 in the oils and 0.9, 0.3 and 0.2 in the seeds. Fatty acid composition was determined by gas chromatography and the major fatty acids in the oils at all stages of ripening were linoleic (40.8–50.2%) followed by palmitic (21.5–25.9%) and oleic (20.5–21.0%).

KEYWORDS: *Chemical composition;* Cucurbita moschata*; Fatty acids; Phospholipids; Seeds developing; Sterols; Tocopherols*

RESUMEN: ***Cambios en la composición de las semillas de calabaza (*Cucurbita moschata*) durante el desarrollo y la maduración***. Se determinaron los cambios en la composición química y en los lípidos de semillas de *Cucurbita moschata* así como en los aceites extraídos en diferentes etapas del desarrollo de las semillas. El contenido de aceite a los 30, 60 y 90 días después de la floración fue de 10,7, 41,1 y 47,1%, respectivamente. La cantidad de proteínas fue del 26,0, 35,9 y 38,2% y el contenido de hidratos de carbono solubles en etanol fue de 9,5, 1,8 y 1,3%. Los contenidos de almidón y fibras fueron 16,3, 6,8, 2,3% y 4,0, 6,9 y 10,0%, respectivamente y el contenido de cenizas fue de 7.2%, 4.7% y 4.5%. Se encontró un contenido total de esteroles del 2,0, 0,8 y 0,6% en los aceites y de 0,2, 0,3 y 0,3% en las semillas. El contenido de tocoferoles fue 2010, 512 y 527 mg·kg^{-1} en el aceite, y 215, 210 y 250 mg·kg^{-1} en las semillas. Los contenidos de fosfolípidos totales fueron de 8,7, 0,8 y 0,4% en los aceites y 0,9, 0,3 y 0,2% en las semillas. La composición de ácidos grasos determinada mediante cromatografía de gases mostró como principal ácido graso de los aceites en todas las etapas de maduración al linoleico (40,8 a 50,2%), seguido por palmítico (21,5 a 25,9%) y oleico (20,5 a 21,0%).

PALABRAS CLAVE: *Ácidos grasos; Composición química;* Cucurbita moschata*; Esteroles; Fosfolípidos; Semillas en desarrollo; Tocoferoles*

Citation/Cómo citar este artículo: Petkova ZhY, Antova GA. 2015. Changes in the composition of pumpkin seeds (*Cucurbita moschata*) during development and maturation. *Grasas Aceites* **66** (1): e058. doi: http://dx.doi.org/10.3989/gya.0706142.

1. INTRODUCTION

The *Cucurbitaceae* family includes more than 900 species. The fruit of this family are a very valuable source of different biologically active compounds. The origin of these plants is Central and South America but they also grow in other places with warm climates. Pumpkins belong to the genus *Cucurbita*, family *Cucurbitaceae*. The genus *Cucurbita* includes five species: *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo*, *Cucurbita ficifolia* and *Cucurbita turbaniformis*. The most valuable pumpkin species in Bulgaria are *Cucurbita moschata*, *Cucurbita pepo* and *Cucurbita maxima*. The pumpkin seeds are used for human consumption in many cultures all over the world. The seeds are by-products of the food industry and are valuable sources of different biological components such as proteins, oils, carbohydrates, microelements and vitamins. The contents of oil and protein in the seeds was 37.8–50.0% and 24.3–41.6%, respectively (Achu *et al*., 2005, Achu *et al*., 2006, Ardabili *et al*., 2011, Fokou *et al*., 2004, Fokou *et al*., 2009, Martin, 1998, Tsaknis *et al*., 1997). The chemical characteristics of various cucurbit seed oils have been reported in many scientific investigations (Kamel *et al*., 1982, Lazos, 1986, Tsaknis *et al*., 1997). The oil content of *Cucurbita moschata* pumpkin seeds is about 50% (45–60%) according to Tsaknis *et al*. (1997). The oil is dark green to red, with a specific aroma and taste, and a high content of free fatty acids (Tsaknis *et al*., 1997). There is a lot of data about the chemical and lipid composition of *Cucurbita moschata* seed oil but the information about the changes in the chemical composition in the seeds and seed oils during development is scarce.

The purpose of the present study is to investigate the changes in composition of *Cucurbita moschata* seeds as well as those in the content and individual composition of biologically active substances (fatty acids, sterols, tocopherols and phospholipids) in the pumpkin seed oil during the development of the seeds. The changes in the fatty acid composition of the major classes of phospholipids during this period were determined as well.

2. MATERIALS AND METHODS

2.1. Samples

The fruits of *Cucurbita moschata* were grown and obtained from the Thracian plane (Central Southern Bulgaria), crop 2012. Samples were taken randomly between the 30th and 90th day after flowering, during the period from July to September, and the whole seeds were removed by hand. Prior to use for analysis, the seeds were air dried for 72 h at 25 °C.

All solvents and reagents were of analytical grade from Merck (Darmstadt, Germany) and were used without additional purification.

2.2. Chemical composition of seeds

The crude proteins were calculated from the nitrogen content according to the Kjeldahl method using factor 6.25 (AOAC, 1996). The method for determining the content of starch is based on the treatment of the plant material with an alcoholic KOH solution and additional acid hydrolysis of starch into glucose. The quantity of glucose is determined by the oxidation with a bivalent copper from a copper reagent and is then converted into starch (BS 13488:1976). The carbohydrates which are soluble in ethanol were identified by their extraction with 80% C_2H_5OH, converted into glucose which is determined on the basis of its copper-alkaline solutions (Islam *et al*., 2008). Crude fiber was determined by the gravimetric procedure of AOAC (1995). Ash content was evaluated by incinerating at 550 °C in a muffle furnace for 6 h (AOAC, 1995).

2.3. Isolation of glyceride oil and determination of oil content

The seeds (50 g sample) were air-dried at room temperature, ground to powder with a mechanical mill and the oil was extracted with n-hexane in a Soxhlet apparatus for 8 h. The solvent was partially removed in a rotary vacuum evaporator, the residue was transferred to pre-weighed glass vessels and the rest of the solvent was removed under a stream of nitrogen to a constant weight to determine the oil content (ISO, 2009).

2.4. Analysis of fatty acids

The fatty acid composition of glyceride oil, as well as the fatty acid composition of phospholipids, was determined by gas chromatography (GC) after transmethylation of the respective sample with 2% H_2SO_4 in absolute CH_3OH at 50 °C (ISO, 2000a). Fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) on 20 × 20 cm plates covered with a 0.2 mm silica gel 60 G (Merck, Darmstadt, Germany) layer with the mobile phase n-hexane:diethyl ether (97:3, v/v). GC was performed on an HP 5890 series II gas chromatograph (Hewlett Packard GesmbH, Vienna, Austria) equipped with a 60 m × 0.25 mm (I.D.) × 25 μm (film thickness) capillary DB – 23 column (Agilent J&W advanced, Agilent Technology, USA) and a flame ionization detector. The column temperature was programmed from 130 °C (1 min), at 6.5 °C·min^{-1} to 170 °C, at 3.0 °C·min^{-1} to 215 °C (9 min), at 40 °C·min^{-1} to 230 °C (1 min); the injector and detector temperatures were kept at 270 °C and 280 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL·min^{-1}; the split ratio was 1:50. The identification of fatty acids was performed by comparison of retention times

with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions (ISO, 2004). The analytical standard of fatty acid methyl esters (Supelco F.A.M.E. Mix C4-C24, purity ~99%) was from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.5. Analysis of sterols

Unsaponifiables were determined by weight after saponification of the glyceride oil and extraction with hexane (ISO, 2000b). The sterol fraction was separated from the unsaponifiable matter by thin-layer chromatography on silica gel 60 G in the mobile phase diethyl ether:hexane (1:1, v/v). The qualitative and quantitative composition of the sterol fraction was determined by GC on an HP 5890 series II gas chromatograph (Hewlett Packard GesmbH, Vienna, Austria) equipped with 25 m × 0.25 mm (I.D.) × 25 μm (film thickness) DB–5 capillary column (Agilent Technologies, J&W Scientific products Proudly, Santa Clara CA, USA) and flame ionization detector. The temperature gradient ranged from 90 °C (held for 2 min) up to 290 °C at a rate of change of 15 °C·min^{-1} and then up to 310 °C a rate of 4 °C·min^{-1} (held for 10 min); detector temperature, 320 °C; injector temperature, 300 °C and carrier gas, hydrogen (H_2), split ratio, 1:50 and software Data Apex Clarity TM 2.4.1.93/2005. A mixture of sterols containing cholesterol (New Jersey, USA), stigmasterol (Acros organics, St. Louis, MO, USA) and β-sitosterol (with ca 10% campesterol, ca 75% β-sitosterol, New Jersey, USA) was used for the determination of the retention times of the individual sterols in the samples and for quantitative calculations (ISO, 1999).

2.6. Analysis of tocopherols

Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) on a "Merck-Hitachi" (Merck, Darmstadt, Germany) instrument equipped with a 250 mm × 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000. The operating conditions were as follows: mobile phase of n-hexane:dioxan (96:4, v/v), a flow rate of 1.0 mL·min^{-1}, excitation 295 nm, emission 330 nm (ISO 9936:2006). 20 μL of a 1% solution of oil in hexane were injected. The individual tocopherols were identified by comparing the retention times with those of standards (reference individual pure tocopherols – DL-α-, DL-β, DL-γ- and DL-δ-tocopherol with ≥98% purity) purchased from Merck (Darmstadt, Germany). The content of tocopherols in the seed oils was calculated by comparing the peak areas obtained for the relevant tocopherols in the sample with those obtained for the standard solutions with known concentrations.

2.7. Analysis of phospholipids

Another part (50g) of the air-dried seeds was subjected to Folch extraction (Folch *et al*., 1957) and polar lipids were isolated from the total lipids by column chromatography (Angelova-Romova *et al*., 2013). The phospholipid classes were isolated using two-dimensional TLC on 20 × 20 cm glass plates with a 0.2 mm silica gel 60 G layer impregnated with aqueous $(NH_4)_2SO_4$ (1% solution in water). In the first phase 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 from Sigma Aldrich: Dragendorff test (detection of choline-containing phospholipids), Ninhydrin spray (for phospholipids with free amino groups) and Shiff's reagent (for inositol containing phospholipids). An additional identification was made by comparing the respective R_f values with those of authentic standards (Sigma, St. Louis, MO, USA) 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 scrapping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid and sulphuric acid (1:1, v/v). The analytical curve was constructed using a standard solution of KH_2PO_4 and in each series of measurements a standard solution of KH_2PO_4 (10 μg·mL^{-1} in water) was used to confirm the validity of calibration. The phospholipid content in the sample was calculated as a percentage of the phosphorus (ISO, 2003).

2.8. Statistical analyses

All the analyses of the studied samples were performed in triplicate. The results were represented as mean ± standard deviation (SD). Differences between mean values were considered significant at $P = 0.05$.

3. RESULTS AND DISCUSSION

3.1. Changes in the chemical composition of pumpkin seeds (*Cucurbita moschata*) during the growing period

The changes of the main compounds of pumpkin seeds during development are presented in Table 1.

The protein contents in the seeds increased throughout the period of growing from 26.0%, at the 30th day after flowering to 38.2%, at the 90th day. This result is in good agreement with the data of other publications where the protein content in

TABLE 1. Changes in chemical composition of pumpkin seeds (*Cucurbita moschata*) during the growing period*

	Growing period, days after flowering		
Compounds (wt %)	**30**	**60**	**90**
Protein	26.0±0.3	35.9±0.2	38.2±0.1
Fat	10.7±0.2	41.1±0.1	47.1±0.3
Carbohydrate			
starch	16.3±0.2	6.8±0.1	2.3±0.1
soluble carbohydrates (in 80% C_2H_5OH)	9.5±0.7	1.8±0.1	1.3±0.2
crude fibres	4.0±0.1	6.9±0.2	10.0±0.1
Ash	7.2±0.1	4.7±0.2	4.5±0.1

*Value for each nutrient is the mean ± SD (standard deviations) of three determinations (n=3 and $p<0.05$).

ripe seeds varies from 25.0% to 51.0% (Aboul-Nasr *et al*., 1997; Achu *et al*., 2005; Bombardelli and Morazzoni, 1997; Kim *et al*., 2012; Nakiae *et al*., 2006).

During the first thirty days, pumpkin seeds have lower lipid contents (10.7%), in the second 30 days the oil content increased rapidly to 41.1% and then more slowly to 47.1%. These results confirm the data reported earlier by Achu *et al*. (2005) and Kim *et al*. (2012) according to whom the oil content was 41.98–54.45%.

The carbohydrate content at the 30th day of flowering was comparatively higher (~29.8%) and in the following days decreased gradually to about 13.6%. The starch content is higher in the first 30 days (16.3% in the seeds) while in the next 60 days decreased by two to seven times. A similar tendency was observed for the soluble carbohydrates in 80% ethanol. Their amount was 9.5% in the first 30 days and decreased with the maturing of the pumpkin seeds to 1.3%. The content of crude fibres in the seeds increased from 4.0% to 10.0%. These results are in good agreement with the data of other publications where the carbohydrate content was 6.05–23.00% and the content of fiber varied from 1.22% to 10.85%. (Achu *et al*., 2005; Al-Khalifa, 1996; Kim *et al*., 2012).

The ash content in the seeds during development decreased slightly from 7.2 to 4.5%. These results were similar to the data of other publications (4.21–5.32%) (Achu *et al*., 2005; Kim *et al*., 2012).

The results from investigations on the general chemical composition of the pumpkin seeds (*Cucurbita moschata*) showed higher content of oil, protein and fibres and lower contents of minerals (ash) and carbohydrates in the final stage of ripening than that in the first and second stages.

3.2. Changes in the lipid composition of pumpkin seeds during the growing period

The data on the change in oil content in the seeds and biologically active components (such as sterols, tocopherols and phospholipids) in the pumpkin oil and the seeds during maturation are shown in Table 2.

The oil content increased with the development of the pumpkin seeds (10.7–47.1%) while the quantity of the unsaponifiable matter in the oil decreased from 16.5% to 2.2%. The content of unsaponifiable matter in the seeds decreased slightly from 1.8% to 1.0%. The quantity of unsaponifiable matter in the mature seeds was higher than that shown in previous publications (Al-Khalifa, 1996).

The same trend has been shown for the sterol fraction. The sterol content in the oil decreased from 2.0% to 0.6% while in the seeds in the first stage it was 0.2% and in the next two stages it was 0.3%. In the beginning the sterol content was 12.0% of the total unsaponifiable matter, after which its quantity increased to 24.3% then; in the final stage of growing the sterol content was 26.6%. Obviously, sterols were synthesized mainly in the beginning of the ripening and the rate of biosynthesis continued to slow down during the next two stages of development.

The total tocopherol content in the seed oil on the 30th day of flowering was extremely high, at 2010 mg·kg^{-1}, but in the next two stages of development it decreased by around four times, to 512 mg·kg^{-1} and 527 mg·kg^{-1}, respectively.

In the first stage of development, the total phospholipids were represented in a higher quantity (8.7% in oil and 0.9% in the seeds) compared with the next two stages where the total phospholipid contents decreased to 0.4% in the oil and 0.2% in the seeds. The high percentage content of phospholipids in the first stage was due to the fact that they were synthesized mainly in the beginning of vegetation. Triacylglycerols were synthesized in a later stage, with the result that the relative amount of phospholipids in the oil significantly decreased.

3.2.1. Fatty acid composition

The data about fatty acid composition of *Cucurbita moschata* seed oil during the growing period is shown in Table 3.

TABLE 2. Changes in content of biologically active substances in the pumpkin oil and seeds during the growing period*

	Growing period, days after flowering		
Compounds	**30**	**60**	**90**
Oil in the seeds, wt %	10.7±0.2	41.1±0.1	47.1±0.3
Unsaponifiable matters			
in the oil, wt %	16.5±0.2	2.4±0.1	2.2±0.3
in the seeds, wt %	1.8±0.04	1.0±0.01	1.0±0.09
Sterols			
in the unsaponifiable matters, wt %	12.0±0.2	24.3±0.3	26.6±0.1
in the oil, wt %	2.0±0.2	0.8±0.3	0.6±0.1
in the seeds, wt %	0.2±0.04	0.3±0.03	0.3±0.03
Tocopherols			
in the unsaponifiable matters, $mg \cdot kg^{-1}$	331.7±4.6	12.3±0.3	11.6±0.6
in the oil, $mg \cdot kg^{-1}$	2010±20	512±10	527±20
in the seeds, $mg \cdot kg^{-1}$	215±4	210±1	250±6
Phospholipids			
in the oil, wt %	8.7±0.2	0.8±0.2	0.4±0.2
in the seeds, w t%	0.9±0.04	0.3±0.02	0.2±0.06

*Value for each nutrient is the mean ± SD (standard deviations) of three determinations (n = 3 and $p<0.05$).

TABLE 3. Changes in the fatty acid composition and saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of pumpkin seed oil during the development of seeds

	Growing period, days after flowering		
Fatty acids*, %	**30**	**60**	**90**
$C_{8:0}$	0.1±0.02	0.1±0.04	–
$C_{12:0}$	0.1±0.01	–	–
$C_{14:0}$	0.3±0.1	0.2±0.05	0.2±0.05
$C_{15:0}$	0.1±0.02	–	–
$C_{16:0}$	25.9±0.4	24.7±0.2	21.5±0.5
$C_{17:0}$	0.1±0.01	0.1±0.02	0.1±0.01
$C_{18:0}$	9.3±0.4	9.2±0.2	6.7±0.5
$C_{18:1}$	20.5±0.2	21.0±0.3	21.0±0.1
$C_{18:2}$, (n-6)	40.8±0.5	43.6±0.2	50.2±0.2
$C_{18:3}$, (n-3)	1.9±0.2	0.3±0.1	0.2±0.05
$C_{20:0}$	0.4±0.1	0.4±0.1	0.1±0.02
$C_{20:1}$	0.1±0.02	0.1±0.02	–
$C_{22:0}$	0.4±0.1	0.3±0.1	–
SFA	36.7	35.0	28.6
UFA	63.3	65.0	71.4
MUFA	20.6	21.1	21.0
PUFA	42.7	43.9	50.4

*$C_{8:0}$ - Caprylic acid; $C_{12:0}$ - Lauric acid; $C_{14:0}$ - Myristic acid; $C_{15:0}$ - Pentadecanoic acid; $C_{16:0}$ - Palmitic acid; $C_{17:0}$ - Margaric acid; $C_{18:0}$ - Stearic acid; $C_{18:1}$ - Oleic acid; $C_{18:2}$ -Linoleic acid; $C_{18:3}$ - Linolenic acid; $C_{20:0}$ - Arachidic acid; $C_{20:1}$ - Gadoleic acid; $C_{22:0}$ - Behenic acid; SFA - saturated fatty acids; UFA - unsaturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.

Linoleic acid prevailed in the seed oil (40.8–50.2%), followed by palmitic (21.5–25.9%), oleic (20.5–21.0%) and stearic (6.7–9.3%) acids. The quantity of palmitic and stearic acids decreased during the ripening process. The level of oleic acid remained the same during development while the quantity of linoleic acid was increasing significantly from 40.8% to 50.2%. The content of the linolenic acid, which represents one of the essential fatty acids, was minimal (on the 30th day of flowering it was 1.9% and after that it decreased to 0.2%). Changes in the fatty acid composition were a consequence of the different stages of biosynthesis of the fatty acids – in the first stage of ripening the saturated fatty acids were accumulated, after that the rate of biosynthesis of the polyunsaturated fatty acids increased. In addition to the main fatty acids in *Cucurbita moschata* seed oil, many others fatty acids such as caprylic, lauric, myristic, pentadecanoic, margaric, arachidic, gadoleic and behenic acids were detected but they are represented in very small amounts. During the process of investigation they did not undergo any significant changes.

The data on the fatty acid composition of *Cucurbita moschata* seed oil is similar to those reported in previous publications. The content of linoleic acid is higher than the other fatty acids and varies from 35.7% to 56.6% followed by oleic acid (14.8–38.1%), palmitic acid (10.7–16.4%) and stearic acid (4.7–11.1%) (Achu *et al*., 2006; Al-Khalifa, 1996; Kim *et al*., 2012).

The ratio between saturated (SFA) and unsaturated (UFA) fatty acids including monounsaturated

and polyunsaturated fatty acids during the growing period is presented in Table 3.

The quantity of SFA decreased during the process of ripening from 36.7% to 28.6% while that of UFA increased from 63.3% to 71.4%. This higher content of UFA is due to the increase in PUFA, i.e. linoleic acid. The ratio between UFA and SFA increased slightly during ripening and in the first stage this ratio was 1.7:1, in the second stage it was 1.9:1 and in the mature pumpkin seeds it was 2.5:1. These results are in a good agreement with data reported by Al-Khalifa (1996) (SFA – 19.1–19.7%, MUFA – 26.64–53.33% and PUFA – 36.1–44.0%), Achu *et al*. (2006) (SFA – 21.7–30.2%, MUFA – 19.4–25.3% and PUFA – 49,38–52,63%), Kim *et al*. (2012) (SFA – 17.47–20.11%, MUFA – 14.9–32.4% and PUFA – 35.72–56.84%).

3.2.2. Sterol composition

The changes in individual sterol composition during the development of seeds are shown in Table 4.

The main component in the sterol fraction was α-spinasterol, which on the 30th day of flowering was 71.9% and after that its quantity decreased to 44.8% on the 90th day. At the same time, the quantity of $\Delta^{7,25}$-stigmastadienol increased from 5.1% to 25.9%. The Δ^{7}-stigmasterol content on the 30th day after flowering was 10.8%, on the 60th day it was 26.2% and its content was 6.9% on the 90th day. The content of stigmasterol remained the same during development and it was around 7.0–8.0%. β-sitosterol was presented in very small amounts (0.3–0.1%). The cholesterol content was minimal (from 0.5% to 0.2%).

These results differ from the data of other publications about the sterol composition from foreign species of pumpkin seeds. The quantity of β–sitosterol at the final growth stage is 277.58 mg·kg^{-1} according to Kim *et al*. (2012) and 249.0 mg·kg^{-1} as published by Ryan *et al*. (2007).

TABLE 4. Changes in individual sterol composition of *Cucurbita moschata* seed oil during the development of seeds

	Growing period, days after flowering		
Sterols, %	**30**	**60**	**90**
Cholesterol	0.5±0.02	0.2±0.01	0.2±0.04
Campesterol	1.8±0.3	0.6±0.1	0.5±0.05
Stigmasterol	7.3±0.1	7.7±0.2	7.2±0.1
α - Spinasterol	71.9±0.2	45.2±0.1	44.8±0.3
β - Sitosterol	0.3±0.02	0.1±0.01	–
Δ^{5} - Avenasterol	0.4±0.05	0.3±0.04	0.3±0.02
$\Delta^{7,25}$ - Stigmastadienol	5.1±0.1	11.6±0.2	25.9±0.1
Δ^{7} - Stigmasterol	10.8±0.3	26.2±0.1	6.9±0.2
Δ^{7} - Avenasterol	1.9±0.1	8.1±0.2	14.2±0.3

The individual sterol composition of the investigated oils differs significantly from the sterol composition of the other vegetable oils (sunflower, rape, safflower, soybean, grapes, maize) in which β–sitosterol predominated (32.6–87.1%) (Codex-Stan 210-1999). In the pumpkin seed oils α-spinasterol (44.8–71.9%) and $\Delta^{7,25}$-stigmastadienol (5.1–25.9%) prevailed while in other vegetable oils these two sterols are represented in very small quantities or they are not found.

3.2.3. Tocopherol composition

The changes in the content of tocopherols in the pumpkin oils are presented in Table 5.

γ–Tocopherol was presented in considerable amounts in all three growth stages. Its quantity at the beginning was 944.7 mg·kg^{-1}, which later decreased to 383.5 mg·kg^{-1} and in the final stage it was 453.7 mg·kg^{-1}. At the same time, the quantity of α–tocopherol decreased significantly from 894.5 to 20.0 mg·kg^{-1} while that of γ-tocotrienol decreased slightly from 120.6 to 52.7–53.3 mg·kg^{-1}. On the 30th day of flowering the amounts of α-tocopherol and γ-tocopherol are the same but in the next two growth stages the quantity of γ-tocopherol decreased by half compared to the first stage. The content of γ-tocopherol in the seeds in the final stage of ripening was 215.3 mg·kg^{-1} while the content of α-tocopherol was 9.5 mg·kg^{-1} in the seeds. These results differ from the data reported by Kim *et al*. (2012). According to these authors, *Cucurbita pepo* and *Cucurbita moschata* seeds contain higher levels of γ-tocopherol (61.65–66.85 mg/kg in the seeds) than *Cucurbita maxima* seeds (28.7 mg·kg^{-1}). The γ-tocopherol content of *Cucurbita pepo* seeds (61.65 mg·kg^{-1} raw weight) and *Cucurbita moschata* seeds (66.85 mg·kg^{-1} raw weight) is two to three times higher than the α–tocopherol content (21.33–25.74 mg·kg^{-1}) (Kim *et al*., 2012).

The individual tocopherol composition of the investigated pumpkin seed oils in the final growth stage differs significantly from that of the various known vegetable oils. α–Tocopherol predominates in the other vegetable oils except for soybean, sesame, and corn germ oils, whose tocopherol

TABLE 5. Changes in the content of tocopherols of *Cucurbita moschata* seed oil during the ripening process

	Growing period, days after flowering		
Tocopherol, mg·kg^{-1}	**30**	**60**	**90**
α - tocopherol	894.5±21.2	75.8±6.3	20.0±2.6
γ - tocopherol	944.7±23.7	383.5±18.5	453.7±17.6
γ - tocotrienol	120.6±12.4	52.7±4.8	53.3±4.3
δ - tocopherol	50.2±5.6	trace	trace

composition is closer to the composition of pumpkin seed oil where γ–tocopherol prevails.

3.2.4. Phospholipid composition and fatty acid composition of the major classes of phospholipids

The composition of the phospholipid fraction of lipids from *Cucurbita moschata* seed oils during development is shown in Table 6.

Phosphatidic acids (38.7%) prevail during the first phase of ripening. Since phosphatidic acids are the first to be biosynthesized and are a precursor for the biosynthesis of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine (Munshi *et al*., 1983), as expected, its amount is higher in the early stages of seed development and then decreases gradually. The other classes of phospholipids are presented in quantities around 10.0–15.0% except for lysophosphatidylcholine, whose amount was 1.5%. There was an abrupt increase in the quantity of phosphatidylinositol from 14.8% in the first stage to 47.6% and 48.4% in the next two growth stages and a slight increase in phosphatidylethanolamine from 11.9 to 13.5–17.8% and in phosphatidylcholine from 9.9 to 11.0–17.1% at the expense of the decrease in phosphatidic acids (14.6–6.6%). Changes in the content of phosphatidylserine from 0.0 to 4.6–4.9% and sphingomyelin from 0.0 to 0.5–0.9% were minimal.

These results differ from the data reported by Raharjo *et al*. (2011) who investigated phospholipids from *Cucurbita moschata* seeds and established the main phospholipids to be phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine.

Palmitic (35.1–61.4%), linoleic (7.1–28.7%), stearic (8.3–13.2%) and oleic (3.0–27.0%) acids were mainly identified in the major individual phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acids) as shown in Fig. 1. These acids prevail in the phospholipid fraction during the entire process of ripening. Myristic, linolenic and the rest of the fatty acids are presented in very small quantities. On the other hand, the fatty acid composition of the triacylglycerols, where linoleic acid prevails (40.8–50.2%), differs from the fatty acid composition of the main phospholipids.

The quantity of palmitic acid in phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine increased on the 60th day and after that on the 90th day remained in the same amount. The phosphatidic acid content was high during the first 30 days only (Fig. 1). The quantity of stearic acid decreased until the second growth phase, and then remained at the same level or dropped in the final stage of development.

The quantity of oleic acid in phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine decreased on the 60th day of flowering, after which it increased again. The amount of oleic acid in phosphatidic acids increased significantly during the investigated period. The quantity of linoleic acid in phosphatidylcholine and phosphatidylinositol increased until the 60th day, and then decreased on the 90th day while in phosphatidylethanolamine and phosphatidic acids (Fig. 1) it increased throughout the period of the study.

The amount of linolenic acid in all the major classes of phospholipids decreased during seed development.

The content of saturated fatty acids was more than 55.0% and that of unsaturated fatty acids – from 16.2% to 44.5% (Fig. 2).

These results are different from the data in other publications about fatty acid composition of separate individual classes of phospholipids. Raharjo *et al*. (2011) established the main fatty acids in phosphatidylcholine to be oleic (38.21%) and palmitic (24.10%) while in the fraction of phosphatidylethanolamine oleic (45.23%) and linoleic (30.44%) acids prevailed; in the phosphatidylserine, palmitic (30.17%), oleic (28.83%) and linoleic (28.22%) acids are presented in almost equal amounts.

TABLE 6. Changes in the phospholipid composition of *Cucurbita moschata* seed oils during development

	Growing period, days after flowering		
Phospholipids, %	**30**	**60**	**90**
Phosphatidylcholine	9.9±0.1	11.0±0.2	17.1±0.1
Phosphatidylinositol	14.8±0.2	47.6±0.3	48.4±0.5
Phosphatidylethanolamine	11.9±0.1	13.5±0.4	17.8±0.5
Phosphatidic acids	38.7±0.3	14.6±0.2	6.6±0.1
Phosphatidylserine	–	4.6±0.2	4.9±0.3
Lysophosphatidylcholine	1.5±0.1	–	–
Sphingomyelin	–	0.5±0.1	0.9±0.2
Monophosphatidylglycerol	10.7±0.3	5.4±0.2	3.1±0.1
Diphosphatidylglycerol	12.5±0.5	2.8±0.3	1.2±0.1

4. CONCLUSIONS

In the maturing process of *Cucurbita moschata* pumpkin seeds, their nutritional value increased in oil content, protein and fiber and decreased in minerals and carbohydrates. Pumpkin oil is a potential source of essential fatty acids such as linoleic, and is rich in lipid-soluble bioactive compounds. The contents of biologically active compounds in the oil and the seeds dropped during growth. The fatty acid profile of glyceride oil changed. During the ripening process the amount of saturated fatty acids decreased from 36.7% to 28.6%, while that of unsaturated fatty acids increased from 63.3 % to 71.4%. The content of essential linoleic fatty acid in pumpkin oil increased from 40.8 to 50.2%.

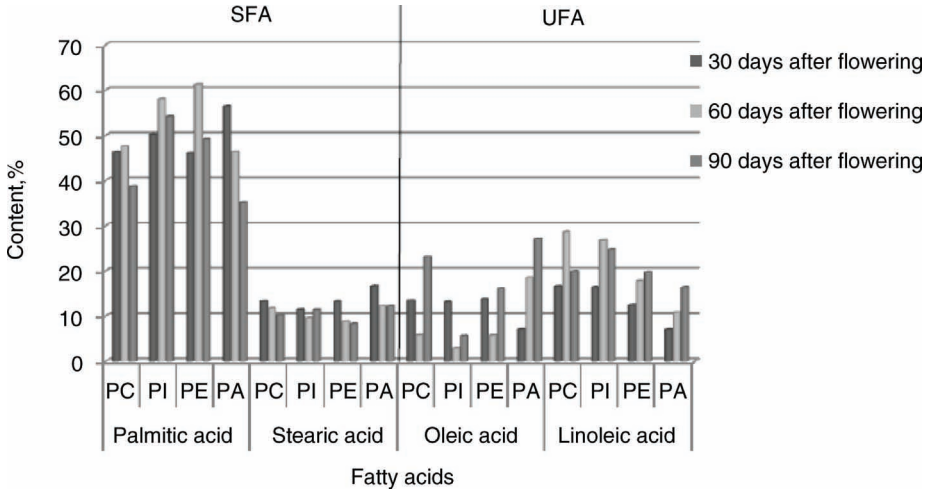

FIGURE 1. Changes in main saturated (SFA) and unsaturated (UFA) fatty acids in the major classes of phospholipids.

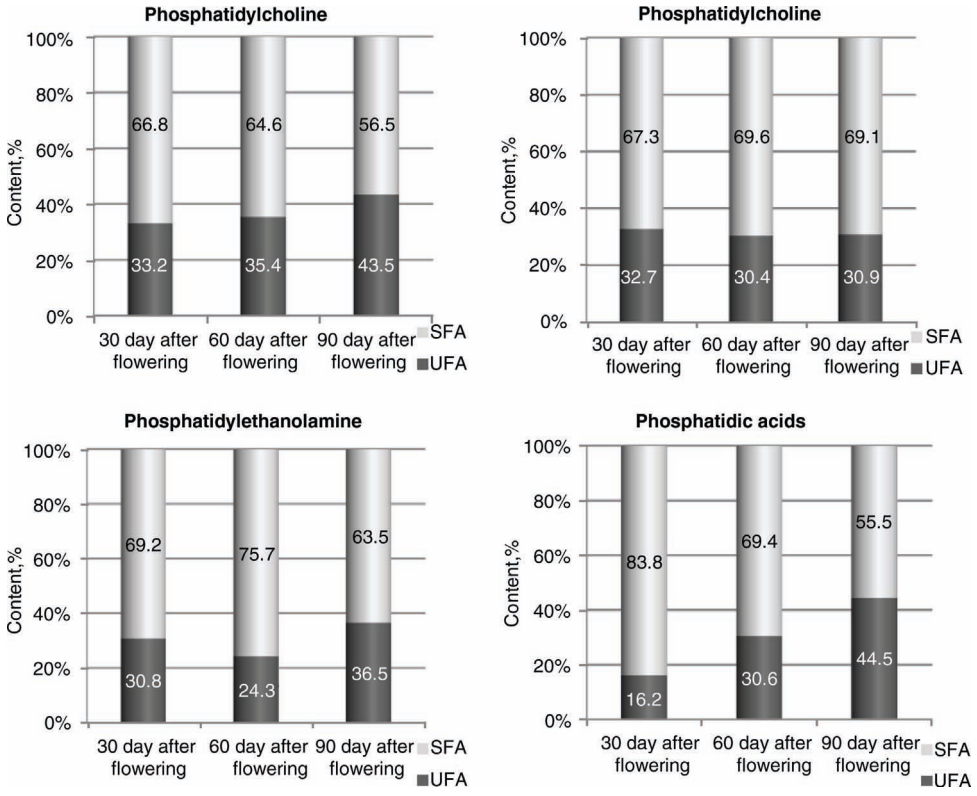

FIGURE 2. Saturated (SFA) and unsaturated (UFA) fatty acids in the main classes of phospholipids.

Phosphatidic acids, phosphatidylinositol, phosphatidylethanolamine, diphosphatidylglycerol and monophosphatidylglycerol predominated in the phospholipid fraction of the oil during the first stage of vegetation, while during the second and third stages of the growing season, the amounts of phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine increased. In the main classes of phospholipids, saturated fatty acids dominated in the three stages of the assay. Because of their high content of unsaturated acids, microelements and vitamins, pumpkin seeds and pumpkin seed oil are very healthy and can be used as a food or in medicine.

ACKNOWLEDGMENTS

The investigations were carried out with the financial support of The Paisii Hilendarski University of Plovdiv, Scientific Research Department (SI 13 FC 006/2013).

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