# THE CHANGES OF LIPID COMPOSITION OF HIGH OLEIC SUNFLOWER OIL DURING GROWING*

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The content and composition of biological active substances – fatty acids, phospholipids, sterols and tocopherols and their changes during growing were studied. The increase of the content of oleic acid during growing from 52.4% to 85.1% at harvest time was observed. Phosphatidylinositol (29.5%), phosphatidylcholine (38.9%) and phosphatidylethanolamine (17.7%) were found to be the main components in the phospholipid fraction. β-sitosterol (78.2% in free form and 46.6% in sterol esters) predominated in the sterol fraction. α-tocopherol (98.0%) was the main component in the tocopherol fraction.

sunflower; high oleic oil; biosynthesis; fatty acids; phospholipids; sterols; tocopherols

## INTRODUCTION

Sunflower oil is a natural food rich in tocopherols (vitamin E) and widely consumed as salad oil, in margarin production and as a cooking and frying oil with a light clean taste. The high qualities of a traditional sunflower oil are the result of high level of unsaturated essential linoleic acid (60.0–80.0%) and low level of saturated acids. However, when sunflower oil is used as frying fat at 160–180 °C it undergoes physical and chemical deterioration as a result of oxidative and polymerization processes. To avoid this undesired effect new varieties of sunflower were selected where the main component in the fatty acid fraction is monosaturated oleic acid, as well as olive oil. Increased interest in high oleic sunflower oil (HOSO) has been spurred by dietary recommendations favoring high monosaturates, low saturates and stable alternatives to hydrogenated oils. Functional applications for this oil include use as a dairy substitute, a spray oil for fruits and cereals, a salad or frying oil, and use in the manufacture of confectionery items, in food and industrial applications where high oxidative stability at termic treating and storage are required (Niemelg et al., 1996). Studies have also shown that monosaturated fatty acid have the ability to reduce the harmful cholesterol in blood.

In Bulgaria a high oleic, hybrid sort of sunflower named "Diamant" was also created and cultivated. The objective of this study is to investigate the main chemical characteristics of the oil obtained from the seeds of the new variety of sunflower and the changes of the fatty acids, phospholipids, sterols and tocopherols during growing of the seeds.

## MATERIALS AND METHODS

**Fruit material**. The investigated high oleic sunflower oil was obtained by the seeds of sunflower "Diamant" variety provided from the Plovdiv region in South Bulgaria, crop 2002. The investigations were carried out on air dried seeds.

**Glyceride oil isolation. Oil content**. The oils were extracted in Soxhlet apparatus with n-hexane for 8 h. After rotation vacuum distillation of the solvent the extracted oils were weighed.

**Fatty acid composition.** The fatty acid composition of triacylglycerols was identified by capillary gas chromatography of their methyl esters. The esterification was carried out by technique of Metcalfe and Wang (1981). Methyl esters were purified by thin-layer chromatography. Determination was accomplished on a Pye Unicam 304 unit, provided with flame-ionisation detector, 30 m capillary column "Innowax" impregnation (Scotia Pharmaceuticals Ltd.) and conditions as follows: column temperature 165 °C to 225 °C, with a change 4 °C/min; detector temperature 300 °C; injector temperature 280 °C; gas-carrier – nitrogen ($N_2$).

**Triacylglycerol structure.** Triacylglycerols classes differing in unsaturation were separated by silver-ion-TLC analysis on 19 x 4 cm glass plates, coated with ca. 0.2 mm silica gel G layer and impregnated by dipping into a 0.5% methanolic solution of silver nitrate (Tarandjiiska, Marekov, 1998). Continuous ascending development with the respective volume of the mobile phase in open cylindrical tanks was performed. The plate was then dried (1 h at 110 °C), and treated consecutively with bromine and sulphurylchloride vapours 30 min each, in closed tanks and in fume-cupboard to ensure the correct quantitative charring (at 180–200 °C) of the separated triacylglycerol classes. Scanning of the chromatograms was performed in the zigzag transmission mode at 450 nm. Beam-slit varied from 0.4 x 0.4 to 1.2 x 1.2 mm and the stage step varied depending on the separation achieved. The quantity of each spot was presented as relative area percentage, as derived from the integrator. Chromatographic conditions for the separation were following: sample amount: 16–20 μg (65–130

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for minor components), mobile phase: light petroleum : acetone : ethyl acetate 100 : 3 : 2 for major components and 100 : 6 : 5 for minor components. Recording of Ag-TLC chromatograms and quantitative measurement of peak areas were performed with a CS-930 densitometer Shimadzu equipped with DR-2 Shimadzu integrator.

**Phospholipid composition.** Lipids were extracted from the seeds by procedure of Folch et al. (1957). Polar lipids were divided from unpolar lipids by column chromatography (Kates, 1972). The phospholipid constituents were separated by two-directional thin-layer chromatography on Silica gel 60 G "Merck", impregnated with 1 g $(NH_4)_2SO_4$ per 100 g water solution (Beshkov, Ivanova, 1972). The first direction was carried out in chloroform : methanol : ammonia 65 : 25 : 5 v/v/v and the second in chloroform : methanol : ammonia : acetic acid : water 50 : 20 : 10 : 10 : 5 v/v/v/v/v. The spots of the separated individual phospholipids were identified by spraying with specific reagents (Kates, 1972). In addition, $R_F$ and standard spots were used for definitive identification. The quantitative evaluation was carried out spectrophotometrically at 700 nm (Beshkov, Ivanova, 1972).

**Sterol composition**. The free and esterified sterols were separated from the other oil constituents by preparative TLC on Silica gel 60 G "Merck" and mobile phase n-hexane : diethyl ether 1 : 1. The esterified sterols were saponified with ethanolic KOH, extracted with light petroleum and purified by TLC. The quantitative evaluation and individual composition was determined by gas chromatography, using HP 5890 A unit with FID, 25 m capillar column impregnated with OV-17 and conditions as follows: column temperature 260–300 °C (6 °C/min); detector temperature 320 °C, injector temperature 300 °C; gas carrier – nitrogen ($N_2$).

Identification was confirmed by retention time comparison of the individual constituents with those of authentic samples. Betuline was used as internal standard for quantitative evaluation of total sterols (ISO 1229, 1999; Homberg, Bielefeld, 1989).

**Tocopherol composition**. Tocopherols were analysed directly in the oil by HPLC with fluorescence detection (ISO 9936, 1989; Ivanov, Aitzetmüller, 1995). "Merck-Hitachi" unit fitted with column "Nucleosil" Si 50-5 250 x 4 mm and fluorescent detector "Merck-Hitachi" F 1000 was used. The operating conditions were as follows: excitation 295 nm, emission 330 nm, mobile phase n-hexane : dioxan 94 : 4, rate of mobile phase 1 $cm^3$/min. The peaks were identified and quantified using authentic individual tocopherols.

## RESULTS AND DISCUSSION

The changes of the general composition of the oil, content of sterols, tocopherols and phospholipids are shown in Table 1. Variation from ca. 20.9% to ca. 65.5% oil at harvest were observed. Sterols and phospholipids

Table 1. Changes of general composition of sunflower seeds during growing*

| Compounds | 15 days after flowering | 30 days after flowering | 45 days after flowering | 60 days after flowering | 75 days after flowering | 90 days after flowering |
|---|---|---|---|---|---|---|
| 1. Oil in kernels, % | 20.9 | 44.6 | 53.7 | 60.6 | 61.8 | 65.5 |
| 2. Phospholipids in oil, % | 0.6 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 |
| 3. Sterols in oil, % | 0.5 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 |
| 4. Tocopherols in oil, mg/kg | 560.0 | 572.0 | 554.0 | 675.0 | 750.0 | 869.0 |

*Mean of three replicates

Table 2. Changes of fatty acid composition of the sunflower oil during growing*

| Fatty acids | 15 days after flowering | 30 days after flowering | 45 days after flowering | 60 days after flowering | 75 days after flowering | 90 days after flowering |
|---|---|---|---|---|---|---|
| 1. $C_{14:0}$ miristic | 0.2 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 |
| 2. $C_{16:0}$ palmitic | 11.3 | 6.6 | 5.6 | 4.8 | 4.4 | 4.3 |
| 3. $C_{16:1}$ palmitoleic | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | tr. |
| 4. $C_{18:0}$ stearic | 10.7 | 6.5 | 4.5 | 4.0 | 3.7 | 3.6 |
| 5. $C_{18:1}$ oleic | 52.4 | 72.5 | 77.0 | 80.3 | 83.3 | 85.1 |
| 6. $C_{18:2}$ linoleic | 1.4 | 8.7 | 9.0 | 8.6 | 7.8 | 6.7 |
| 7. $C_{18:3}$ linolenic | 22.7 | 4.2 | 3.3 | 1.8 | 0.4 | tr. |
| 8. $C_{20:0}$ arachidic | 1.1 | 1.1 | 0.4 | 0.2 | 0.1 | 0.1 |
| Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 |
| Ratio unsaturated : saturated fatty acids | 76.7 : 23.3<br>3.3 | 85.6 : 14.4<br>5.9 | 89.4 : 10.6<br>8.4 | 90.8 : 9.2<br>9.9 | 91.6 : 8.4<br>10.9 | 91.8 : 8.2<br>11.2 |

*Mean of three replicates

were accumulated at the first 30 days, and then their content was relative permanent.

The changes of fatty acid composition of triacylglycerols are listed in Table 2. In the first 15 days saturated palmitic acid (11.3%), stearic acid (10.7%) and essential linolenic acid (22.7%), besides oleic acid, were accumulated. During the second stage (15–60 days) the percentage of these acids decreased, whereas the content of oleic acid increased. In the end of the stage 2 (60–90 days) linolenic and arachidic acids are presented in very small quantities. The percentage of oleic acid in the third stage (60–90 days) continued to growth at expense of the other acids. During the growth of the seeds total content of unsaturated fatty acids continuously increased and proportion unsaturated : saturated acids was changed from 3.3 to 11.2. These results are closed to data reported by Soler et al. (1988) about other vegetable developing oils. Fatty acid composition of the Bulgarian high oleic sunflower oil is similar to composition reported by other authors (Ivanov, 1991).

Monoenoic acids $M_3$ (mainly triolein) – 54.6% followed by dioleins $SM_2$ (mainly palmitdiolein) – 18.6% are the main components in the high oleic sunflower triacylglycerol structure (Table 3). This composition is closed to the olive oil, but the content of triolein in sunflower oil is higher than in the olive oil. On the other hand, the quantity of $SM_2$ and $M_2D$ (26.4% and 12.8%, respectively) in olive oil is higher than sunflower oil.

Fatty acid composition of the main phospholipid classes and of the sterol esters can be seen in Table 4. Oleic acid predominated in all of compounds but the content of saturated palmitic (8.3–22.3%) and stearic (3.6–12.5%) acids is considerably higher than in triacylglycerols. Fatty acid composition varies significantly in the different phospholipid components. This of phosphatidylcholine was found to be closed to the composition

Table 3. Triglyceride composition of high oleic sunflower and olive oil*

| Triglycerides | Sunflower oil | Olive oil |
|---|---|---|
| $S_2M$ | 1.5 | 5.2 |
| $SM_2$ | 18.6 | 26.4 |
| $M_3$ | 54.6 | 43.4 |
| $S_2D$ | 0.5 | 0.2 |
| SMD | 3.8 | 6.8 |
| $M_2D$ | 8.2 | 12.8 |
| $SD_2$ | 3.6 | 0.8 |
| $MD_2$ | 5.8 | 1.8 |
| SMT | – | 0.7 |
| $M_2T$ | – | 1.4 |
| $D_3$ | 3.4 | 0.2 |
| MDT | – | 0.3 |
| Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 |

*Mean of five replicates

Triacilglycerol classes (TAG): Abbreviations:

S – saturated acids ($C_{16:0}$, $C_{18:0}$, $C_{20:0}$)
M – monoenoic acids ($C_{16:1}$, $C_{18:1}$)
D – dienoic acids ($C_{18:2}$)
T – trienoic acids ($C_{18:3}$)
$S_2M$ – TAG with 2 saturated and 1 monoenoic acids
$SM_2$ – TAG with 1 saturated and 2 monoenoic acids
$M_3$ – TAG with 3 monoenoic acids
$S_2D$ – TAG with 2 saturated and 1 dienoic acids
SMD – TAG with 1 saturated, 1 monoenoic and 1 dienoic acids
$M_2D$ – TAG with 2 monoenoic and 1 dienoic acids
$SD_2$ – TAG with 1 saturated and 2 dienoic acids
$MD_2$ – TAG with 1 monoenoic and 2 dienoic acids
SMT – TAG with 1 saturated, 1 monoenoic and 1 trienoic acids
$M_2T$ – TAG with 2 monoenoic and 1 trienoic acids
$D_3$ – TAG with 3 dienoic acids
MDT – TAG with 1 monoenoic, 1 dienoic and 1 trienoic acids

Table 4. Fatty acid composition of phospholipids and sterol esters*

| Fatty acids | PC | PI | PE | PA | Sterol esters |
|---|---|---|---|---|---|
| 1. C $C_{14:0}$ miristic | 0.2 | 0.3 | 0.4 | 1.0 | 3.5 |
| 2. C $_{16:0}$ palmitic | 8.3 | 18.5 | 13.0 | 24.0 | 22.3 |
| 3. C $_{16:1}$ palmitoleic | 0.1 | 2.1 | 0.2 | 0.6 | 0.5 |
| 4. C $_{17:0}$ margarinic | 0.1 | 0.3 | 0.1 | 0.4 | 0.6 |
| 4. C $_{18:0}$ stearic | 3.6 | 8.0 | 5.2 | 11.9 | 12.5 |
| 5. C $_{18:1}$ oleic | 75.6 | 67.2 | 78.4 | 61.5 | 41.7 |
| 6. C $_{18:2}$ linoleic | 12.0 | 3.5 | 2.5 | 0.4 | 16.3 |
| 7. C $_{18:3}$ linolenic | 0.1 | 0.1 | 0.2 | 0.2 | 2.1 |
| 8. C $_{20:0}$ arachidic | tr. | tr. | tr. | tr. | 0.5 |
| Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 |
| Ratio unsaturated : saturated fatty acids | 87.8 : 12.2<br>7.2 | 72.9 : 27.1<br>2.7 | 81.3 : 18.7<br>4.3 | 62.7 : 37.3<br>1.7 | 60.6 : 39.4<br>1.5 |

*Mean of three replicates

PC – phosphatidylcholine
PI – phosphatidylinositole
PE – phosphatidylethanolamine
PA – phosphatidic acids

Table 5. Phospholipid composition of high oleic sunflower (HOSO) and linoleic (LSO) sunflower oil*

| Phospholipids | HOSO, % | LSO, % (Zlatanov et al., 1992) |
|---|---|---|
| 1. Phosphatidylcholine (PC) | 38.9 | 39.8 |
| 2. Phosphatidylinositol (PI) | 29.5 | 20.7 |
| 3. Phosphatidylethanolamine (PE) | 17.7 | 13.8 |
| 4. Phosphatidic acids (PA) | 2.3 | 5.6 |
| 5. Lysophosphatidylcholine | 1.4 | 5.7 |
| 6. Lysophosphatidylethanolamine | 1.5 | 3.6 |
| 7. Phosphatidylserine | 3.5 | 3.2 |
| 8. Others | 5.2 | 7.6 |
| Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 |

*Mean of three replicates

Table 6. Sterol composition of high oleic sunflower (HOSO) and linoleic sunflower (LSO) oil*

| Sterols | HOSO, % | | | LSO, % (Homberg E., 1989) |
|---|---|---|---|---|
| | free sterols | esterified sterols | total sterols | total sterols |
| 1. Cholesterol | 0.1 | 4.9 | 0.8 | 0.1 |
| 2. Campesterol | 6.6 | 4.5 | 6.3 | 9.8 |
| 3. Stigmasterol | 12.5 | 12.1 | 12.4 | 9.7 |
| 4. β-sitosterol | 78.2 | 46.6 | 73.0 | 59.1 |
| 5. $\Delta^5$-avenasterol | 1.0 | 23.4 | 4.7 | 6.8 |
| 6. $\Delta^{7,25}$-stigmasterol | 0.3 | tr. | 0.3 | 1.2 |
| 7. $\Delta^7$-avenasterol | 0.5 | tr. | 0.4 | 5.4 |
| 8. $\Delta^7$-stigmasterol | 0.8 | 8.5 | 2.1 | 7.9 |
| Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 |
| Ratio sterols with 1 : 2 double bonds | 86.4 : 13.6 | 56.0 : 44.0 | 80.0 : 19.2 | 75.6 : 24.4 |
| | 6.4 | 1.3 | 4.2 | 3.1 |

*Mean of three replicates

of triacylglycerols, while considerable higher percentages of palmitic and stearic acids in phosphatidylinositol, phosphatidylethanolamine and phosphatidic acids was observed. The high concentration of saturated acids may be related with different stages of the biosynthesis of the oil constituents. Sterol esters and phospholipids are synthesised in the first stage when the concentration of palmitic and stearic acids is very high and they are incorporated into them. Later, during the growth of the seeds the content of triacylglycerols progressive rises and begins to related unsaturated oleic and linoleic acids. The variation of linolenic acid percentage is different because it is also component of triacylglycerols. Phosphatidylcholine is synthesised after other phospholipids before triacylglycerols and because that this fatty acid composition is similar to triacylglycerols.

Phospholipid composition of the sunflower oil is presented in Table 5.

Phosphatidylcholine (38.9%), phosphatidylinositol (29.5%) and phosphatidyl-ethanolamine (17.7%) were found to be the main constituents in phospholipid fraction. This composition is closed to phospholipid composition of sunflower oil linoleic type.

Sterols in the vegetable oils are found in free form and as sterol esters. The main part of them in investigated oil are free sterols – 83.6%. Sterol fraction in both free and esterified form consisted mainly of β-sitosterol (78.2% and 46.6%, respectively) followed by stigmasterol in free sterols and $\Delta^5$-avenasterol in sterol esters (Table 6). Relatively high level of cholesterol (4.9%) in sterol esters was observed. This content is similar to composition of other vegetable oils reported earlier by Kiosseoglou, Boskou (1989) and Zlatanov et al. (1998, 1999). Higher percentage (44.0%) of sterols with two double bonds was detected in sterol esters than in free sterols (13.6%).

Higher percentage of β-sitosterol (73.0%) in high oleic sunflower oil than in linoleic sunflower oil (59.1%) was established. On the other hand, the content of $\Delta^7$-avenasterol and $\Delta^7$-stigmasterol was lower: 0.4% and 2.1% respectively in high oleic sunflower oil to 5.4% and 7.9%, respectively, in linoleic sunflower oil. The ratio of free sterols to esterified sterols in high oleic sunflower oil (HOSO, %) is 83.6 : 16.4.

α-tocopherol (98.0%) is the predominant constituent in the tocopherol fraction like linoleic type sunflower oil

Table 7. Tocopherol composition of high oleic sunflower (HOSO) and linoleic sunflower (LSO) oil*

| Tocopherols | HOSO, % | LSO, % (Coors) |
|---|---|---|
| 1. α-tocopherol | 98.0 | 94.2 |
| 2. β-tocopherol | 1.6 | 1.9 |
| 3. γ-tocopherol | 0.3 | 2.9 |
| 4. δ-tocopherol | 0.1 | 1.0 |
| Σ = 100% ± 0.1 | Σ = 100% ± 0.1 | Σ = 100% ± 0.1 |

*Mean of three replicates

(Table 7). The other tocopherols were presented with traces or minute amounts. Those data are similar to tocopherol composition of sunflower oil linoleic type.

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ZLATANOV, M. – ANGELOVA, M. (University of Plovdiv, Department of Chemical Technology, Plovdiv, Bulgaria):


**Změny ve složení lipidů vysoce olejnatého slunečnicového oleje během růstu.**


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Předmětem zkoumání byl obsah a složení biologicky aktivních látek – mastných kyselin, fosfolipidů, sterolů a tokorefolů a jejich změny během růstu. Bylo zjištěno zvýšení obsahu kyseliny olejové během růstu z 52,4 % na 85,1 % během sklizně. Fosfatidylinositol (29,5%), fosfatidylcholin (38,9%) a fosfatidyletanolamin (17,7%) jsou hlavní složky ve fosfolipidové frakci. β-sitosterol (78, 2% ve volné formě a 46,6 % ve sterolových esterech) převládal ve sterolové frakci, α-tokoferol (98,0%) byl hlavní složkou v tokoferolové frakci.



slunečnice; vysoce olejnatý olej; biosyntéza; mastné kyseliny; fosfolipidy; steroly; tokoferoly



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