

LIPID COMPOSITION DURING THE GERMINATION OF KAZAKHSTAN MAIZE HYBRID

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Abstract

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The chemical composition of the oil germination of Kazhastan maize hybrids was investigated. The main components in the triacylglycerol fraction were oleic (61.9 – 64.0%) and palmitic (21.9 – 23.1%) acids. γ -Tocopherol (66.3 %) predominated in the tocopherol fraction, and β -sitosterol (54.8 – 61.2%) and campesterol (27.1 – 30.6%) – in the sterol fraction.

Key words: maize hybrids, lipid composition

Introduction

Maize in their homeland was revered as a sacred plant, now people are more pragmatic and introduced it to the list of "bread" products along with wheat and rice. Maize is used as flour to make bread, scones, and pancakes, to produce popcorn, sugary cereals and other foods. Milled maize added to the minced meat or minced vegetables, peeled ears cooked or canned. During preservation in the maize stored many valuable substances. It requires conserving the fresh grains, the so-called milk-wax stage. Precisely such contains a rich complex of minerals. Potassium, for example, is the main constituent element for every living cell. Calcium is essential for bone tissue. Magnesium, which is part of the maize, is actively involved in protein synthesis, and the iron is an essential component of hemoglobin. Phosphorus is so essential organism for muscle contractions provides the biochemical processes in the brain, supports the normal functioning of the nervous system, liver and other organs. In addition, the researchers found that the maize protein contains the essential amino acids lysine (immunomodulator antidiabetic agent) and tryptophan, which is required for the body to produce vitamin B₃ (Niacin). Maize vitamin set represented by a

group of the vitamins B₁, B₂, B₅, involved in tissue respiration and production of the energy and carotene. The fat content of maize grains ranges from 3.5 to 6.5%. The main part of fat consists of fatty acids: 30 - 45% oleic acid, 40 - 55% linoleic, 11.8% palmitic, 3.5% stearic, 0.4% arachic and 0.2% lignoceric acid (Popov and Ilinov, 1986; Shterbakov, 1963).

Important moment in the life of the plant is a period of germination. By its enormous energy intensity, speed and variety of biochemical transformations germination process is unique in nature. Experimentally proved that during the germination of seeds increases the amount of antioxidants that neutralize an excess of free radicals (Shaskol'skaya and Shaskol'skii, 2009; Yashin et al., 2009; Kulazhanov et al., 2010).

In view the development of functional products from plant materials, including maize, have been examined the changes of vitamin, mineral composition and antioxidant activity during germination of Kazakhstan varieties of maize (Nabiyeva et al., 2012; Tnymbaeva and Kizatova, 2010).

The aim of present investigation is to examine the lipid composition of maize hybrid "Arman-689" from Kazhastan.

In order to study the effect of germination on fatty acid composition.

Materials and Methods

Isolation of lipid fraction. The fruits were extracted with n-hexane in Soxhlet for 18 h. The solvent was partly removed in a rotary vacuum evaporator, the residue was transferred to a pre-weighed glass vessel and the rest of the solvent was removed under stream of nitrogen to a constant weight, in order to determine the oil content (ISO 659, 2009).

Fatty acids. The total fatty acid composition of the oil was determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50°C according to Christie, 2003. Fatty acid methyl esters (FAME) were purified by TLC on 20 cm x 20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane: acetone, 100: 8 (by volume). Determination was performed on a gas chromatograph equipped with a 30 m x 0.25 mm x 25 µm (I.D.) capillary EC 30-Wax column (Hewlett Packard GmbH, Vienna, Austria) and a flame ionization detector. The column temperature was programmed from 130°C (hold 4 min), at 15°C/min to 240°C (hold 5 min); injector and detector temperatures were 250°C. Hydrogen was the carrier gas at a flow rate 0.8 ml/min; split was 50:1. Identification was performed by comparison of retention times with those of a standard mixture of FAME subjected to GC under identical experimental conditions (ISO 5508, 2000).

Sterols. Unsaponifiables were determined by weight after saponification of the glycerides oil and extraction with hexane (ISO 18609, 2000). The unsaponifiable matters (100 mg, precisely measured) was applied on 20 cm x 20 cm glass plates (ca. 1 mm thick Silica gel G layer) and developed with n-hexane: acetone, 100: 8 (by volume). Free sterols ($R_f = 0.4$) were detected under UV light by spraying the edges of each plate with 2',7'-dichlorofluorescein, they were then scraped, transferred to small glass columns and eluted with diethyl ether. The solvent was evaporated under a stream of nitrogen and the residue was weighed in small glass containers to a constant weight. Sterol composition was determined by GC using HP 5890 gas chromatograph (Hewlett Packard GmbH, Vienna, Austria) equipped with a 25 m x 0.25 mm DB – 5 capillary column (Agilent Technologies, Santa Clara CA, USA) and a flame ionization detector. Temperature gradient was from 90°C (hold 2 min) up to 290°C at a rate 15°C/min and then up to 310°C at a rate of 4°C/min (hold 10 min); the injector temperature was 300°C and the detector temperature was 320°C. Hydrogen was used as carrier gas at a flow rate 0.8 ml/min; split 50:1. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols (ISO 12228, 1999).

Tocopherols. Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) by a

Merck-Hitachi (Merck, Darmstadt, Germany) unit equipped with a 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and a fluorescent detector Merck-Hitachi F 1000. The operating conditions were as follows: mobile phase n-hexane : dioxan, 96 : 4 (by volume), flow rate 1.0 ml/min, excitation 295 nm, emission 330 nm. 20 µkl 1% solution of crude oil were injected. Tocopherols were identified by comparing the retention times to those of authentic individual pure tocopherols. The tocopherol content was calculated on the base of tocopherol peak areas in the sample vs. tocopherol peak area of the standard tocopherol solution (ISO 9936, 1997).

Phospholipids: Another part (10 g) of air-dried seeds was subjected to Folch extraction according to Christie, 2003. Polar lipids were isolated from the total lipids by column chromatography. Briefly, the sample (100 mg) was applied on 40 cm x 2 cm glass column packed with Silica gel Unisil 100-200 mesh (Clarkson Chemicals Co., USA) and eluted in sequence with chloroform (for neutral lipids, sterols and sterol esters), acetone (sterol glycosides) and with methanol to isolate phospholipids. The phospholipid classes were isolated by a variety of the two-dimensional thin-layer chromatography on 20 cm x 20 cm glass plates with 0.2 mm Silica gel 60 G layer (Merck) impregnated with aqueous $(\text{NH}_4)_2\text{SO}_4$ (10 g kg^{-1}). In the first direction, the plate was developed with chloroform: methanol: ammonia, 65: 25: 5 (by volume) and in the second – with chloroform: acetone: methanol: acetic acid: water, 50: 20: 10: 10: 5 (by volume). The individual phospholipids were detected and identified by spraying with specific reagents according to Christie, 2003. Dragendorff test (detection of choline-containing phospholipids); Ninhydrin spray (for phospholipids with free amino groups), and Shiff's reagent (for inositol containing phospholipids). 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 scrapping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 (by volume). The calibration curve was constructed by using a standard solution of KH_2PO_4 . It was linear in the concentration range 1 – 130 µg ml^{-1} (as phosphorus). In each series of measurements a standard solution of KH_2PO_4 (10 µl ml^{-1} in water) was used to confirm the validity of calibration.

Results

The triacylglycerol fraction was 4.1% in the hybrid and 3.2% in the sprouted. The fatty acid composition is presented

in Table 1, the individual sterol composition - in Table 2, the tocopherol and tocotrienol composition – in Table 3 and phospholipid composition – in Table 4. The distribution of fatty acids in hybrid and sprout is presented in Figures 1 and 2.

Table 1
Fatty acid composition of seed oil

№	Fatty acids	Content, g.kg ⁻¹	
		Hybrid “Arman-689”	Sprouted hybrid “Arman-689”
1	C _{12:0} Lauric	1	1
2	C _{14:0} Myristic	1	1
3	C _{16:0} Palmitic	219	231
4	C _{16:1} Palmitoleic	2	1
5	C _{17:0} Margaric	1	1
6	C _{18:0} Stearic	10	10
7	C _{18:1} Oleic	640	620
8	C _{18:2} Linoleic	115	124
9	C _{20:0} Arachidic	4	5
10	C _{20:1} Gadoleic	4	3
11	C _{22:0} Behenic	3	3

Table 2
Sterol composition of seed oil

Sterols	Content, g.kg ⁻¹	
	Hybrid “Arman-689”	Sprouted hybrid “Arman-689”
Cholesterol	15	11
Brassicasterol	7	6
Campesterol	306	271
Stigmasterol	35	60
β- Sitosterol	548	612
Δ ⁵ - Avenasterol	5	8
Δ ⁷ - Stigmasterol	32	6
Δ ⁷ - Avenasterol	52	26

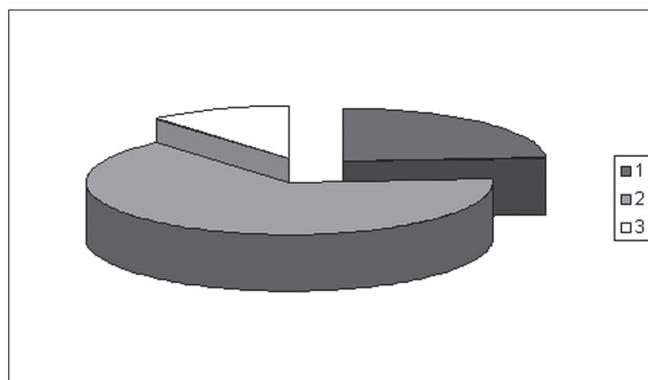


Fig. 1. Distribution of fatty acids in hybrid “Arman-689”
1-saturated fatty acids (239 g.kg⁻¹)
2-monounsaturated fatty acids (646 g.kg⁻¹)
3-polyunsaturated fatty acids (115 g.kg⁻¹)

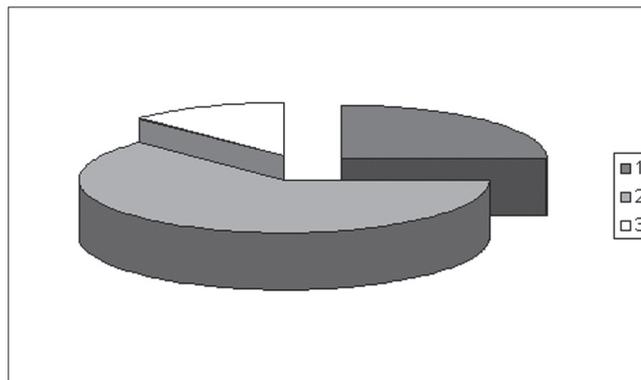


Fig. 2. Distribution of fatty acids in sprouted hybrid “Arman-689”
1-saturated fatty acids (252 g.kg⁻¹)
2-monounsaturated fatty acids (624 g.kg⁻¹)
3-polyunsaturated fatty acids (124 g.kg⁻¹)

Table 3
Tocopherol composition of seed oil

Tocopherols and tocotrienol	Content, g.kg ⁻¹	
	Hybrid "Arman-689"	Sprouted hybrid "Arman-689"
α-Tocopherol	140	268
β-Tocopherol	32	-
γ-Tocopherol	663	-
δ-Tocopherol	112	732
δ-Tocotrienol	53	-

Table 4
Phospholipid composition of seed oil

Phospholipids	Content, g.kg ⁻¹	
	Hybrid "Arman-689"	Sprouted hybrid "Arman-689"
Phosphatidylcholine	273	423
Phosphatidylethanolamine	212	151
Phosphatidylinositol	182	233
Phosphatidic acids	91	65
Phosphatidylserine	121	26
Diphosphatidylglycerol	-	38
Lysophosphatidylethanolamine	121	64

Discussions

Data in Table 1 show that 11 fatty acids were determined, constituting 100% of the total oil content. The main fatty acids in the triacylglycerol fraction were oleic and palmitic acids. In the lipid fraction, according to data from the literature are identified as predominating the same three fatty acids, but in different quantities, which could be explained by the influence of the origin of the raw.

The correlation unsaturated: saturated fatty acids was 74.5-76.1: 23.9-25.3, and their distribution profiles are presented on Figures 1 and 2. Palmitic acid predominated in the fraction of saturated fatty acids and oleic and linoleic were predominant among the unsaturated acids.

Regarding the individual presence of oleic and linoleic acid, the oil from maize seeds was similar to the oils from other nontraditional materials such as grape seeds, watermelon, tobacco and poppy seeds (Popov and Ilinov, 1986; Shterbakov, 1963). Maize seeds oil was found to contain very high amounts of the saturated palmitic acid, which comes close to the levels in other oils (O'Brien et al., 2000).

Sterols are present in the so-called non-saponificated part (11.5% in hybrid and 4.5% in sprout) and their total content in the oil was found to be 0.3% and 0.8%, respectively. β-Sitosterol predominated in the sterol fraction. It is obvious from the data, that regarding its sterol content and composi-

tion, maizen seeds oil was similar to the findings for hawthorn fruits (Angelova-Romova et al., 2010), fruits from the *Apiaceae* family (Zlatanov and Ivanov, 1995) and for cottonseed oil (Codex, 2003).

The total content of tocopherols in the oils was comparatively low – 281 mg/kg for oil from hybrid and 25 mg/kg for oil from sprout hybrid. The γ-tocopherol predominated in the oil from hybrid, followed by α-tocopherol and δ-tocopherol. In the oil from sprout hybrid predominated δ-tocopherol, followed by α-tocopherol. The oil from hybrid with higher content of γ-tocopherol proved superior to a number of common food oils, for example – corn oil (50.0 – 62.0%) and soya oil (60.0 – 85.0%), thus showing similarity to some non-traditional oils, such as pyrene oils of morello (93.1%) and apricot (96.0%) (Popov and Ilinov, 1986).

The total content of phospholipids in the oils was comparatively low – 0.3-0.8 %. The phosphadilholin, phosphadilethanolamin and phosphadilinozitol predominated in the oils.

Conclusion

Maize hybrid "Arman-689" can be used as a non-traditional material for producing the oil which is rich in biologically active substances as sterols and tocopherols for nutritive purposes, as well as for an additive in food products in order to enrich them with valuable nutrients.

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