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REGULAR ARTICLE

POPPY SEED (*PAPAVER SOMNIFERUM* L.): EFFECT OF GENOTYPE AND YEAR OF CULTIVATION ON VARIABILITY IN ITS LIPID COMPOSITION

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ABSTRACT

Poppy seeds have a high nutritive value and are used as a food and a source of edible oil. This oil is a rich source of polyunsaturated fatty acids. It is known that polyunsaturated fatty acids present not only basic nutriments for human body, but its taking to the organism is very important in term of protection against cardiovascular diseases, heart attacks and many inflammatory diseases. The goal of the study was to determine lipid content and fatty acids composition in eight selected poppy genotypes grown in experimental fields of the Plant Production Research Centre Piešťany – Research and Breeding Station at Malý Šariš (Slovak Republic) in two years. Seed oils were analyzed by gas chromatography (GC-FID) from prepared methylesters of fatty acids. The highest lipid content in 2007 was detected for genotype Opál (49.9%). In 2009, genotype ZB-6 contained the highest lipid content (50.1%). Linoleic acid was dominant fatty acid in all analyzed poppy oils. Its highest level contained the genotype ZB-5 (68.1%) in 2007 and ZB-1 (66.5%) in 2009. Other major fatty acids were palmitic and oleic acids. As minority fatty acids were presented stearic, *alpha*-linolenic and palmitoleic acids. Myristic, arachidic and gadoleic acids were observed in trace amounts. Furthermore, the effect of year of cultivation on the fatty acids content in poppy seed oils was examined by Student *t*-test and appropriate non-parametric Mann-Whitney test.

Keywords: lipid, poppy seeds, oil, fatty acids, FID, ALA, OA

INTRODUCTION

Poppy (*Papaver somniferum* L.) is very important crop growing mainly for production of seed. According to **FAOSTAT (2009)** database, areas of poppy seeds cultivated in the world achieved in 2009 year 140 534 ha, with total production quantity of 98 835 tons. Quality and nutritive value of poppy seeds is based on lipid content and mainly polyunsaturated fatty acids. Generally, poppy seeds contain 50% of edible oil (**Singh *et al*., 1990**). This value is influenced by genotype and color of the seed (**Eklund and Agreen, 1975**). **Özcana and Atalay (2006)** found out lipid content in poppy seeds in the range between 32.4% and 45.5%.

Polyunsaturated fatty acids (PUFAs) are considered to be necessary components of cell membranes and play a key role in many cell processes. Fatty acids (n-3) have an anti-inflammatory effect because of decreasing the production of cytokines and adhesion molecules. Diseases as rheumatoid arthritide, Crohn´s disease, ulcerative colitis, cystic fibrosis, asthma, diabetes, allergic diseases, sclerosis multiplex, atherosclerosis, obstructive pulmonary disease are possible therapeutic purposes of n-3 fatty acids (**Abbate *et al*., 1996; Calder, 2001, 2003**). Currently, effect of poppy seed oil in control of iodine absence in human body is studied by many researchers. **Untoro *et al*. (2006)** detected higher concentration of iodine in urine in comparison with placebo. Poppy oil is also suitable alternative for sunflower oil in dietary products (**Kusmenoglu *et al*., 2002**).

Plants are important renewable sources of fatty acids because many species accumulate the fatty acids in the form of triglycerides as the main substances in seeds. Therefore it is also very important to understand which factors limit the accumulation of fatty acid structures in seeds (**Thelen and Ohlrogge, 2002**). In higher plants, PUFAs are synthesized by both prokaryotic (chloroplast) and eukaryotic (endoplasmic reticulum) pathways (**Roughan *et al*., 1980; Browse *et al*., 1986**). Synthesis of < 18C fatty acids in the cell matrix proceeds in the presence of NADPH as hydrogen donor. Carbon chain of fatty acid acyl requires the presence of NADPH and O_2. They are present in eukaryotic cells, the phospholipid is a carrier for acyl, phosphatidylcholine, phosphatidylethanolamine; and phosphatidyl inositol acts as substrates. Stearoyl-ACP desaturase catalyzes the production of 18:1 fatty acids in plants (**Shanklin and Somerville, 1991**). Phosphoenolpyruvate is one of several transporters in the synthesis of fatty acids and other pathways from the cytosol to the plastid. Currently, research is more oriented on the identification of genes modulating the

unsaturated fatty acids and the study of metabolism and synthesis at both transcriptional and post-transcriptional levels.

According to authors **(Erinc *et al*., 2009; Azcan *et al*., 2004)**, poppy seed oils contain linoleic acid in the highest level (56.4-69.2%), oleic acid in the level of 16.1%-19.4% and palmitic acid in the range of 10.6%-16.4%. Smaller amounts represent stearic and *alpha*-linolenic acids.

The objective of our study was to determine lipid content and fatty acids composition in poppy seed samples grown in the Slovak Republic and to evaluate the effect of genotype and year on their amounts.

MATERIAL AND METHODS

As biological material we used eight poppy genotypes, which were grown in experimental field of the Plant Production Research Centre Piešťany – Research and Breeding Station at Malý Šariš (Slovak Republic) in two years (2007 and 2009). Six genotypes were obtained from the collection expeditions (ZB-1, ZB-3, ZB-4, ZB-5, ZB-6, and ZB-7). The variety Opál is listed on the List of Registered genotypes of Slovak origin and Budha has a Hungarian origin.

For determination of the lipid content, a Soxhlet method according to the norm (**STN 46 1011–28**) for testing of cereals, legumes and oil crops was used. From obtained oil, methylesters of fatty acids were prepared according to **Christopherson and Glass (1969)**. Fatty acids were analyzed as their methyl esters by gas chromatography (GC–6890 N, Agilent Technologies) using a capillary column DB–23 (60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies) and a FID detector (constant flow, hydrogen 35 ml/min, air 350 ml/min, 250°C) under a temperature gradient (130°C for 1 min; 130–170°C at program rate 6.5°C/min; 170–215°C at program rate 2.7°C/min; 215°C for 7 min; 220–240°C at program rate 2°C/min) with hydrogen as a carrier gas (flow 2.1 ml /min, velocity 49 cm/s, pressure 174 kPa) and a split ratio of 1/50 (Inlets: heater 230°C, total hydrogen flow 114 ml/min, pressure 174 kPa) according to **Čertík *et al*. (2005)**. The fatty acid methylesters peaks were identified by authentic standards of C4–C24 fatty acid methylesters mixture (Supelco, USA) and evaluated by ChemStation 10.1 (Agilent Technologies). The degree of fatty acid unsaturation (IU) was calculated in D/mole from the equation: IU = [1 (% monoene) +2 (% diene) + 3 (% triene)]/100 (**Čertík and Šajbidor, 2006**) and oleic acid/linoleic acid (OA/LA) and *alpha*-linolenic acid / linoleic acid (ALA/LA) ratios were also evaluated in this study.

The comparison of samples groups by year of cultivation (the target factor) was performed by the Student *t*-test and its non-parametric analogue Mann-Whitney test which is appropriate in case of violation of the validity of normal distribution and equality of variances of each population from which the sample is taken. Statistical data treatment was performed using program package SPSS v. 15.0.

RESULTS AND DISCUSSION

Lipid content in evaluated poppy genotypes grown in 2007 is shown on the figure 1. Opál, which is listed on the List of registered varieties, contained the highest lipid content (49.9%), followed by ZB-6 (46.7%), Budha (46.2%) and ZB-7 (44.3%). Other genotypes contained lower, but very similar lipid amounts in the order: 43.7% (ZB-3), 43.6% (ZB-4), and 43.4% (ZB-1). In given year of cultivation, genotype ZB-5 contained the lowest lipid content. Its value was 43.3%.

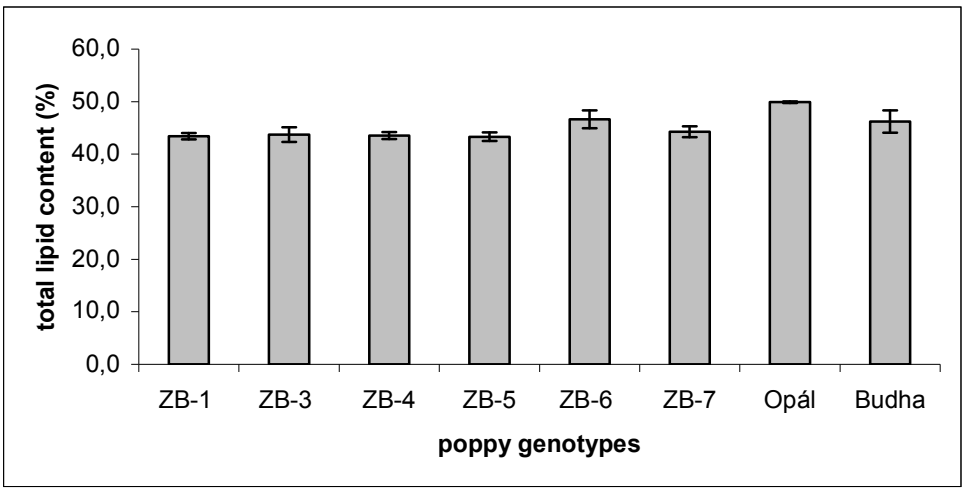

Figure 1 Total lipid content (in %) in poppy genotypes grown in 2007 year

Lipid content in genotypes grown in 2009 is displayed in the figure 2. In 2009, genotype ZB-6 contained the highest lipid content (50.1%). Similarly, we observed relatively high lipid content in genotype ZB-5 (48%). Genotypes ZB-3 and ZB-7 contained levels of 46.9% and 45.4%, respectively. We detected similar lipid content in two genotypes (Budha and ZB-4). Lipid values of both genotypes were similar, 44.5%. The lowest lipid content was determined in variety Opál (40.8%).

These results indicate that there are differences in lipid content between years of cultivation. Whereas in 2007, Slovak variety Opál contained the highest lipid content (49.9%), in 2009 was its value the lowest (only 40.8%). On the other hand, genotype ZB-6 disposed by high lipid levels in both years of cultivation, 46.7% in 2007 and 50.1% in 2009, respectively. Differences between years present the genotype ZB-5. In 2007, it contained the lowest lipid content compared to other genotypes (43.3%). However, in 2009 was its value relatively high (48%).

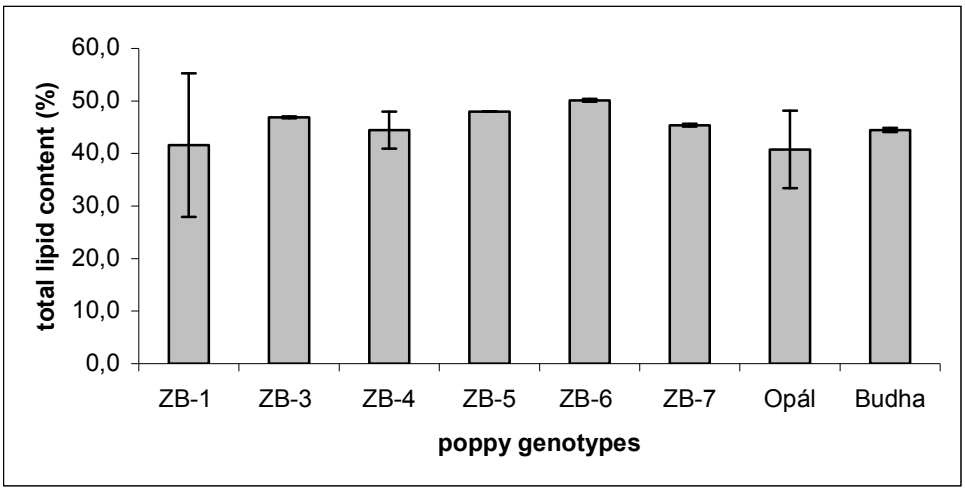

Figure 2 Total lipid content (in %) in poppy genotypes grown in 2009 year

Major fatty acids in all analyzed oil samples were palmitic (C 16:0), oleic (C 18:1) and linoleic acids (C 18:2). As minority fatty acids were characterized stearic acid (C 18:0) and *alpha*-linolenic acid (C 18:3). The most abundant fatty acid was linoleic acid. Minority fatty acids in all oil samples were palmitoleic (C 16:1), myristic (C 14:0), arachidic (C 20:0) and gadoleic acids (C 20:1). The fatty acids composition of analyzed poppy oils in 2007 is shown on the figure 3. The levels of palmitic acid ranged from 14.4% (ZB-6 and Opál) to 17.8% (Budha). Genotype ZB-4 contained relatively high level of palmitic acid, 16%. In other genotypes, values of palmitic acid were similar in the order 15.8% (ZB-3) > 15.6% (ZB-7) > 15.3% (ZB-1) > 15.1% (ZB-5). Our results indicated that Budha and Opál presented samples with the highest and the lowest level of palmitic acid.

Genotypes Opál and ZB-6 were characterized by the highest level of oleic acid (17.5% for both genotypes). On the other hand, genotype ZB-5 contained the lowest level of oleic acid. Its value was 13.7%. Values of oleic acid in other genotypes were similar in the range between 14.1% (ZB-7) and 15.5% (Budha).

We observed that the most abundant fatty acid was the linoleic acid. Genotype ZB-5 contained the highest level of linoleic acid (68.1%), followed by ZB-7 (67.1%) and genotypes ZB-3, ZB-1, ZB-4 with levels of 66.1%, 66.2%, and 66.3%. Smaller amounts of linoleic acid contained genotypes ZB-6 and Opál (64.8%). The lowest level of linoleic acid contained the genotype Budha (63.4%).

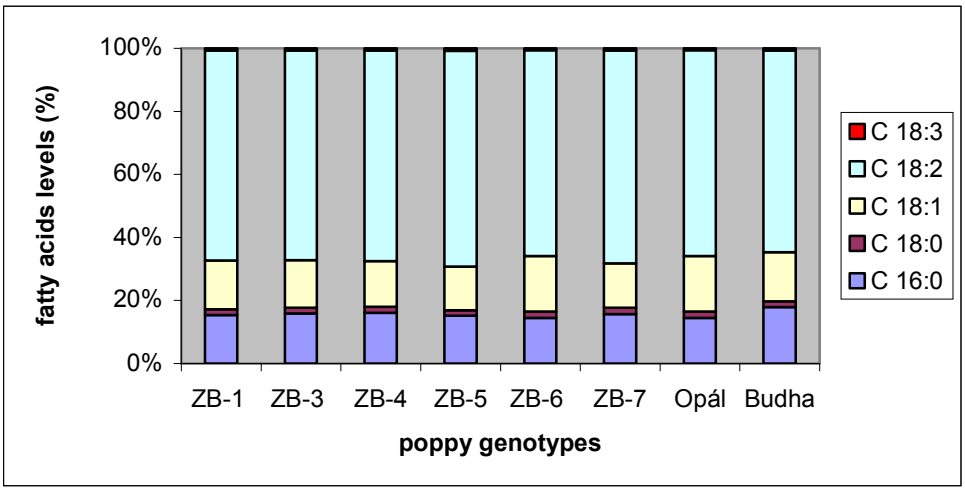

Figure 3 Major fatty acids of poppy seed oils grown in 2007

As minority fatty acids were presented in oils palmitoleic, stearic, and *alpha*-linolenic acids. Levels of stearic acid were very similar among genotypes ZB-1, ZB-3, ZB-4, and Budha with the level of 1.8% and for ZB-6, ZB-7 and Opál it was 2%. The lowest level of stearic acid contained the genotype ZB-5 (1.7%). *Alpha*-linolenic acid was presented in the poppy-seed oil in smaller amounts. Its values ranged from 0.6% (ZB-6 and Opál) to 0.8% (ZB-1, ZB-4 and ZB-5). Other genotypes contained 0.7% of *alpha*-linolenic acid. Palmitoleic acid was presented in levels between 0.3% (ZB-3) and 0.5% (Budha). Myristic, arachidic, and gadoleic acids were presented in very small amounts. Their values ranged from traces (<0.1%) to 0.2%.

The fatty acids profile in genotypes, which were grown in 2009, is displayed on the figure 4. The highest levels of palmitic acid contained the genotype ZB-7 with the value of 16.3%, followed by ZB-3 (15.5%), ZB-6 (15.4%), and Budha (15.3%). Smaller levels of palmitic acid contained genotypes ZB-1 and ZB-4 (14.9% and 14.8%). Genotype Opál contained 14.5% of palmitic acid. The lowest level represented the genotype ZB-5 (14.4%).

The lowest level of oleic acid represented genotype ZB-1 (15.5%). We determined higher levels in genotypes ZB-7 (16.8%), ZB-5 (16.6%), and ZB-4 (16.4%). The highest level

of given fatty acid was determined in the genotype Budha (17.8%). Genotypes Opál and ZB-6 represented values of 17.1% and 17%. Similarly, in the first year of cultivation - 2007, these genotypes contained relatively high levels of oleic acid.

Linoleic acid levels ranged from 63.8% (ZB-7 and Budha) to 66.5% (ZB-1). Genotypes Opál, ZB-4 and ZB-5 contained levels of linoleic acids as follows: 65.6%, 65.7% and 65.9%, respectively. Smaller levels were detected in genotypes ZB-6 (64.6%) and ZB-3 (64.2%). The values of stearic acid were very similar among genotypes and were in the range between 1.7% (ZB-6) and 1.9% (ZB-3, ZB-5, ZB-7, Opál, and Budha). Genotypes ZB-1 and ZB-3 contained 1.8% of stearic acid. Other detected minority fatty acid was *alpha*-linolenic. Its levels were in the majority of genotypes in value of 0.7%; besides genotypes ZB-3 and Budha, which contained 0.6% of given fatty acid.

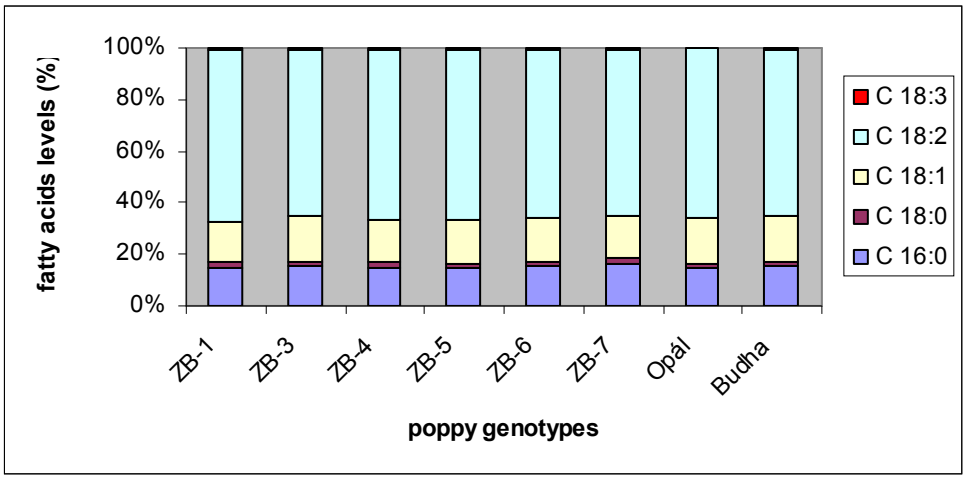

Figure 4 Major fatty acids of poppy seed oils grown in 2009

We observed very strong differences in Opál. In 2007, the level of *alpha*-linolenic acid was 0.6%. In 2009, we found out markedly decrease in the level of this fatty acid (0.2%). Palmitoleic acid was presented in levels between 0.3% and 0.4%. Myristic, arachidic, and gadoleic acids were presented in very small amount in both years. Their values ranged from traces (<0.1%) to 0.2%.

Unsaturation index of fatty acids (UI) in analyzed poppy genotypes for 2007 year of cultivation is reported in the figure 5. From these results we can conclude that the highest UI was observed in ZB-5 (1.53) and ZB-7 (1.51). Genotypes ZB-1, ZB-3, and ZB-4 had similar UI, 1.5. Lower value of UI had Opál and ZB-6 (1.49). We detected the lowest UI in the genotype Budha. Its value was 1.45.

Figure 6 represents oleic/linoleic acids ratio in genotypes grown in 2007. The lowest ratio was detected in the genotype ZB-5 (0.2) and the highest in two genotypes (ZB-6 and Opál, 0.27). Budha contained the value of this ratio of 0.24. Values of this ratio in other genotypes were in the range from 0.21-0.23.

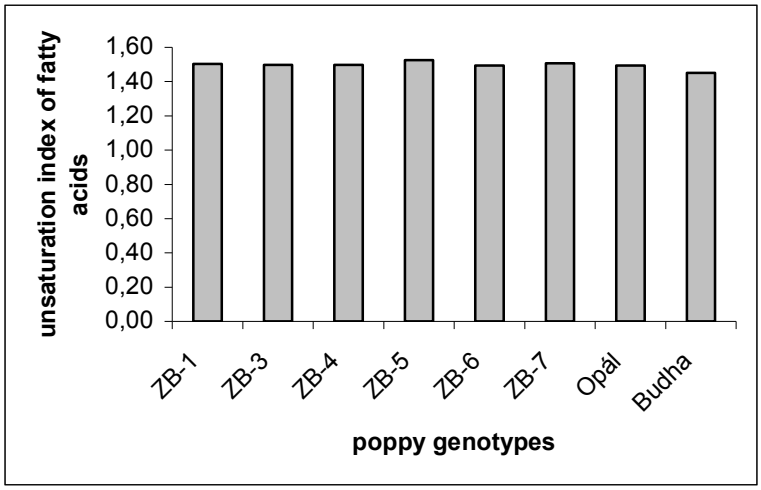

Figure 5 Unsaturation index of fatty acids in poppy genotypes grown in 2007

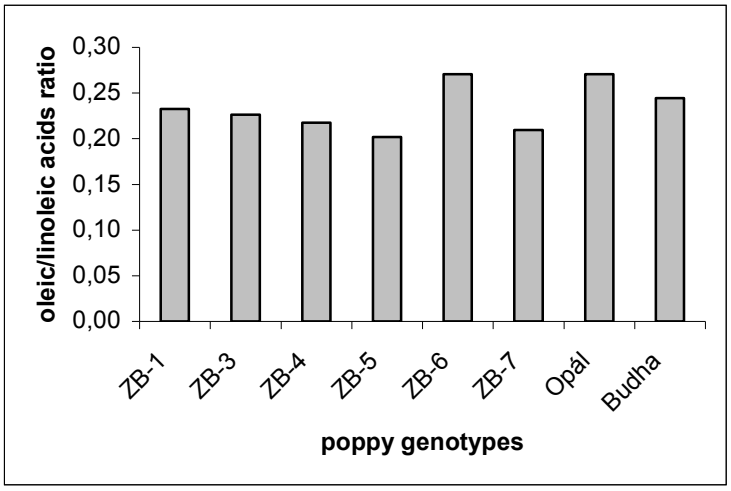

Figure 6 Oleic/linoleic acids ratio in poppy genotypes grown in 2007

In 2009, the highest unsaturation index (1.51) was observed in genotypes ZB-1 and ZB-5 (Fig 7). Smaller value was detected in genotype ZB-4 (1.5), followed by ZB-6 and Opál (1.49). The lowest ratio was calculated for the genotype ZB-7 (1.47). Figure 8 represents oleic/linoleic acids ratio for genotypes grown in 2009. Budha was characterized by the highest value of this ratio (0.28). We investigated a smaller ratio in genotypes ZB-3 (0.27), followed by ZB-6, ZB-7, and Opál (0.26). The lowest ratio had the genotype ZB-1 (0.23).

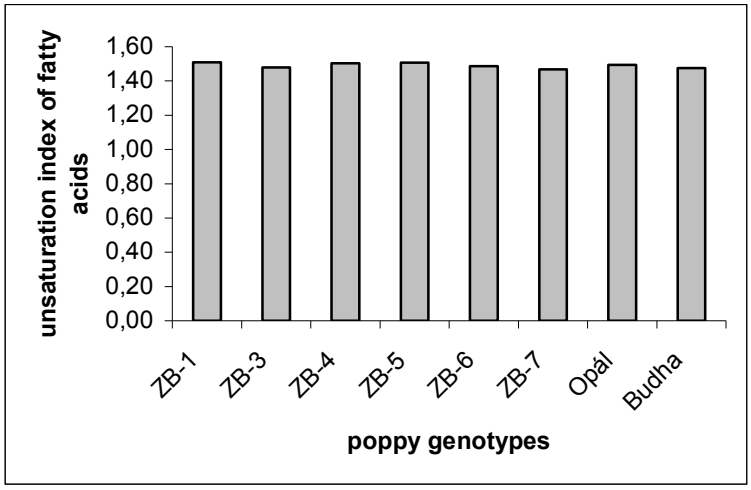

Figure 7 Unsaturation index of fatty acids in poppy genotypes grown in 2009

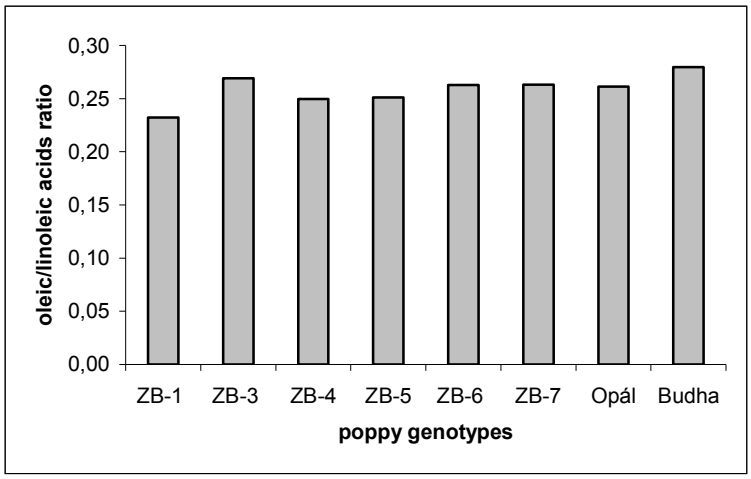

Figure 8 Oleic/linoleic acids ratio in poppy genotypes grown in 2009

Together sixteen poppy samples were divided into two categories according to their year of cultivation (the target factor). The effect of *Year* on the fatty acid composition was examined by Student *t*-test and the nonparametric Mann-Whitney test. Palmitoleic acid (C 16:1) content was found by Mann-Whitney significantly ($p < 0.05$) influenced by *Year*; and oleic acid (C18:1, OA) content was determined by *t*-test also significantly affected ($p < 0.05$). In addition, the linoleic (C 18:2, LA) and *αlpha*-linolenic (C 18:3, ALA) acid contents as well as the ratios OA/LA and ALA/LA were also found slightly affected by year of production with $p < 0.1$. In general, both of the performed tests resulted in similar observations. The poppy samples originated from year 2007 exhibit higher concentrations of C 16:1, LA and ALA, as well as higher ratio ALA/LA compared to the samples from 2009. In contrast the samples from 2009 contained higher OA concentration as well as the OA/LA level (Tab 1).

Table 1 Summary of experimental results for determination of fatty acids in the poppy samples and composition for two years of cultivation.

Descriptor	Year of cultivation		*p*-value	
	2007	2009	Student *t*-test	Mann-Whitney test
Lipid content (%)	45.13 ± 2.32	45.21 ± 3.14	0.95	0.64
C 14:0[a]	0.14 ± 0.016	0.14 ± 0.011	0.53	0.72
C 16:0[a]	15.55 ± 1.08	15.13 ± 0.61	0.36	0.51
C 16:1[a]	0.39 ± 0.052	0.33 ± 0.028	**0.05**	**0.005**
C 18:0[a]	1.86 ± 0.10	1.86 ± 0.060	0.82	1.0
C 18:1, OA[a]	15.39 ± 1.45	16.80 ± 0.70	**0.03**	**0.09**
C 18:2, LA[a]	65.83 ±1.46	65.02 ± 1.07	0.23	0.20
C 18:3, ALA[a]	0.702 ± 0.066	0.60 ± 0.17	0.13	**0.07**
C 20:0[a]	0.088 ± 0.030	0.070 ± 0.0069	0.11	0.38
C 20:1[a]	0.052 ± 0.015	0.052 ± 0.011	0.94	0.44
UI	1.50 ± 0.021	1.49 ± 0.016	0.56	0.57
OA/LA	0.23 ± 0.026	0.26 ± 0.014	**0.04**	0.11
ALA/LA	0.011 ± 0.00092	0.0092 ± 0.0026	0.16	**0.09**

Legend: [a]Values are expressed as % and represent means ± standard deviation.

Note: Statistically significant *p*-values are marked in bold.

Our results indicate that dominant fatty acids in poppy oils were palmitic, oleic and linoleic acids. Our results are in agreement with many authors (**Erinc *et al*., 2009; Bezáková *et al*., 1994** and **Luthra and Singh, 1989**), who detected that poppy oil contains high content of linoleic, oleic and palmitic acids. Similarly, **Azcan *et al*. (2004)** observed that linoleic acid was presented in the highest level (56.4-69.2%). Other quantitatively dominant fatty acids were oleic (16.1-24.7%) and palmitic (10-13%).

According to **Nergiz and Otles (1994**), poppy oil contained 50-60% of linoleic acid, 30% of oleic acid and 6-9% of palmitic acid. **Murphy (1993)** mentioned that the ratio of unsaturated fatty acids to saturated fatty acids is 2:1, eventually ratio 1:1:1 for saturated, monounsaturated and polyunsaturated fatty acids has been observed.

Poppy seed is very suitable crop for food industry due to high level of linoleic and low level of linolenic acid (**Singh *et al*., 1998**). High level of *alpha*-linolenic acid is unsuitable for food industry because its instability and modification associated with autooxidation (**Green, 1986**). In our study, minority fatty acids were palmitoleic, stearic and *alpha*-linolenic. Decrease of palmitic and *alpha*-linolenic acids in poppy oil is probably caused by accumulation of triacylglycerols. It is documented that leucoplasts are specialized in conversion of carbohydrates to fatty acids in mature oil seeds (**Miernyk, 1985**).

Our results are in agreement with **Bajpai *et al.* (1999).** Presence of linoleic and oleic acids determines the quality of oil and its end-use. It is known, that the presence of linoleic acid decreases cholesterol concentrations in blood and thus helps to prevent atherosclerosis and heart attacks. According to **Thies (1970)**, accumulation of *alpha*-linolenic acid typically occurs in oil plants which have active chloroplasts during development, because *alpha*-linolenic acid is one of dominant fatty acids of chloroplast thylakoid membrane. Our results indicate that poppy oil is suitable crop for food industry due to high levels of linoleic and low levels of *alpha*-linolenic acids.

Modification in fatty acids composition is probably caused by environmental effects. Many researchers confirmed that plants produce polyunsaturated fatty acids during cold stress. To other factors, which influence the fatty acids composition, belong salt and drought. According to **Zhang *et al.* (2005)**, non-tolerant plants subjected to salt stress usually show decreasing in levels of *alpha*-linolenic acid in their membranes. **Mikami and Murata (2003)** demonstrated that tolerance of plants to salt and drought is strongly dependent on the inheritance of fatty acids levels. Similarly, water stress causes the inhibition of lipid biosynthesis and stimulation of lipolytic and peroxidative activities (**Matos *et al.*, 2001**). **Jemal *et al.* (2000)** confirmed the decrease of unsaturation in leaves with plants exposure by heavy metals. The effect of temperature on lipid content and fatty acids composition in various oil seeds was studied by Canvin (1965). Their results showed that amounts of high polyunsaturated fatty acids were decreased due to increasing of temperature. This decrease was caused by increase of oleic acid level.

According to **Luthra and Singh (1989)**, palmitic acid positively correlated with stearic and oleic acids. On the other hand, negative correlation between linoleic and *alpha*-linolenic acids was confirmed by authors. According to **Singh *et al.* (1998)**, significant negative correlation was found between oleic and linoleic acids. This result might be explained by desaturation reaction of the oleic acid which is considered to be the basic pathway for synthesis of linoleic and *alpha*-linolenic acids. These authors confirmed positive correlation between lipid content and other component of oil. Correlations between fatty acids are interpreted on the basis of the biosynthetic pathway. On the basic of our fatty acids composition in poppy seed oil we can assume the pathways of given fatty acids. Pathway starting with acetyl-CoA, through FAS complex and the end product is palmitic acid. Then, desaturation by Δ^9 desaturase causes the conversion of palmitic acid to palmitoleic acid. The end product of elongation of palmitic acid is stearic acid, from which due to desaturation by Δ^9, Δ^{12} and Δ^{15} are formed oleic, linoleic and *alpha*-linolenic acids. Elongation of stearic acid

leads to formation of arachidic acid and elongation of oleic acid leads to formation of gadoleic acid.

CONCLUSION

In this study, eight selected poppy genotypes were analyzed for lipid content and fatty acids composition and statistically evaluated for the effect of genotype and year. To conclude the results, we can assume that the total lipid content ranged from 49.9% (Opál) to 43.3% (ZB-5) in 2007 and from 50.1% (ZB-6) to 40.8% (Opál) in 2009. All seeds were very rich in linoleic acid (in the average of 65.8% in 2007 and 65% in 2009). Other major fatty acids were presented oleic and palmitic acids. As minority fatty acids were observed *alpha*-linolenic, palmitoleic, and stearic acids. Arachidic and gadoleic acids levels ranged from trace amounts to 0.1%. The highest unsaturation index of fatty acids was detected for genotype ZB-5 (1.53) in 2007 and for ZB-1 and ZB-5 (1.51) in 2009. Genotypes ZB-6 and Opál were characterized by the highest oleic/linoleic acids ratio in 2007 (0.27) and Budha (0.28) in 2009. On the basic of statistic evaluations, we can conclude that oleic and oleic acids content was significantly affected by the year of cultivation. In addition, the linoleic (C 18:2, LA) and *αlpha*-linolenic (C 18:3, ALA) acid contents as well as the ratios OA/LA and ALA/LA were also found slightly affected by the year of cultivation.

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