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# DEVELOPMENT AND UTILIZATION OF SUNFLOWER GENOTYPES WITH ALTERED OIL QUALITY

# STVARANJE I KORIŠĆENJE GENOTIPOVA SUNCOKRETA SA PROMENJENIM KVALITETOM ULJA

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## ABSTRACT

*Sunflower oil is among the highest quality oils of plant origin. The oil of standard sunflowers has an average of 10% saturated fatty acids, 20-30% oleic acid and 60-70% linoleic acid. The total content of tocopherols in standard sunflower oil is 700-1000 mg/kg with the predominant being alpha-tocopherol (vitamin-E). Following the trends of the food and non-foodindustries sunflower breeders have been able to significantly change the fatty acid composition of the oil. The oil of high-oleic hybrids (more than 80% of oleic acid in sunflower oil) has excellent nutritional properties,as well as oxidative stability, and is a suitable raw material for many derivatives of the chemical industry and for the production of high quality biodiesel. In addition to creating high-oleic sunflower hybridis also possible to direct selection towards increasing or decreasing other fatty acids content (linoleic, palmitic and stearic). Achievements in sunflower breeding have allowed changes in the type and content of tocopherols in the oil.*

***Key words:*** *sunflower, oil quality, oleic acid.*

## REZIME

*Suncokretovo ulje spada među najkvalitetnija ulja biljnog porekla.Ulje standardnog suncokreta u proseku ima 10% zasićenih masnih kiselina, 20% oleinske kiseline i oko 60-70% linolne kiseline. Ukupan sadržaj tokoferola u ulju standardnog suncokreta je 700-1000 mg/kg, a preovladava alfa-tokoferol (vitamina E). Prateći zahteve prehrambene i drugih industrija oplemenjivači suncokreta uspeli su da bitnije izmene sastav masnih kiselina ulja. Ulje visokooleinskih hibrida suncokreta ima odlične nutritivne karakteristike, pogodna je sirovina za proizvodnju mnogih derivata hemijske industrije i za proizvodnju kvalitetnog biodizela. Pored stvaranja visokooleinskih hibrida suncokreta moguće je selekciju usmeriti i na povećanje ili smanjenje sadržaja drugih masnih kiselina (linolne, palmitinske i stearinske). Dostignuća u oplemenjivanju suncokreta omogućila su promenu tipa i sadržaja tokoferola u ulju.*

***Ključne reči:*** *suncokret, kvalitet ulja,oleinska kiselina.*

## INTRODUCTION

The sunflower (*Helianthus annuus* L.) is one of the world's four most important oilseed crops, after soybean,rapeseed and palm seed. Sunflower is cultivated on 23 million hectares, with an annual production rate of about 30 million tons of seed, with a slight tendency to increase both in terms of area of growing and in terms of the total amount of seed due to an increase in seed yield per area unit (*Kaya et al., 2012*). It is the main oilseed crop in Serbia, accounting for over 80% of the total amount of edible oil produced from plant origin. According to data from the Statistical Office of the Republic of Serbia (www.webrzs.staserb.sr.gov.rs), over the last decade, cultivable sunflower land comprised approximately 170.000 hectares, with the gross production of 380 000 tonnes of grain per year *(Babić et al. 2012)*. Sunflower hybrids are used in the commercial production and the main directions in the creation of hybrids in the world and in Serbia are increasedproductivity (seed and oil yield per unit area) and resistance to diseases and stressful conditions (*Cvejić, 2011*). Great attention is paid to increasing the adaptability, stability of hybrids and attractiveness to pollinators (*Balalić et al., 2012*). In addition to basic directions in breeding, work is on-goingon the creation of highly productive hybrids for special purposes; hybrids with different oil quality, hybrids tolerant to certain herbicides and confectioneryhybrids (*Škorić et al., 2006*).

In contrast to other vegetable oils, approximately 90% of the entire sunflower oil production is used for food, and only 10% for the production of biodiesel and for industrial purposes.Recently, the issue of oil quality has become one of the main challenges put before science by the market. The optimal quality of sunflower oil depends on the purpose of its use in food or non-food industry. In food industry, sunflower oil is used as salad oil, for frying, or for producing margarine and other products. In non-food industry, sunflower oil is used for production of biodiesel, different lubricants and in cosmetic industry. The desirable characteristics of sunflower oil for one purpose are frequently not desirable for some other purposes; therefore, breeding for oil quality improvement in sunflower has divergent paths. Creating various types of oil plays a significant role in future development of sunflower as an agricultural crop (*Cvejić et al., 2014*).

## MATERIAL AND METHOD

Genetic material described in the paper was developedat the Institute of Field and Vegetable Crops in Novi Sad, Serbia. In developing high-oleic sunflower inbred lines we used line which is donor of *Ol*and *tph*genes and crossed with the commercial lines to obtain new B-lines.By series of back-crossing, cytoplasm mail sterile lines (CMS) were created. New restorer (Rf) lines were developed by crossing commercial restorer lines with line serving as donor of desirable gene. The creation of hybrids was achieved by crossing CMS lines with Rf lines.

These hybrids were tested for their general and specific combining abilities (GCA and SCA) for seed and oil yield (*Jocić et al., 2000; Škorić et al., 2008*).

Moreower, we applied mutation breeding in aim to improve the quality of seed oil in sunflower. Seeds of 15 inbred lines were treated with the follow mutagens: gamma (*γ*) rays, fast neutrons (*Nf*) and ethyl methane sulfonate (*ems*) at different treatment doses (*Cvejić et al., 2011a*). Mutant lines were selected based on changes in fatty acid composition in comparation to untreated lines. Selected mutants (in $M_6$ generation) and untreated lines (original lines) were planted in comparative trail in order to test their productivity and stability, as well as morphological and biological characteristics.

The oil content was determined by nuclear magnetic resonance (NMR) analyser and the content of fatty acids was determined by gas chromatography(*AOCS Official Method Ce 1-62, 1993*). Quantitative and qualitative analysis of tocopherols was determined by HPLC- high performance liquid chromatography (*AOCS Official Method Ce 8-89, 1993*).

## RESULTS AND DISCUSSION

### Sunflower oil composition and development of altered oils

Sunflower oil is among the highest nutritive quality oils of plant origin, with high oil content in seeds, and is considered a species susceptible to genetically changing oil quality (*Cvejić et al., 2012*). Standardhigh-oil hybrids contain 40-60% of oil in the seed. Sunflower oil belongs to the group semi-dry oils (*Karlović and Andrić, 1996*). Standard sunflower oil mostly contains unsaturated fatty acids that are liquid at room temperature. It has the highest content of polyunsaturated linoleic acid (C 18:2), about 70%. Linoleic acid is an essential fatty acid which *cannot*be synthesized by the human body, and must be taken through food. The next is the monounsaturated oleic acid (C 18:1) with 20%. Although, the amount of these two higher fatty acids can vary under the influence of environmental factors, sunflower oil typically contains about 90% of these two fatty acids. In addition, a percentage of other saturated fatty acids is also detected, such as palmitic acid (C 16:0) with 4-9%, and stearic acid (C 18:0) with 1-7%. Sunflower oil also contains traces of other fatty acids, such as myristic (C 14:0), myristoleic (C 14:1), palmitoleic (C 16:1), arachidic (C 20:0) and behenic (C 22:0). All these fatty acids account for about 10% of the total fatty acid content in oil. Total amount of tocopherol in standard sunflower oil is about 700-1000 mg/kg. Natural tocopherols are present in four isomers: α (5,7,8-trimethyltocol), β (5,8-dimethyltocol), γ (7,8-dimethyltocol) and δ (8-methyltocol). Standard sunflower oil mostly contains α-tocopherol (95%), β-tocopherol (3%) γ-tocopherol (2%) (*Škorić et al*., 2008).

The quality of oil is linked to its nutritional and functional value. There is no optimal oil quality, as this depends on its final use. The main parameters for defining oil quality are the composition of higher fatty acids, the distribution of higher fatty acids in triacylglycerol molecule and the total content and profile of a several polyisoprenoid lipids present in oil, mostly tocopherols and sterols (*Fernandez-Martinez et al., 2004*). From a nutritional perspective, saturated fatty acids are considered undesirable in the human diet and have a deleterious effect because they increasethe content of total cholesterol level and LDL (low density lipoprotein) in comparison with the mono-and poly-unsaturated fatty acids (*Mensink et al., 1994*). From a technological perspective, the main aspects of fats with saturated fatty acidsare plasticity (hardness) and oxidation resistance, especially at high temperatures. Standard linoleic type sunflower oil contains predominantly unsaturated fatty acids, which are liquid at room temperature. In order for the oil to be used in the food industry (margarines)it needs to be previously hydrogenatedin order to become solid or semi-solid. However, hydrogenating induces cis-trans isomerization of fatty acids (*Tatum and Chow, 1992*), resulting in the production of trans-fatty acids, which are associated with the causes of heart disease (*Willett and Ascherio, 1994*).

The existence of genetic variability is an essential prerequisite for changing oil quality. Increasing genetic variability is achieved by use of mutations (spontaneous or induced) or the recombination of genes. Induced mutations are frequently used in breeding programmes in order to change the quality of sunflower seed oil (*Table 1*).

*Table 1. Oil quality mutations in sunflower*

| Authors | Mutagens | Changes in traits |
|---|---|---|
| *Borodulina and Kharchenko, 1976* | Different chemicalmutagency | Increased content of oil in seed |
| *Chandrappa, 1982* | Gamma-rays, ems, des | Increased oil content in seed and seed yield |
| *Kharchenko and Soldatov, 1976* | Different chemicalmutagency | Increased oleic acid content in oil |
| *Soldatov and Surokivin, 1975* | nmu, neu, dms | Oil content, early maturity |
| *Soldatov, 1976* | dms | Increased oleic acid content in oil (PERVENEC) |
| *Surokivin, 1973 and 1977* | nmu, neu, dms, ei | Increased content of oil in seed, early maturity and plant height changes |
| *Ivanov et al., 1988* | Gamma-rays | Increased content of palmitic acid |
| *Mancha et al., 1994* | X-rays | Increased content of palmitic acid |
| *Osorio et al., 1995* | ems | Increased content of saturated fatty acids |
| *Demurin, 1993* | Spontaneous | Changed form and content of tocopherols |
| *Cvejić, 2009* | Gamma-rays,fast neutrons, ems | Altered fatty acid content |

*Chemical tags, as well as international abbreviations (according to IAEA 1977): ei = ethylene-imine, ems = ethyl methane-sulphate, des = di-ethyl sulphate, dms = di-methyl-sulphate, neu = N-nitroso-N-ethylurea, nmu = N-nitroso-N-methylurea

### The high-oleic sunflower genotypes

Following the trends of the food industry and non-food industries, sunflower breeders succeeded in significantly changing the quality of oil in sunflower seeds, especially the composition of fatty acids and tocopherols (*Demurin et al., 1996*). The most significant contribution is increasing the content of oleic acid(more than 80%) and decreasing the content of linoleic acid (less than 20%) in the oil, with which high-oleic and medium oleic (mid-oleic) genotypes were created. High- and mid-oleic hybrids have become prevalent in the regions of sunflower production in France and the U.S., while in Spain, Italy and Argentina they are increasing significantly (*Alonso, 2014*). This increase of high-oleic sunflower is a result of transition to Mediterranean nutrition (which uses oils rich in oleic acid), and the needs of industry producing biodiesel, as sunflower oil with high oleic acid content is favourable for the production of biodiesel from standard sunflower oil.

The most significant induced mutation that led to a change in sunflower oil composition, and the first source of high-oleic acid content, was made by *Soldatov (1976)*, who treated the seed of the VNIMK 8931 variety with 0.5% DMS solution. In the $M_3$ generation, Soldatov selected single plants containing over 70% oleic acid, developing the Pervenec variety with 80-90% oleic acid in oil. High-oleic acid content in this variety proved to be stable during processing at different temperatures and the trait can easily be transferred to another genotype using conventional breeding methods. Pervenec has been used worldwide as a high-oleic source (*Ol*-gene) in breeding programmes (*Jocić et al., 2000; Škorić et al., 2006*). In the U.S., larger numbers of mid-oleic sunflower hybrids were developed under the generic name "NuSun" containing the *Ol*-gene from the Pervenec variety.

Inbred lines and hybrids with high-oleic content, obtained by classical breeding methods have also been created through the breeding programme of the Novi Sad Institute of Field and Vegetable Crops (*Jocić et al., 2000*). Finding the source for high-oleic acid in the Pervenec variety opened up possibilities for creating new high-oleic hybrids.The Institute of Field and Vegetable Crops from Novi Sad was among the first in Europe to create hybrids with high oleic acid content in sunflower oil. These are the hybrids *Olivko* and *Oliva* in Serbia, hybrids*Goleador*and*Olinca* registered in Italy and *Saša* in Russia (*Škorić et al., 2006*).Recently new high-oleic hybrids were developed which can compete with standard oleic sunflower hybrids on the basis of productivity and agronomic characteristics (*Cvejić et al., 2014a*). The new high oleic hybrids have also high oil content and high seed yield. According to seed and oil yield, these new hybrids are better than the standard high-oleic hybrids and even standard oil hybrids. These hybrids are under the official variety testing process and are expected to soon enter mass production.

High-oleic sunflower oil has found wide applications in the food industry, in the preservation of vegetables and fish, storing various types of margarine, mayonnaise and confectionary and baked goods, as well as in direct human consumption and cooking (frying). It is also used as a salad oil for seasoning fresh salad.High-oleic sunflower seed oil has excellent nutritional properties and a particularly favourable effect on the cholesterol metabolism. Oleic acid decreasesthe total cholesterol level and LDL (low density lipoprotein) and triglycerides in the blood, thereby reducing the risk of coronary heart disease (*Mensink et al., 1994*).As such, this kind of oil is often used in the diet.

The special properties of oleic acid make high-oleic sunflower oil a choice ingredient for cosmetic formulations. Research has indicated that the oil is not skin-irritating or sensitising. It may be used in sun-tanning products and cosmetics with a high content of natural lipids, such as bath oils, massaging oils, skin-care products, lipstick and cosmetic cream bases (*Grompone, 2005*).

Sunflower oil, either regular or high-oleic, is a very good raw material for the production of high quality biodiesel (*Vanozzi, 2006*). The constant rise of petrol prices on the world market has led to an increase of alternative energy sources such as biofuels. It is expected that in the next 10-15 years the share of biodiesel in total transportation fuels will reach as much as 20%.Analysing the possibilities of biodiesel production in Serbia, the results of *Tešić et al. (2009)* point out that with the average sunflower yield of 1.79 t/ha and oil content of 40%, from 1 ha, sunflower provides 716 kg of oil, or 816 l of biodiesel.There is only a theoretical potential of biodiesel production in Serbia, while the reality depends on a number of agro-technical, economic and even political factors. Respecting the rules of crop rotation and the required area under oilseeds for human consumption, the needs of livestock and seed production for reproduction, the area available for the production of raw materials for biodiesel in Serbia is 350.000 ha and the largest part (about 90%) of this potential area is located in Central Serbia.

## Increased content of saturated fatty acids

In addition to creating high-oleic hybrids, the selection can be directed to the increase of other fatty acids (*Cvejić et al., 2014*). Compared to standard sunflower oil, sunflower oil with high saturated fatty acids content is more used due to its higher oxidative stability. This type of sunflower oil has a semi-solid state at room temperature and can be used directly in margarine production without prior hydrogenation (*Fernandez-Moya et al., 2002*). Hydrogenation of vegetable oils creates trans fatty acids, closely connected to coronary diseases (*Willet and Acherio, 1994*). Higher stearic acid content (>25%) was achieved by using ems and sodium azide (NaN3), and increased palmitic acid content by the use of physical mutagens (X-rays and γ-rays). On the other hand, a decreased content of fatty acids have been induced by the use of chemical mutagens ems and nmu. A review of induced sunflower mutants with changed saturated fatty acid content and the used mutagens is given in Table 25.1.

*Table 2. Fatty acid composition of the principal induced mutants of sunflower in comparison with the standard types*

| **Mutant line** | **Oil type** | **Fatty acid composition (%)** | | | | | **Mutagenic treatment** |
|---|---|---|---|---|---|---|---|
| | | **16:0** | **16:1** | **18:0** | **18:1** | **18:2** | |
| Standard | | 5.7 | | 5.8 | 20.7 | 64.5 | |
| **Low content in saturated fatty acids** | | | | | | | |
| LS-1 | Low 18:0 | 5.6 | | 4.1 | 20.2 | 67.4 | NMU (4-8 $gkg^{-1}$) |
| LS-2 | Low 18:0 | 8.6 | | 2.0 | 10.8 | 75.0 | NMU (4-8 $gkg^{-1}$) |
| LP-1 | Low 16:0 | 4.7 | | 5.4 | 23.8 | 63.7 | EMS (4-8 $gkg^{-1}$) |
| **High content in palmitic acid** | | | | | | | |
| 275HP | High 16:0 | 25.1 | 6.9 | 1.7 | 10.5 | 55.8 | γ-rays (1550 R) |
| CAS-5 | High 16:0 | 25.2 | 3.7 | 3.5 | 11.4 | 55.1 | X-rays (150Gy) |
| CAS-12 | High 16:0 | 30.7 | 7.6 | 2.1 | 56.0 | 3.1 | X-rays (150Gy) |
| CAS-37 | High 16:0-16:1 | 29.5 | 12.3 | 1.4 | 5.4 | 38.7 | X-rays (150Gy) |
| NP-40 | High 16:0 | 23.9 | 3.4 | 2.0 | 20.4 | 50.7 | EMS (70 mM) |
| **High content in stearic acid** | | | | | | | |
| CAS-3 | High 18:0 | 5.1 | | 26.0 | 13.8 | 55.1 | EMS (70 mM) |
| CAS-4 | Medium 18:0 | 5.4 | | 11.3 | 34.6 | 48.0 | $NaN_3$ (2-4 mM) |
| CAS-8 | Medium 18:0 | 5.8 | | 9.9 | 20.4 | 63.8 | $NaN_3$ (2-4 mM) |
| CAS-14 | Very high 18:0 | 8.4 | | 37.3 | 12.4 | 38.0 | $NaN_3$ (2-4 mM) |

16:0=palmitic acid; 16:1=palmitoleic acid; 18:0=stearic acid; 18:1=oleic acid; 18:2=linoleic acid.

Mutations with higher palmitic (*Ivanov et al., 1988; Osorio et al., 1995*) and stearic acid concentration (*Osorio et al., 1995*), as well as mutant lines with lower palmitic acid concentration, were developed using physical and chemical mutagens. In the breeding programme of the Institute of Field and Vegetable Crops in Novi Sad, genotypes with a high content of saturated fatty acids were developed (*Cvejić, 2009*), which is a valuable material for the creation of special purposehybrids. The agronomic and production characteristics are the same as the original phenotype, with a change of only one trait (level of saturated fatty acids), *table 3*.

*Table 3. Comparison of agronomic traits and oil quality between the original line (control) and new lines (MU-4 and MU-6)*

| Genotype | Plant Height | Earliness | 1000 Seed Mass | Oil content | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid |
|---|---|---|---|---|---|---|---|---|
| | (cm) | (days) | (g) | (%) | (g/100 g ulja) | | | |
| Control | 165.02 | 62.00 | 40.03 | 45.81 | 6.31 | 4.84 | 24.96 | 62.84 |
| MU-4 | 164.47 | 62.50 | 39.63 | 46.46 | 6.20 | 7.24** | 27.30 | 58.06 |
| Control | 160.47 | 57.00 | 55.10 | 51.84 | 5.16 | 5.43 | 24.48 | 62.94 |
| MU-6 | 162.24 | 57.10 | 55.37 | 51.27 | 6.88** | 7.53** | 30.39 | 51.75 |

*t-test at the level of significance P=0.05; **t-test at the level of significance P=0.01

## Changed form and content of tocopherols

Achievements in sunflower breeding allowed a change to the form and content of tocopherols (vitamin E) in the oil. Tocopherols are considered as natural antioxidants, showing different *in vivo* and *in vitro* antioxidative activities. Alpha-tocopherol shows maximum *in vivo* activity, so-called vitamin E activity. Contrary to that, γ- and δ-tocopherol, and to a lesser extent β-tocopherol, are very powerful antioxidants, but with a low amount of vitamin E bioactivity (*Packer and Obermuller-Jevic, 2002*). Sunflower is thought to be one of the most promising plant species for genetic modifications of oil quality, being the crop with high oil content in seed (*Škorić, 1989*). Following the trends of food and other industries, sunflower breeders have managed to modify the oil quality to suit different uses (*Demurin et al., 1996*). Mutants with high, intermediate and low levels of saturated fatty acids, mid and high levels of oleic acid, as well as high levels of β-, γ-, and δ-tocopherol have been developed, providing more variability for fatty acid and tocopherol profiles in sunflower oil than in any other oilseed crop (*Fernandez-Martinez et al., 2009*). A significant contribution in sunflower breeding for oil quality was made by *Demirin (1993)*, who discovered a spontaneous mutation that changes the form and level of tocopherols. Changing forms of tocopherols is achieved by substitution of the alpha-tocopherol, which is dominantly present in the sunflower oil, with the beta-, gamma-and delta-tocopherols, which have a greater value and oxidative stability. Studies have shown that much of the same genotype is possessed by genes for high oleic acid and a high beta and gamma tocopherol content. These genes come to a certain synergy that ensures that this kind of oil has 15-16 times higher oxidative stability than standard sunflower oil (*Demurin, 2012*). The following combinations have been made: high-oleic type with alpha and beta tocopherols; high-oleic type with alpha and gamma tocopherols and high-oleic type with alpha, beta, gamma and delta tocopherols.

## CONCLUSION

A significant contribution to altering seed oil quality, used for various purposes, has been made in sunflower breeding programmes. New high-oleic sunflower hybrids have been developed that present a range of possibilities for both food and non-food uses of this oil crop. The new types of sunflower oil could also be used for medicinal purposes, as prevention against cardiovascular disease. Moreover, the combination of several quality traits in a single phenotype will enable tailoring speciality oils providing essentially “new oilseed crops” for specific uses in the food and non-food industry, thus guaranteeing a promising future for the sunflower on the global market. To define the parameters of future innovative sunflower oils, geneticists, breeders, physiologists, but also sunflower oil producers and consumers, should make a joint multidisciplinary effort with doctors, nutritionists and specialists from other fields in order to find the answers for future uses of sunflower oil.

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