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INSTITUTE OF FOOD TECHNOLOGY IN NOVI SAD

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# GENOTYPIC VARIATION OF FATTY ACID COMPOSITION IN SAFFLOWER (*Carthamus tinctorius L.*) OIL

Ana Marjanović Jeromela[1], Nada Grahovac[1], Zvonko Sakač[1], Snežana Kravić[2], Zorica Stojanović[2]*, Ana Đurović[2], Ankica Kondić Špika[1], Dragana Miladinović[1]

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## ABSTRACT

Safflower is a multipurpose crop with quality oil and brilliantly colored flowers which are used as a source of natural dyes for food and fabrics. In addition, different parts of the safflower plant have medicinal properties and are useful in treating many chronic diseases. Further, safflower oils are rich in polyunsaturated fatty acid (FA) with a fraction of some very desirable essential fatty acids (EFA). The increased interest for healthier food related with the consumption of long-chain n-3 fatty acids has conducted to the sale of supplements and fortified foods containing these fatty acids. Safflower oils can be considered functional foods without any biochemical additions. The objective of this study was to assay the fatty acid composition of oils obtained from eight safflower cultivars, which belong to the alternative oil crops collection of the Institute of Field and Vegetable Crops, Novi Sad. Oil samples were obtained by pressing seeds in hydraulic press. Fatty acid composition was determined by gas chromatography (Konik HRGC 4000) coupled with a flame ionizing detector, after derivatization to their volatile methylesters (FAME). In order to chemically convert FA to FAME, 10 µl of oils were subjected to transesterification using 190 µl methanolic trimethylsulfonium hydroxide solution (0.2 mol/dm$^3$). Peak identification was performed by comparing the relative retention times with those of a commercial standard mixture of FAME and FA contents are expressed as weight percentages of total FAME. The results have shown significant differences among the safflower cultivars regarding the fatty acids composition. In average, linoleic acid represented the most FA (74.96%) followed by oleic (15.15%), palmitic (5.79%), stearic (2.77%) and α-linolenic (0.40%) acids. Based on obtained results safflower oils were characterized by high proportion of polyunsaturated fatty acids (mean value 75.48%) versus 15.32% of monounsaturated and 8.87% of saturated ones, indicating that the safflower is rich sourse of polyunsaturated EFA (linoleic and α-linolenic).
***Keywords**: fatty acid, safflower, seed oil, GC, flame ionizing detector*

## INTRODUCTION

Safflower (*Carthamus tinctorius L.*) is a deep-rooted crop that belongs to the Cynareae tribe, subfamily of *Tubulifloreae* and *Asteraceae* family. It is very old crop and had survived for over 4000 years (Gupta, 2015). Safflower is crop that producing quality oil, rich in polyunsaturated fatty acids (PUFA) (Tonguc et al., 2012), and giving brilliantly colored flowers which are used as a source of natural dyes for food and fabrics (Purdy et al., 1959). In addition, different parts of the safflower plants also have medicinal properties and are useful in treating many chronic diseases. Safflower plants at a tender age are consumed as a leafy vegetable. They are rich in vitamin A, iron, phosphorus and calcium. Additionally, the safflower oil contains *N*-(*p*-coumaroyl) serotonin (CS), a potent antioxidant compound.
Safflower is an edible and biodiesel oilseed crop that is recommended to plant in dry and marginal regions of the world (Mihaela et al., 2013). The cultivation of safflower under various climatic conditions in different countries, suggests a very high sustainability of the crop. Seed of safflower contains 27% – 40% oil with mostly linoleic, oleic, stearic and palmitic acids (Lata and Prakash, 1984; Knowles, 1989) and 15.6% to 21.5% protein (Ahmadzadeh et al., 2014). The oil obtained from the seeds of safflower is healthy, because of its high unsaturated oil content, while the residual meal is rich in proteins (Pavlov and Todorov, 1996; Corleto et al., 1997). In recent years availability of safflower meal as an import protein

supplement increased due to the popularity of safflower oil in human diets. Safflower meal from the unhulled seed has approximately 20% protein and is relatively low in energy, while meal from the well-hulled seed is much higher in protein (~40%) and energy. Safflower is considered one of the best oilseed crops for human nutrition, due to its oil contains high levels of polyunsaturated or monounsaturated fatty acids (Shirvani et al., 2016; Mokhtari et al., 2013). Cultivated safflowers contain either very high levels of linoleic (87-89%) or very high levels of oleic acid (>85%) (Fernandez-Martinez et al., 1993). Medicinal properties of linoleic acid were first reported in the 1960s indicating its usefulness in lowering serum cholesterol levels in laboratory tests on animals and humans and reducing the risk of heart attacks. Omega-3 PUFA play an important role in modulating human metabolism and have nutritional importance (Trautwein, 2001). Thus omega-3 PUFA has been broadly accepted as one of the cornerstones of healthy nutrition.

The objective of this study was to assay the fatty acid composition of eight safflower cultivars and to investigate the variation within these cultivars. This information can be important for improving fatty acid composition in safflower breeding programs. Genetic variation of fatty acid composition of safflower oil is essential for genetic improvement of the oil quality and developing new cultivars.

## MATERIAL AND METHODS

Eight safflower cultivars were cultivated on the experimental field of the Institute of Field and Vegetable Crops, Rimski Šančevi (Vojvodina Province, Serbia) Safflower oils were obtained by pressing of safflower seeds in a hydraulic press (Sirio, Mikodental 10 tons stregth, cc 400 bars). Analysis of triacylglycerols of safflower oils was carried out in first step by their transesterification with trimethylsulfonium hydroxide (TMSH) (Shantha & Napolitano, 1992; Brondz, 2002; Garcés & Mancha, 1993; Ruiz-López et al. 2003), chemicaly converting the fatty acids into the their volatile esters and further analysis were done by gas chromatograph equipped with a flame ionizing detector (GC-FID).

### Preparation of fatty acids methyl esters

Fatty acid methyl esters were prepared according to AOCS official method Ce 2-66 (AOCS, 1992) with some modifications. Samples of 10 µl of oils were placed into reaction vial. An aliquot (190 µl) of 0.2 mol/dm$^3$ methanolic trimethylsulfonium hydroxide (TMSH) solution was directly added to the oil. The reaction vial was capped and vigorously shaken by the vortex for 60 s. The reaction is complete upon dissolution of the oil. After for an hour standing the aliquot of 1 µl prepared fatty acid methyl esters was taken for GC-FID analysis.

### Gas chromatography analysis

The analysis of fatty acid methyl esters were performed on a Konik HRGC 4000 gas chromatograph coupled with a FID detector. A fused silica capillary column Omegawax® 250 (30 m length, 0.25 mm ID, film thickness 0.25 µm) with poly(ethylene glycol) stationary phase was used. This process was operated at an oven temperature of 150°C, which was then raised to 250°C at a rate of 12°C/min and then kept at 250°C for 8 min. The injector and detector temperatures were 250°C. The carrier gas was helium with constant flow rate of 1 ml/min and split ratio was 1:70. Identification of the individual fatty acids was performed by comparing relative retention times with those of the pure commercial standard mixture of FAME. Reference multistandard from Supelco (Cat. No. 07756-1AMP, Bellefonte, PA, USA) containing the methyl esters of 11 fatty acids (myristic C14:0, palmitic C16:0, stearic C18:0, oleic C18:1, linoleic C18:2, linolenic C18:3, arachidic C20:0, eicosenoic C20:1, behenic C22:0, erucic C22:1 and lignoceric C24:0) was used. The results were processed by the ChemStation software.

### Statistical analysis

Standardized trait mean values were used to perform the cluster analyses using Statistica software (Statistica 13). Cluster analyses was conducted based on Euclidean distances and

applying the unweighted pair group method with arithmetic mean (UPGMA). In addition, cluster analysis was used to expose similarity and diversity between investigated safflower cultivars.

## RESULTS AND DISCUSSION

The fatty acid composition of the investigated safflower cultivars is shown in Table 1. Fatty acid contents are expressed as weight percentages of total FAME content. Values are displayed as the mean ± standard deviation (SD) of three replicates.
Linoleic acid was major fatty acid, followed by oleic, palmitic, and stearic acid ranged between 60.9% – 80.5%, 9.4% – 29.8%, 5.2% – 6.3%, and 2.1% – 3.5%, respectively (Table 1). These four fatty acids represent 97% of safflower total fatty acids. Linoleic acid is essential fatty acid that humans must take for good health because their body requires them for different biological processes but might not biosynthesize them from other food components (Kaur et al., 2014).

*Table 1. Fatty acid composition (% of total fatty acid) of safflower oil*

| Fatty acid | Sample | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NS-L-1 | NS-L-24 | NSL-25 | NS-L-31 | NS-L-32 | NS-L-33 | NS-L-34 | NS-L-53 |
| 14:0 | 0.1 ± 0.00 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 |
| 16:0 | 5.5 ± 0.03 | 5.5 ± 0.15 | 6.3 ± 0.03 | 5.9 ± 0.26 | 5.6 ± 0.09 | 6.0 ± 0.12 | 5.9 ± 0.11 | 6.0 ± 0.10 |
| 18:0 | 2.4 ± 0.17 | 2.4 ± 0.04 | 3.5 ± 0.03 | 3.4 ± 0.05 | 2.5 ± 0.05 | 2.1 ± 0.05 | 3.2 ± 0.13 | 3.4 ± 0.14 |
| 18:1 | 11.5 ± 0.06 | 16.1 ± 0.43 | 16.0 ± 0.42 | 14.3 ± 0.1 | 29.5 ± 0.44 | 13.2 ± 0.38 | 9.6 ± 0.19 | 15.2 ± 0.54 |
| 18:2n-6 | 79.6 ± 0.19 | 74.6 ± 0.72 | 72.5 ± 0.88 | 75.2 ± 0.3 | 61.2 ± 0.45 | 77.7 ± 0.32 | 80.2 ± 0.26 | 73.0 ± 0.28 |
| 18:3n-3 | 0.2 ± 0.01 | 0.5 ± 0.03 | 0.6 ± 0.04 | 0.2 ± 0.01 | 0.2 ± 0.05 | 0.2 ± 0.05 | 0.2 ± 0.05 | 0.9 ± 0.40 |
| 20:0 | 0.2 ± 0.02 | 0.3 ± 0.02 | 0.4 ± 0.01 | 0.4 ± 0.02 | 0.3 ± 0.02 | 0.3 ± 0.01 | 0.3 ± 0.02 | 0.4 ± 0.02 |
| 20:1 | 0.2 ± 0.02 | 0.2 ± 0.02 | 0.2 ± 0.01 | 0.2 ± 0.01 | 0.2 ± 0.02 | 0.1 ± 0.02 | 0.2 ± 0.01 | 0.4 ± 0.14 |
| 22:0 | 0.1 ± 0.04 | 0.2 ± 0.02 | 0.2 ± 0.00 | 0.2 ± 0.02 | 0.2 ± 0.01 | 0.2 ± 0.01 | 0.2 ± 0.02 | 0. 2± 0.01 |
| 24:0 | 0.1 ± 0.02 | 0.1 ± 0.01 | 0.1 ± 0.00 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 |
| SFA | 8.5 ± 0.21 | 8.6 ± 0.13 | 10.6 ± 0.02 | 10.0 ± 0.20 | 8.8 ± 0.04 | 8.8 ± 0.08 | 9.8 ± 0.08 | 10.3 ± 0.14 |
| MUFA | 11.6 ± 0.07 | 16.3 ± 0.45 | 16.2 ± 0.45 | 14.5 ± 0.10 | 29.7 ± 0.46 | 13.3 ± 0.40 | 9.8 ± 0.21 | 15.6 ± 0.68 |
| PUFA | 79.8 ± 0.20 | 75.1 ± 0.96 | 73.1 ± 1.34 | 75.4 ± 0.29 | 61.4 ± 0.50 | 77.9 ± 0.38 | 80.5 ± 0.31 | 73.9 ± 1.68 |

Results are given as mean ± standard deviation (n=3);
SFA – saturated fatty acids;
MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

Contents of myristic (C14:0), linolenic (C18:3), arachidic (C20:0), eicosenoic (C20:1), behenic (C22:0) and lignoceric (C24:0) were less than 0.5% of the total fatty acid content for all investigated safflower cultivars.
The contents of fatty acids are in agreement with reported results for cultivated safflower with respect to the predominant fatty acids (Sabzalian et al. 2008). As can be seen from the obtained results safflower cultivars were characterized by a high proportion of polyunsaturated fatty acids (ranged between 61.4 - 80.5%) in opposition to saturated (ranged between 8.5% - 10.6%) and monounsaturated (ranged between 9.8%-29.7%) ones.

According to the cluster analysis dendrogram of safflower cultivars based on fatty acid composition (Figure 1) safflower cultivars were grouped in two clusters, the first with 2 cultivars and the second larger cluster with 6 cultivars divided into two groups (consisted of 5 and 1 cultivars).

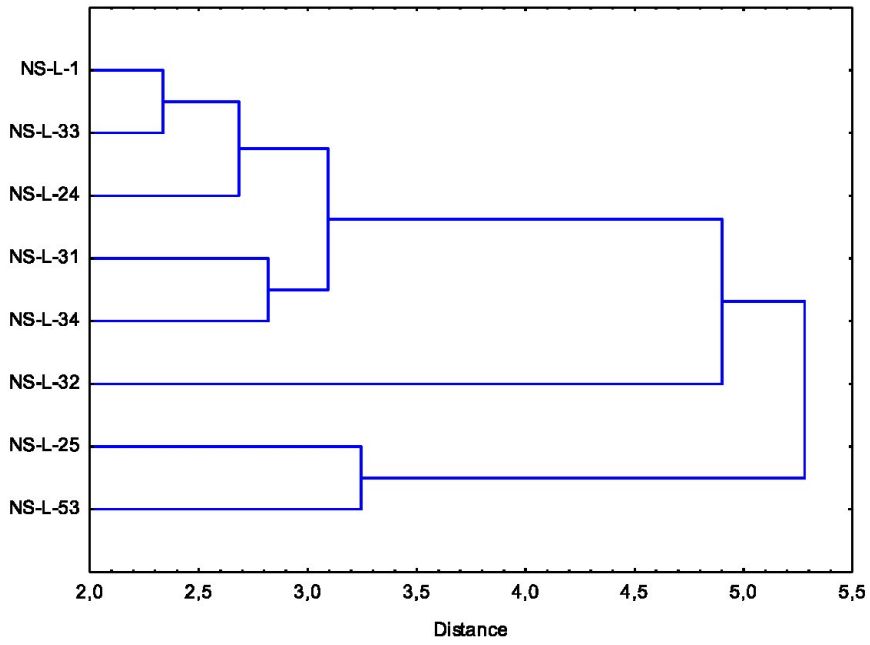


*Figure 1. Dendrogram of safflower cultivars based on fatty acid composition by using the Euclidean distance coefficients*

## CONCLUSIONS

The fatty acid composition of safflower oil for different cultivars was determined by gas chromathography with a flame ionizing detector. Obtained resultalts showed that the safflower is rich source of polyunsaturated essential fatty acid, linoleic acid. All tested safflower cultivars were linoleic type. These results suggest that safflower cultivars contain important health-beneficial compounds and could be of great interest for safflower breedes. Furthermore, the variability between the cultivars detected by the study indicates the possibility for an additional enhancement of the fatty acid composition.

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