



# Seed nutrients and bioactive compounds of underutilised oil crop *Carthamus tinctorius* L.

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**Summary:** Seeds of seven different genotypes of underutilised oil crop *Carthamus tinctorius* L. (*Asteraceae*) from alternative oil species collection of the Institute of Field and Vegetable Crops (Novi Sad, Serbia), were analysed for their protein, oil, fatty acids, tocopherols and total phenolics contents, with a view to test their diversity and potential as an alternative source of these valuable compounds. Seeds of the tested safflower genotypes had total protein (determined by Kjeldahl method) content from 11.5 to 16.0%, while total oil content (determined by Soxhlet method) ranged from 16.8 to 24.5% of dry matter, on average. Two main unsaturated fatty acids in safflower seeds, oleic and linoleic acids, represent approximately 90% of the total fatty acid content (determined by gas chromatography). Linoleic acid was the dominant fatty acid in all genotypes (61.2-80.2% of oil), while oleic acid was in a negative correlation with linolenic acid content and ranged from 9.6 to 29.5% of oil. The amount of saturated fatty acids ranged from 5.5 to 6.05% for palmitic, and 2.1 to 3.5% for stearic acid. Safflower seed is a source of  $\alpha$ -tocopherol (determined by high performance liquid chromatography method with fluorescence detection) and its amount ranged from 358.8 to 461.8 mg/L of oil. The content of total phenolics (determined spectrophotometrically) ranged from 4.0 to 6.0 mg of gallic acid equivalents/g of dry weight. This comprehensive screening of valuable chemical compounds of safflower seeds shows the importance of this alternative oil seed crop as a good source of important nutrients and bioactive constituents.

**Key words:** alternative oil crops, *Carthamus tinctorius* L., fatty acids, nutritional quality, oils, proteins, safflower, tocopherols, total phenolics,  $\alpha$ -tocopherol

## Introduction

Safflower (*Carthamus tinctorius* L.) is an annual crop which belongs to *Asteraceae* (Compositae) family. It is a highly branched, herbaceous, thistle-like annual, usually with many long sharp spines on the leaves and bracts or without them (Figure 1 A and B). Plants are 30-150

(210) cm tall, with globular flower heads (capitula), which comprise of brilliant yellow, orange or red flowers (Dajue & Mündel, 1996). Each flower head contains 15 to 20 seeds per head (Figure 1C). Achenes are smooth, four-sided and generally lack of pappus. It develops a deep taproot (up to 1 m depth) which enables it to thrive in arid environments with seasonal rain. It has an ability to withstand drought, strong winds, hail storms and flooding. Safflower flowers have been used like colorant of food and fabrics, especially before less expensive aniline dyes became available. Today, safflower is used as an oilseed crop, mostly used for edible oil (salad oil, oil for cooking and margarine) and as birdseed, livestock forage, tea, biodiesel production, etc. Its maximum production cycle is equal to 130-140 days, so it can be intercropped in temperate climates or have two crops in semi-arid climate (Herdrich, 2001).

It is grown mostly in Asia and Americas, with over half of its production in India (mainly for the domestic vegetable oil market) (FAOSTAT, 1994-2019). An average global yield of safflower seeds reaches up to 900

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Figure 1. Safflower plants (A, B) and seeds (C)

kg/ha, however, in the recent years, harvest area and production is lower than in the past (FAOSTAT, 1994-2019).

The hull fraction of safflower seeds is 35-45%. Depending on the hull size, the oil content of the seed ranges between 20 and 47% (Cosge et al., 2007; Yeloojeh et al., 2020; Kutsenkova et al., 2020). The whole seeds in normal hull types contain 27-32% of oil, 5-8% of moisture, 14-17% of protein, 2-7% of ash, and 32-40% of crude fiber (Gecgel et al., 2007, Kutsenkova et al., 2020).

Safflower has multiple usage possibilities, but its potential depends of growing region, the reason of which it is important to test their growing capacity in agroecological conditions of interest.

The aim of this paper was to analyse seeds of seven different genotypes of underutilised oil crop *Carthamus tinctorius* L., grown under agroecological conditions of Serbia, on their protein, oil, fatty acids, tocopherols and total phenolics contents, with a view to test their biochemical diversity and potential as an alternative source of these valuable compounds.

## Material and methods

Safflower genotypes (Table 1) used in this research are a part of the collection of alternative oil species of the Institute of Field and Vegetable Crops (Novi Sad, Serbia).

Seed samples analysed in this paper were harvested in 2017. Each plot in the experiment consisted of 4

rows, which were 3.6 m long, with 0.25 m between rows. The harvest area of individual plot was 3.6 m<sup>2</sup>. Plants were grown under isolation. Sowing and harvest were performed manually.

All seeds were pre-dried at 50 °C for 24h to obtain moisture content below 8% (moisture content determined gravimetrically, by drying at 103 °C, for 3h).

Seeds were ground in a laboratory mill (IKA®, A11 basic, Germany) and sieved into 1 mm fractions. Total oil content in seeds was determined by Soxhlet extraction procedure. Protein content was determined by Kjeldahl method, according to the manufacturer's instructions (Gerhardt, 2003). The fatty acid profiles of safflower seed oil were measured by gas chromatography method described by Dimitrijević et al. (2017). Components of safflower seed oil saponifiable fractions was esterified using trimethylsulfonium hydroxide solution to their volatile fatty acid methyl esters (FAMES). The results were expressed as the percentage of individual fatty acids of the content of the oil. Tocopherol content was performed by high performance liquid chromatography (HPLC) with fluorescence detection, according to an AOCS standard method (Ce 8-89). The results were expressed as milligram of individual tocopherol per litre of the oil (mg/L). Total phenolics contents was determined by The Folin-Ciocalteu reagent, spectrophotometrically (Makkar, 2003) and expressed as mg of gallic acid equivalents per g of dry seed.

Values of the biochemical parameters were expressed as means ± standard error of determinations

Table 1. Name and source of the genotype, year of introduction into collection, flower colour and presence of spines (+ present, - absent)

|   | Genotype           | Source             | In collection since | Flower colour | Spines |
|---|--------------------|--------------------|---------------------|---------------|--------|
| 1 | Sunčana – СОНЯЧНИЙ | Ukraine            | 2004                | red           | +      |
| 2 | Bird 1             | bird food 1        | 2009                | orange        | +      |
| 3 | Liman              | Liman local market | 2009                | orange        | -      |
| 4 | Bird 2             | bird food 2        | 2011                | orange        | +      |
| 5 | 'Remzibey'         | Turkey             | 2014                | yellow        | +      |
| 6 | 'Dinçer'           | Turkey             | 2014                | orange        | +      |
| 7 | 'Yenice'           | Turkey             | 2014                | red           | +      |

made in triplicates and tested by ANOVA followed by comparisons of means by the Duncan's test ( $p < 0.05$ ). All statistical analyses were performed using STATISTICA for Windows version 13 (Dell Software, Dell Inc., Aliso Viejo, CA, USA).

## Results and discussion

Recently there has been alternation between drought and flood seasons in Serbia and other regions, which is why the importance of plant adaptation and agrobiodiversity has become one of the primary focuses. Being adaptable to arid conditions, with seasons of heavy rain, safflower has a potential in sustainable plant production.

Depending on the genetics of the safflower line, three types of oil can be produced: high in linoleic acid (>70%), high in oleic acid (>70%), and almost equal amounts of oleic and linoleic acids in oil (Ahmadzadeh et al., 2014).

The oil content in the tested seeds was 16.8-24.5% (Figure 2), but published data show values up to 38%, mostly for cultivars from Turkey that are grown in the Mediterranean regions of the country during winter (Samancı & Özkaynak 2003; Coşge et al., 2007; Esendal et al., 2008; Coşge et al., 2008). The same data showed 5% higher values of oil content in seeds sown in winter, compared to these sown in spring, however spring sowing results are similar to findings reported in this paper.

Frost during kernel filling can radically lower yields and oil content, or destroy the seed completely, due to sensitivity of genes for fatty acid synthesis to low temperatures (Dajue & Mündel, 1996), which could result in reduction of stearic and oleic acids and increase

in linoleic acid (Ladd & Knowles, 1971). The three genes that control production of oleic, linoleic and stearic acids (ol ol, li li and st st, respectively), appear to be the major recessive genes at different loci. In certain genotypes, synthesis and accumulation of stearic acid is negatively correlated to accumulation of oleic or linoleic acid or both (Ladd & Knowles, 1971).

Dajue & Mündel (1996) proposed five groups of safflower lines and possible genotypes according to fatty acid composition and content, which positions genotypes tested in this research into three groups: very high linoleic ('Remzibey'), high linoleic ('Yenice') and high stearic (the rest of the tested genotypes).

High oleic acid content in safflower is inherited and controlled by a partially recessive allele, 'ol' at a single locus 'OL' (Hamdan et al., 2009). According to Hamdan et al. (2012), microsomal oleoylphosphatidylcholine desaturase FAD2-1 gene underlies the 'OL' locus. FAD2 enzyme catalyses conversion of oleic acid into linoleic acid during lipid biosynthesis (Okuley et al., 1994). There are data on the development of molecular marker assay applied to predict high oleic acid content in safflower. It is based on the fact that the DNA sequence varies between large introns situated in the 5' UTR of CtFAD2-1 and CtFAD2-1', fatty acid desaturase gene and its affected variant, respectively (CtFAD2-1' conventionally designated as 'ol' allele) (Liu et al., 2013).

Variation of safflower oil content depends on the process and technique of oil extraction (Han et al., 2009). In general, all safflower genotypes in our collection have high amount of linoleic acid (polyunsaturated fatty acid, PUFA) content (Table 2). Oleic and linoleic acids were the two major unsaturated fatty acids in these safflower seeds. According to the

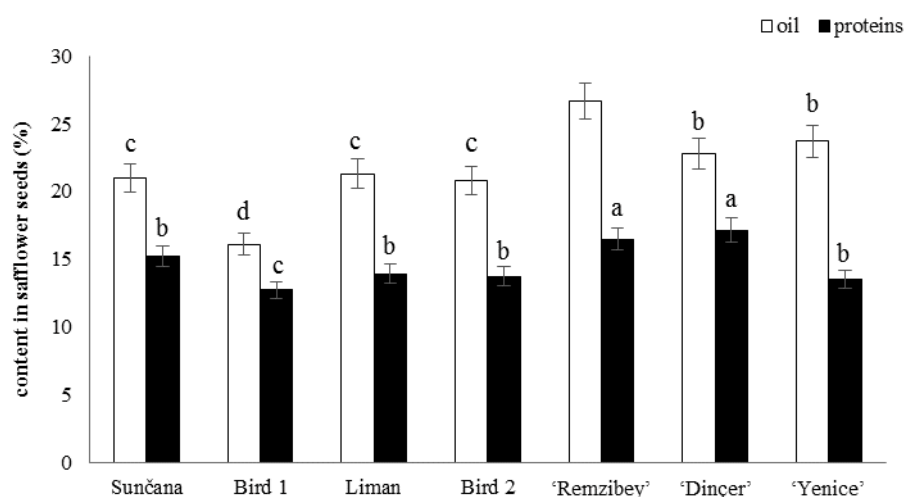


Figure 2. Oil and protein content in safflower seeds. Values marked with the different letter differ significantly among genotypes at  $p < 0.05$  (Duncan's test).

Table 2. Average fatty acid content (% of oil) in safflower seeds (mean  $\pm$  standard error of mean). Values marked with the different letter differ significantly among genotypes at  $p < 0.05$  (Duncan's test). \*Coşge et al., 2007; Mailer et al., 2008; Sabzalian et al., 2008; Ben Moumen et al., 2013; Kostik et al., 2013; Al Surmi et al., 2015; Kutsenkova et al., 2020; Yeloojeh et al., 2020)

|                  | Myristic          | Palmitic          | Stearic           | Oleic              | Linoleic           | Linolenic          | Arachidic         | Eicosapentaenoic  | Behenic           | Lignoceric         |
|------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|-------------------|-------------------|-------------------|--------------------|
| Sunčana          | 0.13 $\pm$ 0.00 a | 5.54 $\pm$ 0.02 b | 2.43 $\pm$ 0.10 c | 11.46 $\pm$ 0.03 e | 79.62 $\pm$ 0.11 a | 0.19 $\pm$ 0.01 c  | 0.24 $\pm$ 0.01 c | 0.15 $\pm$ 0.01 c | 0.13 $\pm$ 0.03 d | 0.07 $\pm$ 0.01 ab |
| Bird 1           | 0.12 $\pm$ 0.01 a | 6.27 $\pm$ 0.02 a | 3.53 $\pm$ 0.02 a | 15.98 $\pm$ 0.24 b | 72.48 $\pm$ 0.51 d | 0.62 $\pm$ 0.27 a  | 0.40 $\pm$ 0.00 a | 0.20 $\pm$ 0.01 a | 0.24 $\pm$ 0.00 a | 0.09 $\pm$ 0.00 a  |
| Liman            | 0.12 $\pm$ 0.00 a | 5.28 $\pm$ 0.04 c | 2.38 $\pm$ 0.07 c | 13.90 $\pm$ 0.39 c | 77.16 $\pm$ 0.62 b | 0.43 $\pm$ 0.17 ab | 0.26 $\pm$ 0.01 c | 0.18 $\pm$ 0.01 b | 0.18 $\pm$ 0.01 c | 0.06 $\pm$ 0.01 b  |
| Bird 2           | 0.12 $\pm$ 0.00 a | 5.91 $\pm$ 0.15 a | 3.36 $\pm$ 0.03 b | 14.34 $\pm$ 0.06 c | 75.24 $\pm$ 0.16 c | 0.19 $\pm$ 0.00 c  | 0.35 $\pm$ 0.01 b | 0.19 $\pm$ 0.00 b | 0.20 $\pm$ 0.01 b | 0.09 $\pm$ 0.01 a  |
| 'Remzibey'       | 0.11 $\pm$ 0.00 a | 5.62 $\pm$ 0.05 b | 2.52 $\pm$ 0.03 c | 29.53 $\pm$ 0.25 a | 61.18 $\pm$ 0.26 e | 0.22 $\pm$ 0.03 b  | 0.31 $\pm$ 0.01 b | 0.20 $\pm$ 0.01 a | 0.21 $\pm$ 0.00 b | 0.08 $\pm$ 0.00 ab |
| 'Dinçer'         | 0.12 $\pm$ 0.01 a | 6.03 $\pm$ 0.07 a | 2.14 $\pm$ 0.03 d | 13.19 $\pm$ 0.22 d | 77.66 $\pm$ 0.19 b | 0.23 $\pm$ 0.03 b  | 0.25 $\pm$ 0.00 c | 0.12 $\pm$ 0.01 d | 0.17 $\pm$ 0.01 c | 0.07 $\pm$ 0.01 ab |
| 'Yenice'         | 0.12 $\pm$ 0.01 a | 5.92 $\pm$ 0.07 a | 3.18 $\pm$ 0.08 b | 9.60 $\pm$ 0.11 f  | 80.24 $\pm$ 0.15 a | 0.22 $\pm$ 0.03 b  | 0.31 $\pm$ 0.01 b | 0.15 $\pm$ 0.01 c | 0.18 $\pm$ 0.01 c | 0.08 $\pm$ 0.01 ab |
| Literature data* | 01-0.5            | 4-8.6             | 1.7-2.80          | 8.1-18.4           | 71.5-83.7          | 0.07-0.1           | 0-0.4             | 0-0.2             |                   |                    |

literature, similar findings and values were reported by various authors (Coşge et al., 2007; Mailer et al., 2008; Sabzalian et al., 2008; Ben Moumen et al., 2013; Kostik et al., 2013; Al Surmi et al., 2015; Khalid et al., 2017; Kutsenkova et al., 2020; Yeloojeh et al., 2020).

Although beneficial for human consumption, edible oil that is rich in PUFA is prone to oxidation, which leads to instability and short shelf life. To prevent deterioration of oil, process of oil hydrogenation is mostly applied, however it results in the formation of *trans* fats. Contrary to this, high oleic acid (monounsaturated fatty acid, MUFA) is more stable (a heat-tolerant cooking oil), has extended shelf life and is preferable to the food industry for production of margarines, salad dressings, infant formulas and food coatings. All these facts make oleic acid-rich oil crop varieties more interesting, which stands for safflower genotypes, as well.

Tocopherols found in tested samples belong to  $\alpha$ -tocopherol group (358.8-461.8 mg/L of oil). Compared to the literature data (Mailer et al., 2008; Aydeniz et al., 2014; Matthaus et al., 2015; Topkafa, 2016; Celenk et al., 2018; Ergönül & Özbek, 2018), it can be concluded that tocopherols values of tested genotypes are within the range of published data ( $\alpha$ -tocopherol 46-1310 mg/kg). However, some of the cited literature data point to presence of  $\beta$ -tocopherols (4-21.6 mg/kg),  $\gamma$ -tocopherols (4.5-25 mg/kg),  $\gamma$ -tocotrienols (9.7 mg/kg) and d-tocopherol (1.0 mg/kg) that were absent in our samples.

Protein content was up to 16% (Figure 2), which was similar to the available literature (Khalid et al., 2017; Kutsenkova et al., 2020). Cold pressed cakes from safflower seeds are mostly used as feed and production of biochar fertilizer (Angın, 2013).

Total phenolics content in tested safflower seed samples ranges from 4-6 mg GAE/g while literature

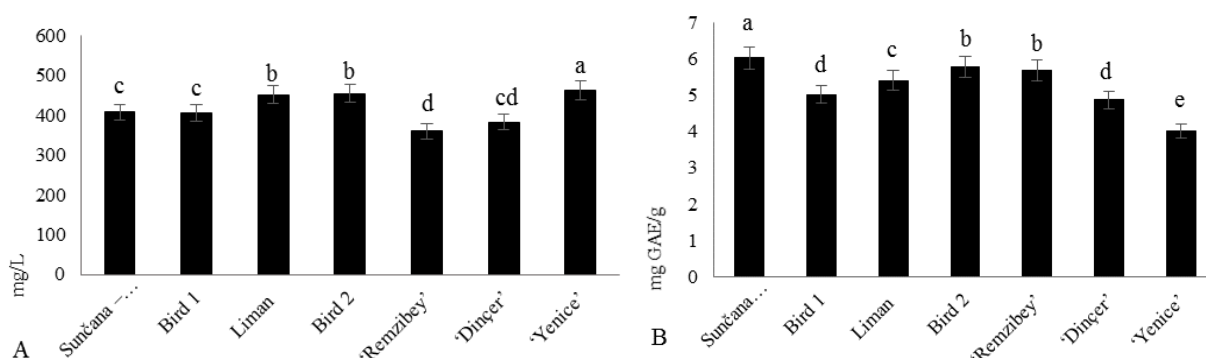


Figure 3. Tocopherol ( $\alpha$ -tocopherol) (A) and total phenolics (B) contents in seeds of safflower seeds. Values marked with the different letter differ significantly among genotypes at  $p < 0.05$  (Duncan's test).



data point to much higher values, which depends on extraction procedures, experiment and genotype, as well. Ergönül & Özbek (2018) revealed the effect of variety on bioactive compounds of cold pressed safflower seeds and reported values for total phenolic content were up to 53.9 mg/g. The major phenolic compounds detected in safflower seeds were: flavones - apigenin (as the main phenolic compound) and luteolin, hydroxybenzoic acids (vanillic acid and syringic acid), hydroxycinnamic acids (p-coumaric acid, cinnamic acid, sinapic acid, and ferulic acid), as well as flavanols (epigallocatechin and gallocatechin) (Mailier et al., 2008; Yu, et al., 2013; Moumen et al., 2015; Ergönül & Özbek, 2018).

Due to its bioactive compounds, safflower has antioxidant, antitumor, cardio-protective, hepatoprotective, phytoestrogens related, anti-diabetic and anti-obesity, and skin health properties (reviewed in Khalid et al., 2017).

The analysed data show that safflower cultivars have higher amounts of oil, oleic and linoleic acids contents, as well as  $\alpha$ -tocopherols content. Further breeding objectives would include higher seed oil contents without affecting oil and protein content in seed, as well as disease resistance/tolerance and tolerance to abiotic factors. In recent years, the only infectious disease that was detected in the tested safflower collection was viral infection (alfalfa and cucumber mosaic viruses), both reported by Milosević et al. (2015 and 2020).

From the aspect of sustainable agricultural production, minor or underutilized oil crops could have an added value and could contribute to a better crop rotation (short cycle oil crop), growing at small areas/households, additional income to small producers, agrobiodiversity, food diversity, etc.

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## Hranljive materije i bioaktivna jedinjenja semena manje gajene uljane vrste *Carthamus tinctorius* L.

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**Sažetak:** Seme sedam različitih genotipova manje gajene biljne vrste *Carthamus tinctorius* L. (*Asteraceae*), dela kolekcije alternativnih uljanih vrsta Instituta za ratarstvo i povrtarstvo (Novi Sad, Srbija), je analizirano na sadržaj proteina, ulja, masnih kiselina, tokoferola i ukupnih fenola, kako bi se ispitala njihova raznovrsnost i potencijal upotrebe ove vrste kao alternativnog izvora ovih, značajnih jedinjenja. Sadržaj proteina (određen metodom po Kjeldahl-u) u semenu šafranike bio je od 11.5 do 16.0%, dok je ukupan sadržaj ulja (određen metodom po Soxhlet-u), u proseku bio od 16.8 do 24.5% suve materije. Dominantne nezasićene masne kiseline u semenu šafranike bile su oleinska i linolna kiselina, koje predstavljaju približno 90% ukupnog sadržaja masnih kiselina (određen gasnohromatografski). Najzastupljenija masna kiselina u svim genotipovima je linolna kiselina (61.2-80.2% ulja), dok je oleinska kiselina bila u negativnoj korelaciji sa sadržajem linoleinske kiseline i bila je u rasponu od 9.6 do 29.5% ulja. Količina zasićenih masnih kiselina kretala se u rasponu od 5.5 do 6.05% za palmitinsku i od 2.1 do 3.5% za stearinsku kiselinu. Seme šafranike je značajni izvor  $\alpha$ -tokoferola (određen metodom tečne hromatografije visokih performansi sa fluorescencijom detektorom) i njegova količina se kretala od 358.8 do 461.8 mg/l ulja. Sadržaj ukupnih polifenola varirao je između 4 i 6 mg ekvivalenata galne kiseline/g suve materije. Analiza datih genotipova semena šafranike ukazuje na značaj ove alternativne uljarice kao dobrog izvora važnih hranljivih sastojaka i bioaktivnih jedinjenja.

**Ključne reči:** alternativne uljane kulture, *Carthamus tinctorius* L., masne kiseline, nutritivni kvalitet, proteini, šafranika, tokoferoli, ukupni fenoli, ulja,  $\alpha$ -tokoferol

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