

Variations in seed oil and chemical composition among the safflower genotypes (*Carthamus tinctorius* L.)

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Abstract

Seeds of oil crops are both important sources of edible oils and bioactive compounds. The present study was carried out to determine variations in oil content, bioactive compounds (total phenolic, flavonoid, carotenoid, xanthophyll and tocopherol) and fatty acid compositions in the seeds of 20 safflower genotypes. Relationships among these parameters were also examined with correlation and principal coordinate analysis. Significant differences for seed oil content and bioactive compounds were detected among the safflower genotypes. Total phenolic and flavonoid contents of the genotypes ranged from 5.10-14.55 mg g⁻¹ and from 4.60-7.25 mg g⁻¹, respectively. Total carotenoid and xanthophyll contents of seeds ranged from 3.75-19.17 mg g⁻¹ and from 5.25-20.13 mg g⁻¹, respectively. Total tocopherol content of safflower genotypes was between 43.56-76.42 mg 100 g⁻¹. Oil content of the genotypes were between 24.07-34.53% with average value of 29.22%. Fatty acid analysis revealed that 16 genotypes were linoleic and 4 were oleic type and they had generally high palmitic acid content ranging from 5.6-12.89%. Total unsaturated/total saturated fatty acids ratios were between 4.55-11.59, and 'Olas' and 'Linaz' had the highest values. Correlation analysis revealed 45 correlations, four were positively and 7 were negatively correlated with each other. Principal coordinate analysis showed that some genotypes were clustered separately based on their seed contents. Our results also confirmed that seed oil content, fatty acid compositions and seed chemical compositions among the safflower genotypes varied significantly and could be exploited for higher oil yield and oil quality.

Keywords: carotene; oil quality; phenolic compounds; seed; tocopherol

Introduction

Safflower (*Carthamus tinctorius* L.) has been cultivated traditionally as an oil crop in the world. Even though its cultivation is centuries old, total production and its yield is low compared to other oil seed crops and it has become a minor oil crop species for edible oil production in Turkey and in the world (Fernandez-Cuesta *et al.*, 2014; Kadakoglu and Karli, 2019). However, safflower is an alternative oil seed crop due to its tolerance to drought and salinity and could be grown in arid and semi-arid regions, hence it has a good potential to be used as an oilseed crop in Mediterranean countries (Yau, 2007; Fernandez-Cuesta *et al.*, 2014).

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Safflower seeds contain approximately 25-40% oil with high amounts of unsaturated fatty acids. Safflower oil is used for both human consumption and industrial purposes due to its high oleic and linoleic acids contents, which are essential in human diets (Weiss, 2000). In addition, safflower is also used in cosmetics and pharmaceutical industries and its flowers is a source of natural dyes (Corleto *et al.*, 1997; Sirinivas *et al.*, 1999). Breeding researchers have been focused on increasing oil content and changing fatty acid compositions of seeds to increase oleic acid content, thus quality of oil (Weiss, 2000). The quality and quantity of oil depends on many factors, such as genotype and growth conditions. Safflower seeds contain not only fatty acids but also phenolic compounds, tocopherols, carotenoids and other bioactive compounds (Ergönül and Özbek, 2018; Chakradhari *et al.*, 2019; Yeloojeh *et al.*, 2020).

Different storage molecules accumulate in the endosperm and cotyledons depending on plant species and the cotyledons are the main storage organs of safflower seeds. The majority of tocopherols in safflower seeds is α -tocopherol and accounts more than 95% of total tocopherol content (Johnson *et al.*, 1999), but germplasm screens revealed different tocopherol accumulating mutants of safflower (Velasco *et al.*, 2005). Carotenoids are pigments that give plants and seeds yellow color and act as antioxidants, hormone and vitamin precursors and divided into two subgroups depending on oxygen in their structure: carotenes and xanthophylls, which cannot be synthesized by humans and animals. Therefore, they must be obtained from dietary sources (Howit and Pokson, 2006). Phenolic compounds are secondary metabolites and present in fruits, leaves and seeds with many health benefiting and disease prevention mechanisms (Parkinson and Cicerale, 2016) and present in varying kinds and quantities in safflower seeds (Kim *et al.*, 2007; Ergönül and Özbek, 2018; Chakradhari *et al.*, 2019; Yeloojeh *et al.*, 2020). Seeds are good sources of different tocopherols, phenolic compounds and carotenoids, with antioxidant and free radicals scavenging activities (Kim *et al.*, 2007). These compounds prevent peroxidation of oils, protect integrity of membranes within seeds and maintain seed viability and vigor (Galleschi *et al.*, 2002; Calucci *et al.*, 2004) and increase oxidative stability of oils (Fernandez-Cuesta *et al.*, 2014; Moumen *et al.*, 2015). Total phenolic content increase in seeds under abiotic stress conditions (Yeloojeh *et al.*, 2020) and phenolic compounds protect seeds from herbivore and insect damage, and from different abiotic stresses (Mierziak *et al.*, 2014).

Seed oil content, fatty acid and chemical compositions affect oil yield and oil quality in safflower. Therefore, the aim of the present study was to evaluate seed chemical properties by measuring oil content, fatty acid and chemical compositions influencing seed oil yield and oil quality of safflower genotypes.

Materials and Methods

Plant material

Eighteen cultivars and two breeding lines of safflower, originating from five countries, were evaluated for their seed chemical and fatty acid compositions, and oil contents in the present study. Cultivars Olas and Linas were provided by Trakya Agricultural Research Institute, Turkey. Safflower genotypes, their registration status and country of origins were given Table 1.

Plants were grown at the research farms of Faculty of Agriculture, Isparta University of Applied Sciences, (37 ° 45' N and 30 ° 33' E, 997 m) in 2020. Soil physical and chemical properties of the research area were assessed according to the method of Rowell (1996). The soil was clay-loam, total lime was 7.1% (Schiebler calcimeter method), total nitrogen content was 0.18% (macro Kjehldal method), exchangeable K and P contents were 137 mg kg⁻¹ and 10.6 mg kg⁻¹, respectively (Olsen method). Soil organic content was 1.2% (Walkley-Black method) with a pH value of 7.8. The total precipitation, mean humidity, minimum and maximum temperatures and long-term averages for the experimental area were obtained from State Meteorological Service and were given in Table 2.

Seeds were sown by hand on March 29, 2020. Sowing norm was 0.50×0.15 m. The plot length was 5 m and each plot contained 5 rows. Experimental area was fertilized with 8 kg da^{-1} of P [diammonium phosphate (18% N, 46% P)] and 10 kg da^{-1} of N [ammonium nitrate (33% N)]. Weed control was performed by mechanical rotary tillage and hoeing. Three middle rows from each plot were harvested on the last week of August and seeds were collected for analyses.

Table 1. Safflower cultivars and lines used as plant material in the study

	Genotypes	Accession number	Country	Registration status
1	'AC Sunset'	PI 592391	Canada	Cultivar
2	'Lesaf 414'	PI 603206	Canada	Breeding line
3	'FO-2'	PI 506426	China	Cultivar
4	'Yuyao'	PI 514631	China	Cultivar
5	'Ziyang'	PI 514632	China	Cultivar
6	'Quiriego 88'	PI 537110	Mexico	Cultivar
7	'San Jose 89'	PI 561703	Mexico	Cultivar
8	'Gila'	PI 537692	USA	Cultivar
9	'Royal'	PI 537694	USA	Cultivar
10	'Ole'	PI 537695	USA	Cultivar
11	'Montola 2000'	PI 538025	USA	Cultivar
12	'Us-10'	PI 572414	USA	Cultivar
13	'UC-1'	PI 572434	USA	Cultivar
14	'Leed'	PI 572436	USA	Cultivar
15	'Sidwill'	PI 572471	USA	Cultivar
16	'4022'	PI 572465	USA	Breeding line
17	'Dincer-5-18-1'	TR 69497	Türkiye	Cultivar
18	'Linaz'	-	Türkiye	Cultivar
19	'Olas'	-	Türkiye	Cultivar
20	'Remzibey-05'	TR 69499	Türkiye	Cultivar

Table 2. The climatic data for average of long years and for 2020 in Isparta province

Month	Long Years (1929-2020)			2020 Year		
	Total Precipitation (L/m ²)	Mean temperature (°C)	Mean humidity (%)	Total Precipitation (L/m ²)	Mean temperature (°C)	Mean humidity (%)
January	81.0	1.8	75.3	74.1	1.4	70.6
February	67.6	2.9	71.7	71.4	3.8	75.2
March	58.8	6.0	65.9	41.3	7.7	64.8
April	52.1	10.7	61.3	24.2	11.6	58.6
May	57.0	15.4	59.2	92.1	16.1	57.1
June	34.3	19.9	52.7	42.6	20.3	52.3
July	15.9	23.4	45.6	1.9	27.0	36.5
August	14.3	23.3	46.3	24.8	25.6	39.2
September	18.5	18.9	52.2	1.0	24.1	42.9
November	38.4	13.4	62.3	48.7	17.4	58.2
October	44.8	7.8	69.9	26.5	9.3	63.1
December	86.7	3.6	76.0	35.4	6.9	81.1
Total	569.4	-	-	484.0	-	-
Mean	-	12.3	61.5	-	14.3	58.3

Determination of total soluble phenolic and flavonoid contents

Total soluble phenolic and flavonoid extraction was carried out by combining 0.2 g sample with 10 mL of 80% methanol. Then samples were stirred on a shaker for 15 min and centrifuged for 10 min at 4000 rpm. Supernatants were transferred to clean tubes and pellets were re-extracted twice as described, and supernatants were pooled and the final volume was adjusted to 25 mL with 80% methanol. For soluble phenolic determination, Folin-Ciocalteu reagent (100 μ L), 2.5 mL dH₂O and 500 μ L 20% w/v sodium carbonate solution were added to 100 μ L extract and incubated in dark for 30 min. Absorbance was determined at 760 nm and gallic acid was used as standard (Folin and Ciocalteu, 1927). Flavonoid content of the samples was determined by the method of Zhang *et al.* (2015). Reaction contained 75 μ L 5% sodium nitrate, 150 μ L 10% aluminum chloride, 1.25 mL dH₂O and 500 μ L 1 M sodium hydroxide solutions were added to the 250 μ L extract. Samples were incubated for 15 min in dark and the absorbance was measured at 510 nm. Standard curve was prepared with (+)-catechin.

Determination total carotenoid and xanthophyll contents

Seeds were dried in an incubator at 35 °C until reaching constant weight prior to analysis, and then ground in a coffee grinder. Total carotenoid and xanthophyll content of seeds was determined by the methods of the Association of Official Analytical Chemists (AOAC, 1984). Seed samples (1 g) were mixed with 10 ml acetone-hexane solution (4:6) and vortexed. Then samples were centrifuged at 10.000 rpm for 5 min, and supernatants were transferred to clean tubes and extracted with an equal amount of 20% NaCl solution twice. The upper phases were used to measure absorbance values of the samples at 436 and at 474 nm for carotenoids and xanthophylls, respectively.

Determination of total tocopherol content

Total tocopherol analysis was carried out according to modified Emmerie-Engel method (Backer *et al.*, 1980). Samples (0.5 g) were mixed with 10 mL of acetone-methanol (7:3) and were shaken for 15 min. Then the mixture was centrifuged at 10.000 rpm for 20 min, and 1 mL supernatant was mixed with 1 mL 0.5% w/v 2,2'-bipyridine and 1 mL 0.2% w/v iron chloride. After 60 s of incubation, absorbance was measured at 522 nm and standard curve was prepared with known concentrations of α -tocopherol.

Determination of oil content and fatty acid compositions

The oil content and fatty acid compositions of the genotypes were determined by Nuclear magnetic resonance and Gas chromatography, respectively. The oil content of the seeds was measured by using 2.5 g seed samples in a nuclear magnetic resonance (NMR) device (Brüker mq_{one}, Germany), and the results were given as percentage of seed weight. Seeds were mixed with hexane-methanol (4:1) solution for oil extraction. After evaporating the solvent, oil samples were converted to fatty acid methyl esters for determination of fatty acid composition. The samples were analyzed with a gas chromatography (Shimadzu GC-2025, Japan) device equipped with flame emission detector (GC-FID) (Agilent 7890A, Agilent Technologies, USA) and the analysis was carried out as described by Erbaş *et al.* (2016).

Statistical analysis

Analysis of seed chemical compositions was carried out with three replications. Data was subjected to analysis of variance (ANOVA) using SPSS Statistics 22.0 software (IBM, USA). Duncan's multiple range test ($p \leq 0.05$) was used to separate differences between the means. Pearson correlation coefficients was calculated to generate heatmap to show the relationship between the examined parameters, using OriginPro version 2021 (OriginLab, Northampton, MA). The mean values of the examined parameters were used for principal component analysis (PCA).

Results and Discussion

Study material consisted of 18 cultivars and two breeding lines of safflower from five countries (Table 1). Total soluble phenolic, flavonoid, carotenoid, xanthophyll, tocopherol, oil content and fatty acid compositions of safflower seeds were determined. The results showed significant differences among 20 safflower genotypes for the examined parameters and given in Table 3.

Table 3. Seed oil content and chemical composition of 20 safflower genotypes used in the study

Genotypes	Phenolics (mg g ⁻¹)	Flavonoid (mg g ⁻¹)	Carotenoids (mg g ⁻¹)	Xanthophylls (mg g ⁻¹)	Tocopherol (mg 100 g ⁻¹)	Oil content (%)
'AC Sunset'	12.99±0.38 bc	4.98±0.07 j-l	8.97±0.86 f-h	10.23±0.60 e-g	75.06±2.16 a-c	24.43±1.01 h
'Lesaf 414'	10.06±0.56 f	5.22±0.26 i-k	8.93±0.05 f-h	8.37±0.14 h	68.16±0.8 i	29.33±2.16 c-f
'FO-2'	8.84±0.38 g	6.33±0.07 d	10.53±1.34 f	11.60±0.36 d	76.42±0.17 a	26.37±1.15 f-h
'Yuyao'	11.63±0.80 de	6.91±0.07 bc	4.00±0.82 l	6.90±0.70 i	71.94±0.15 ef	25.70±1.52 gh
'Ziyang'	10.81±0.48 ef	6.81±0.13 c	9.93±0.10 fg	5.25±0.04 k	75.28±0.20 a-c	24.07±0.58 h
'Quiriego 88'	8.09±0.92 gh	4.84±0.07 lm	16.73±1.12 b	20.13±0.90 a	49.36±0.45 k	29.17±1.92 c-f
'San Jose 89'	5.10±0.38 j	4.63±0.13 m	3.77±0.52 l	6.67±0.41 ij	68.96±0.34 hi	30.97±1.40 b-d
'Gila'	11.36±0.86 de	5.37±0.22 hi	7.00±0.72 i-k	9.10±0.27 gh	76.12±0.26 ab	29.20±0.54 c-f
'Royal'	10.54±1.00 ef	6.19±0.07 de	6.53±1.21 jk	6.25±0.04 i-k	72.81±0.19 de	29.90±0.41 ce
'Ole'	10.54±0.64 ef	5.61±0.20 gh	18.97±0.37 a	16.55±0.49 b	74.42±0.71 b-d	30.67±3.62 b-d
'Montola 2000'	14.55±0.76 a	7.25±0.34 a	14.90±0.91 cd	10.00±0.82 fg	61.33±1.53 j	30.83±0.86 b-d
'US-10'	8.70±0.38 g	5.66±0.09 gh	13.27±1.02 e	10.83±0.68 d-f	70.59±0.27 f-h	30.03±0.94 c-e
'UC-1'	7.31±0.47 hi	4.60±0.07 m	19.17±0.65 a	14.03±1.43 c	72.71±2.00 de	30.33±1.08 c-e
'Leed'	14.01±0.38 ab	7.15±0.15 ab	15.90±0.18 bc	15.13±0.76 c	73.77±0.41 cd	31.60±1.30 a-c
'Sidwill'	6.73±0.55 i	4.93±0.13 kl	9.30±0.68 f-h	8.50±0.18 h	73.10±0.28 de	27.07±2.49 e-h
'4022'	12.92±1.07 bc	6.04±0.07 d-f	6.10±0.98 k	11.35±0.04 de	70.21±0.05 f-h	27.90±2.21 d-g
'Dincer-5-18-1'	7.68±0.38 g-i	5.90±0.15 e-g	14.10±0.98 de	19.40±1.09 a	43.59±0.34 l	28.43±0.34 c-g
'Linas'	7.21±0.28 hi	5.27±0.20 ij	8.00±0.89 h-j	8.37±0.29 h	69.15±0.94 g-i	33.63±1.63 ab
'Olas'	12.31±0.11 cd	5.75±0.20 fg	3.75±0.22 l	5.62±0.03 jk	70.85±0.64 fg	34.53±0.69 a
'Remzibey-05'	12.97±0.09 bc	6.09±0.13 de	8.40±0.24 g-i	10.43±0.46 d-f	61.40±1.16 j	30.27±0.10 c-e
Mean	10.21	5.79	10.41	10.74	68.76	29.22
Error	0.35	1.03	0.63	0.55	1.11	0.39
CV (%)	5.59	2.44	8.22	4.25	0.96	4.47

Total phenolic content among 20 safflower genotypes changed between 5.10-14.55 mg g⁻¹. 'Montola 2000', 'Leed', 'Remzibey-05', 'AC Sunset' and '4022' had the highest and 'San Jose 89', 'Sidwill', 'Linas' and 'UC-1' had the lowest levels of total soluble phenolic contents in the study (Table 3). Seed phenolic content of safflowers were similar to previous reports, but we have observed a wider range for phenolic content, hence there were more safflower cultivars in our study (Yeloojeh *et al.*, 2020). Total flavonoid content among the safflowers were lower than the total phenolic content and was within 4.60-7.25 mg g⁻¹ range. 'Montola 2000' (7.25 mg g⁻¹), 'Leed' (7.15 mg g⁻¹) and 'Yuyao' (6.91 mg g⁻¹) had the highest and 'UC-1' (4.60 mg g⁻¹), 'San Jose 89' (4.63 mg g⁻¹) and 'Quiriego 88' (4.84 mg g⁻¹) had lowest flavonoid content among the safflower genotypes. Yeloojeh *et al.* (2020) reported the range of flavonoid content was between 5.58-13.42 mg g⁻¹ among the genotypes and induction of drought decreased flavonoid content in seeds. Flavonoids were mainly accumulated in hull, and dark-colored seeds had higher flavonoid content and seed hulls were a good source of flavonoid compounds in safflower (Karami *et al.*, 2018). Composition and accumulation of phenolic and flavonoids were depended on genotypes and growth conditions (Kim *et al.*, 2007; Ergönül and Özbek, 2018; Yeloojeh *et al.*, 2020). Both phenolic compounds isolated individually from safflower seeds and total flavonoids showed good radical scavenging activity against superoxide and hydroxyl radicals (Kim *et al.*, 2007; Karami *et al.*, 2018).

Reports also indicated that safflower oil contained lesser amounts of phenolic compounds per gram of oil than seed extracts (Moumen *et al.*, 2015; Ergönül and Özbek, 2018), but presence of phenolic compounds in safflower oil increased oil quality by raising oxidative stability of safflower oil (Moumen *et al.*, 2015).

Carotenoid and xanthophyll content of safflower seeds were measured and significant differences were detected (Table 3). Carotenoid content of genotypes changed between 3.75-19.17 mg g⁻¹, and xanthophyll content was between 5.25-20.13 mg g⁻¹, respectively. Among the safflower genotypes, 'UC-1', 'Ole', 'Quiriego 88' and 'Leed' had the highest and 'Olas', 'San Jose 89' and 'Yuyao' had the lowest amounts of carotenoids contents. Xanthophyll content of genotypes showed a similar pattern, genotypes with high carotenoid content had also had high xanthophyll content in the study. 'Quiriego 88', 'Dinçer 5-18-1', 'Ole', 'Leed' and 'UC-1' had the highest amounts of xanthophyll contents and 'Olas' and 'Ziyang' had lowest amounts of xanthophyll contents among the safflower genotypes. Chakradhari *et al.* (2019) detected 6 carotenoid compounds in oils of safflower and *C. oxyacanthus*. Levels of individual carotenoid compounds did not differ in safflower, but it was different in *C. oxyacanthus* and the wild species had higher amounts of carotenoids in its oil. Carotenoids and xanthophylls in seeds and oils act as antioxidants and prevent oxidation process, which reduce oil quality and cause seed aging by disrupting the integrity of cell membranes. Presence of reactive oxygen species increased by 2.5-fold in seeds whose carotenoid content is halved, and consequently, seeds lost the germination ability (Galleschi *et al.*, 2002). Total phenolic and carotenoids content of seeds and oils were also shown to be affected by fertilizer applications, growth conditions, years and locations (Criado *et al.*, 2004; Karakurt *et al.*, 2015; Tonguç, 2019; Yeloojeh *et al.*, 2020), therefore their contents could also be manipulated to increase their levels in safflower seeds.

Tocopherols are oil soluble antioxidants present in seed oils and α -tocopherol is the most common tocopherol type in safflower and acts as vitamin E in seeds (Johnson *et al.*, 1999; Fernandez-Cuesto *et al.*, 2014). In the present study, total tocopherol content of 20 safflower genotypes changed between 43.59-76.42 mg 100 g⁻¹ (Table 3). 'Dinçer 5-18-1' (43.59 mg 100 g⁻¹) and 'Quiriego 88' (49.36 mg 100 g⁻¹) had the lowest amounts of tocopherol contents among the safflower genotypes. Five genotypes had 60-70 mg 100 g⁻¹ and the rest of the genotypes had over 70 mg 100 g⁻¹ tocopherol content in their seeds. The highest tocopherol content was observed in 'FO-2', 'Gila', 'AC Sunset' and 'Ziyang'. Total tocopherol content of 9 safflower genotypes were between 47.29-69.74 mg 100 g⁻¹, and 'Remzibey-05' had 62.87 mg 100 g⁻¹ tocopherol content, which is very close to value found in our study for the same cultivar (61.40 mg 100 g⁻¹) (Matthaus *et al.*, 2015). Other reports indicated that tocopherol content of safflower oil changed between 112.67-131.84 mg 100 g⁻¹ (Ergönül and Özbek, 2018), 57.9-58.2 mg 100 g⁻¹ between oil of two *Carthamus* species (Chakradhari *et al.*, 2019), and between 46.1-49.9 mg 100 g⁻¹ (Moumen *et al.*, 2015). Tocopherol contents was between 19.3-53.1 mg 100 g⁻¹ among seed extracts of 6 *Carthamus* species and variation for γ -tocopherol levels was observed in a population of *C. oxyacanthus* (Velasco *et al.*, 2005). Tocopherol content of safflower seed extract was lower than tocopherol content of oil (Bozan and Temelli, 2008), suggesting that tocopherol content could be increased with different oil extraction methods.

Total oil content of the genotypes varied between 24.07-34.53% and the mean oil content was 29.22% among the genotypes. 'Olas' (34.53%) and 'Linas' (33.63%) had the highest, whereas 'Ziyang' (24.07%) and 'AC Sunset' (24.43%) had the lowest oil contents observed in the study. High oil content is necessary to increase oil yields of the cultivars and germplasm screens revealed that oil content of safflower genotypes could change from 11% to 46% (Johnson *et al.*, 1999). Safflower cultivars usually contain not less than 20% oil (Pascual-Villalobos and Albuquerque, 1996; Erbaş *et al.*, 2016) and breeding lines could contain up to 55% oil in the seeds (Rubis, 2001). Oil content has moderate heritability and is influenced by genotype and environmental factors (Çamaş and Esendal, 2006), and our previous (Erbaş *et al.*, 2016) and present results confirm that variation for oil content exist and oil content could change between the years for the same safflower cultivars.

Table 4. Seed fatty acid profile and distribution of total saturated and unsaturated fatty acids contents among the safflower genotypes

Genotypes	Palmitic acid (C _{16:0})	Stearic acid (C _{18:0})	Oleic acid (C _{18:1})	Linoleic acid (C _{18:2})	Σ TUFA	Σ TSFA	TUFA/TSFA
'AC Sunset'	9.91±0.20	3.70±0.08	13.00±0.26	73.37±1.49	86.37	13.61	6.35
'Lesaf 414'	10.15±0.21	3.71±0.08	22.10±0.45	64.02±1.30	86.12	13.86	6.21
'FO-2'	14.27±0.29	3.72±0.07	12.16±0.25	69.61±1.41	81.77	17.99	4.55
'Yuyao'	11.92±0.24	3.60±0.05	23.17±0.47	61.30±1.24	84.47	15.52	5.44
'Ziyang'	10.26±0.21	4.09±0.11	18.31±0.37	67.31±1.36	85.62	14.35	5.97
'Quiriego 88'	10.68±0.22	4.09±0.07	18.69±0.38	66.39±1.35	85.08	14.77	5.76
'San Jose 89'	11.16±0.23	5.03±0.07	15.17±0.31	68.61±1.39	83.78	16.19	5.17
'Gila'	12.50±0.25	3.94±0.09	14.54±0.29	68.62±1.39	83.16	16.44	5.06
'Royal'	13.44±0.27	4.34±0.12	13.10±0.27	67.61±1.37	80.71	17.78	4.54
'Ole'	8.43±0.17	3.15±0.04	63.50±1.29	24.90±0.50	88.40	11.58	7.63
'Montola 2000'	8.90±0.18	3.13±0.08	60.80±1.23	27.15±0.55	87.95	12.03	7.31
'US-10'	12.73±0.26	3.27±0.07	16.97±0.34	67.00±1.36	83.97	16.00	5.25
'UC-1'	10.08±0.20	2.92±0.03	66.00±1.34	20.68±0.42	86.68	13.00	6.67
'Leed'	9.03±0.18	3.03±0.06	12.45±0.25	75.46±1.53	87.91	12.06	7.29
'Sidwill'	10.65±0.22	4.03±0.07	14.87±0.30	70.42±1.43	85.29	14.68	5.81
'4022'	10.65±0.22	3.20±0.08	21.67±0.44	64.17±1.30	85.84	13.85	6.20
'Dincer-5-18-1'	12.89±0.26	4.23±0.04	13.00±0.26	69.45±1.41	82.45	17.12	4.82
'Linas'	5.60±0.14	2.30±0.09	29.20±0.59	62.40±1.27	91.60	7.90	11.59
'Olas'	5.90±0.16	2.10±0.07	40.30±0.82	51.00±1.03	91.30	8.10	11.27
'Remzibey-05'	12.07±0.26	3.20±0.09	18.11±0.37	66.61±1.35	84.72	15.27	5.55

Fatty acid compositions of the safflower genotypes were also determined by GC-MS analysis in the present study (Table 4). Safflower genotypes usually had high palmitic acid content; only two cultivars ('Olas' and 'Linas') contained less than 6.0% palmitic acid among the genotypes and palmitic acid content changed between 5.6 to 14.27%. The highest amount of palmitic acid was found in 'FO-2', 'Royal' and 'Dincer-5-18-1', and the lowest amount of palmitic acid found in 'Linas', 'Olas' and 'Ole'. Stearic acid content exhibited a distribution from 2.1% to 5.03%, and 'San Jose 89', 'Royal' and 'Dincer-5-18-1' had the highest, whereas 'Olas', 'Linas' and 'UC-1' had the lowest content of stearic acid among the genotypes. Analysis of 797 safflower genotypes revealed that fatty acid composition was between 3.9-7.0% for palmitic acid, 1.1-4.5% for stearic acid, 6.0-82.0% for oleic acid and 11.0-83.0% for linoleic acid (Johnson *et al.*, 1999). Other germplasm screens showed that palmitic and stearic acid contents of safflower cultivars were in between 4.9-6.7% and 0.7-2.4%, respectively (Pascual-Villalobos and Alburquerque, 1996). However, our previous results showed higher palmitic (9.1-13.7%) and stearic (2.4-5.6%) acids contents for cultivars, even though some of them, such as 'Gila', 'Ole' and 'Royal' were common at both studies (Erbaş *et al.*, 2016). Most of the genotypes were linoleic type and only four cultivars were high ('Ole', 'Montola 2000' and 'UC-1') and intermediate ('Olas') type for oleic acid content. Oleic acid content was between 12.16-66.0%, and linoleic acid content was between 20.68-73.37% among the safflower genotypes. High oleic type genotypes had the least amount of linoleic acid content. Genotypes with the highest amount of linoleic acid content were 'AC Sunset', 'FO-2' and 'Dincer-5-18-1'. Oleic acid synthesis controlled by three alleles, *Ol* high linoleic acid, *ol* high oleic acid and *ol'* intermediate levels of oleic acid, and *st* allele produce high stearic acid (Knowles, 1972). Fatty acids contents were also affected by temperature changes during growth, location, sowing and harvest times and years (Knowles, 1972; Geçgel *et al.*, 2007; Erbaş *et al.*, 2016). Low temperatures increase linoleic acid, and high temperatures increase stearic and oleic acid contents (Knowles, 1972) and high temperatures have inhibitory effects on desaturase enzymes (Hamrouni *et al.*, 2004), which might explain differences observed for fatty acids contents in this study.

TUFA and TSFA contents of the genotypes changed from 80.71-91.60% and 7.90-17.99%, respectively, and Linas and Olas had the highest TUFA/TSFA contents observed among the safflower genotypes. Oleic acid

and TUFA/TSFA contents are important determinants for oil quality. Oxidative stability of oils is negatively affected by high amounts of linoleic acid content (Moumen *et al.*, 2015). Altering seed chemical composition, such as increasing phenolic or oleic acid contents (Moumen *et al.*, 2015) or changing tocopherol composition (Fernandez-Cuesta *et al.*, 2014) also increase oxidative stability of oils, thus help to increase quality and health benefiting effects of safflower oil.

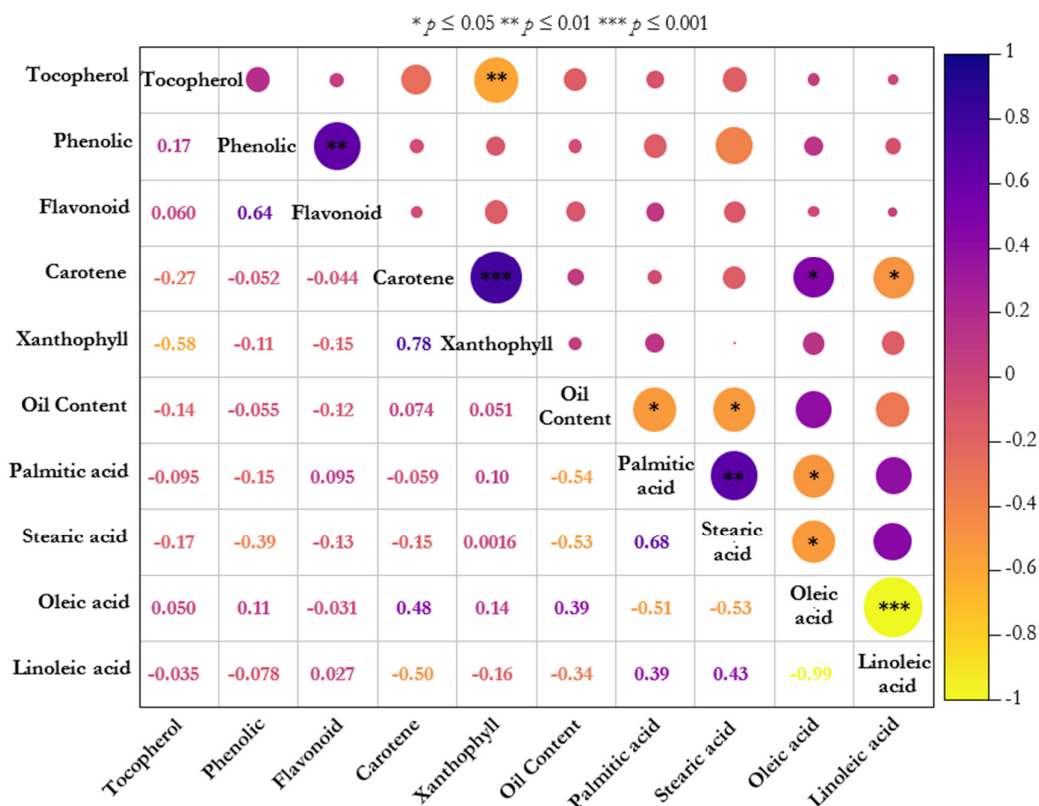


Figure 1. Relationships and significance levels between oil content, fatty acid and seed chemical compositions generated by heatmap

The results of correlation analysis between seed chemical, oil content and fatty acid compositions is presented in Figure 1. Among the 45 Pearson correlation coefficients, 11 were significant. Of these, four were positively and 7 were negatively correlated with each other. Phenolic content correlated positively with flavonoid (0.64), carotenoid content correlated positively with xanthophyll (0.78), oleic acid (0.48) and negatively with linoleic acid (-0.50) contents. Oil content had negative correlation with palmitic (-0.54) and stearic (-0.53) acids. Oleic and linoleic acid contents showed negative correlation (-0.99) with each other. Arslan (2007) reported that palmitic acid was positively, but stearic acid was negatively correlated with oil content and oleic and linoleic acid contents were highly negatively correlated with each other as well. Tocopherol content was negatively correlated with xanthophyll content (-0.58), a similar result was observed in *Pinus brutia* seeds whose tocopherol and xanthophyll contents showed significant negative correlation with each other (Tonguç *et al.*, 2022). Principal component analysis was performed to show linear relationships between examined parameters and genotypes. Results revealed that PC1 (33.75%) and PC2 (22.90%) explained 56.65% of the total variation (Figure 2).

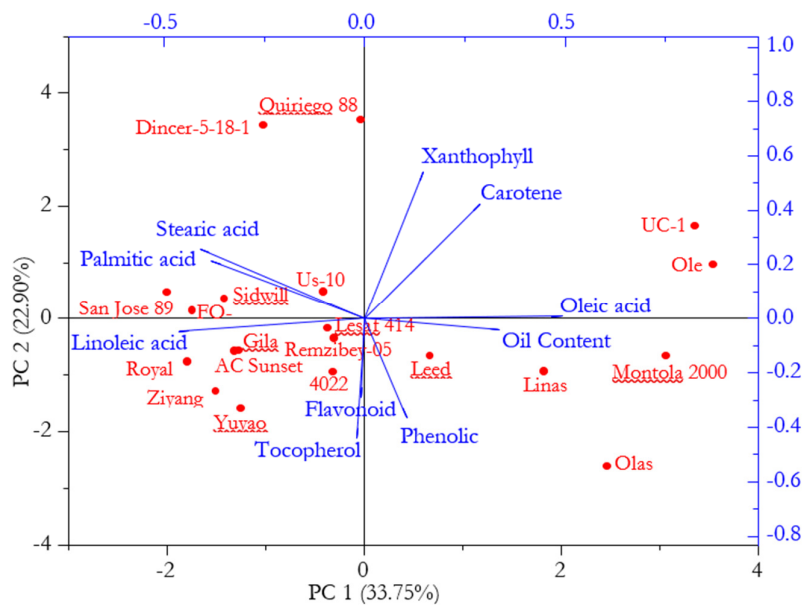


Figure 2. PCA analysis of safflower genotypes and lines based on seed chemical compositions, oil and fatty acids content

Six of the genotypes were grouped on the right side of PC1. ‘UC-1’ and ‘Ole’, which had high amounts of oleic acid, carotenoid and xanthophyll contents and positively associated with them, formed a separate cluster. ‘Linás’, ‘Olas’ and ‘Montola 2000’ formed another cluster and associated with oil and phenolic contents. The other genotypes clustered on PC2 and ‘Dinçer-5-18-1’ and ‘Quireiego 88’ were clustered together with stearic and palmitic acids and carotenoid and xanthophyll contents. ‘US-10’, ‘Remzibey-05’ and ‘Lesaf 414’ were very close to center, indicating that they were not distinguished significantly based on the measured parameters. Karami *et al.* (2018) used PCA and separated *Carthamus* species based on their radical scavenging, flavonoid content and constituents two sub-groups.

Conclusions

Results of the present study showed that variation among safflower genotypes exist for seed chemical compounds. Phenolic and flavonoid contents showed close relationship with each other. The same phenomenon was also observed for total carotenoid and xanthophyll contents. Total tocopherol contents of seed extracts were usually high. Some of the seed chemical composition is known to be controlled by genetic factors and others are affected by cultural practices and growth conditions. Therefore, it is possible to change seed chemical composition of safflower genotypes through plant breeding and manipulating cultural practices and growth conditions to increase the levels these compounds along with suitable oil extraction techniques for improving oil yield and quality.

Authors' Contributions

Conceptualization and Investigation: MT and SÖ; Writing - original draft: MT; Resources: MT and SE; Writing - review and editing: SÖ and SE. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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