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Classification of Turkish safflower oils based on their fatty acid and sterol profiles using multivariate techniques

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ABSTRACT

The aim of the current study was to classify Turkish safflower oils based on sterol and fatty acid composition. For this purpose, 37 samples from five different safflower varieties (Dinçer, Linas, Remzibey-05, Balcı and Olas) grown in the same agricultural and environmental conditions were obtained. Seeds were evaluated for their oil, water and ash content. Oils of seeds were extracted by solvent extraction and oils were analyzed for their sterols and fatty acids. Results have shown that Linas and Olas varieties' oil contents were significantly higher than others'. There were clear differences in fatty acid compositions of various cultivars. Remzibey-05 and Olas varieties were different from others by their higher oleic and relatively lower linoleic acid ratios. Total sterol contents of oils ranged among 2700-3626 mg/kg and the main phytosterol was β-sitosterol covering 43.27-48.16 % of the total sterols.

Introduction

Safflower (*Carthamous tinctorius* L.) is a precious oily seed and cultivated in various parts of the world due to its high adaptability against forceful winds, hail storms, drought etc. Safflower is cultivated in more than 60 countries, where USA, Mexico, Argentina, India and Kazakhstan are the major producers. There's an increasing trend all around the world for safflower production and the cropland expansion is 4.9% per year (Khalid et al., 2017).

Safflower seeds contain 35-50 % oil, 15-20 % protein and 35-45 % hull (Rahamatalla et al., 2001). Safflower oil contains linoleic and oleic acids as unsaturated; stearic and palmitic acids as saturated fatty acids (Ashrafi and Razmjoo, 2010). The stearic acid ratio is approximately 2-3 %, whereas palmitic, oleic and linoleic acids are found among 6-8 %, 16-20 % and 71-75 %, respectively (Sabale and Deokar, 1997; Ashrafi and Razmjoo, 2010). On the other side, high linoleic safflower oil contains about 87-89 % linoleic acid, while high oleic safflower oil contains higher than 85 % oleic acid (Dajue, 1993; Ashrafi and Razmjoo, 2010).

Previous researches have shown that different commercial safflower cultivars were successfully cultivated in different regions of Turkey (Baydar and Turgut, 1999; Samancı and Özkaynak, 2003; Özel et al., 2004; Camas et al., 2007). Various works demonstrated that the chemical properties of safflower oil is highly affected by genotypic variation of safflower seeds. Genotype has been reported to be the most important factor that affect the oil content and chemical properties of the seed (Ashrafi and Razmjoo, 2010).

There are seven types of safflower varieties, namely Dinçer, Remzibey, Balcı, Yenice, Linas, Ayaz, Olas, cultivated in Turkey. The safflower oil extracted from Dinçer, Yenice and Remzibey varieties were analyzed for their fatty acid composition, seed and oil yields (Camas et al., 2007), Remzibey for its fatty acid distribution (Eryılmaz et al., 2014a), Dinçer for its fatty acid composition (Eryılmaz et al., 2014b),

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Yenice, Dinçer and Remzibey for their oil content and fatty acid profile (Arslan and Küçük, 2005), Remzibey for its fatty acid and tocopherol profile (Matthäus et al., 2015) in earlier studies.
The aim of the current work was to determine differences among cultivars based on fatty acid and sterol profiles. To the best of the authors' knowledge, there is no report on the classification of Linas, Remzibey-05, Balcı and Olas safflower cultivars based on their fatty acid and sterol compositions. This is the first article evaluating the sterol content and composition of Linas, Remzibey-05, Balcı and Olas safflower cultivars.

Materials and methods

Plant material

The plant material consisted of five safflower cultivars, namely Dinçer, Linas, Remzibey-05, Balcı and Olas. The seeds were grown in the field crop area of Menemen International Agricultural Research and Training Center. The soil of the growing area was clay, low in organic matter (1.72%), phosphorus content (2.1 ppm), potassium content (243 ppm) and a pH of 8.1. The climate of the growing area is Mediterranean. Average rainfall is 616 mm and average temperature is 19 °C. Seedings were made on 11 November 2014 and the harvest date was 13 July 2015.

Extraction of safflower oil

The extraction of the oil was performed using Soxhlet extraction. The oil samples were stored in dark bottles at -18 °C under nitrogen atmosphere until analysis.

Chemical analysis

Analysis of seed properties

The water content of safflower seeds was determined using a moisture analyzing instrument (Precisa XM 60, Switzerland). The oil content of safflower seeds was determined according to AOCS Official Methods Am 2-93 (AOCS, 2003) by extraction with *n*-hexane in a Soxhlet apparatus. The 1000 seed weight was determined by multiplying the average weight of 100 random selected grains with 10 and the ash content of safflower seeds was determined according to AOAC Official method 923.03 (AOAC, 1990).

Fatty Acid Composition

The fatty acid methyl esters were prepared according to the method of International Union of Pure and Applied Chemistry (IUPAC, 1987) and subsequently quantified with a gas chromatograph (GC 2010, Shimadzu/Japan) using a DB-23 fused silica capillary column (60 m, 0.25 mm internal diameter and 0.25 μm film thickness, J&W Scientific, USA) and flame ionisation detector. The temperatures of the detector, column and injector were 240 °C, 195 °C and 230 °C, respectively. Nitrogen was the carrier gas with a flow rate of 1.0 mL min^{-1} and the split ratio was 80:1.

Sterol analysis

The sterol profile of safflower oils were determined according to AOCS Official method Ch 6-91 (AOCS, 2003). The sterols were initially derivatized and their silyl ethers were then analyzed utilizing a gas chromatograph (GC 2010, Shimadzu, Japan) with flame ionisation detector and HP-5 fused silica capillary column (30 m, 0.25 mm internal diameter and 0.25 mm film thickness, Chrom Tech., Apple Walley, MN, USA). Detector, injector and column temperatures were 290 °C, 280 °C and 260 °C, respectively. The carrier gas was nitrogen with a 50:1 split ratio and 0.8 ml min^{-1} of flow rate.

Statistical Analysis

Statistical evaluation was carried out using SPSS 15 statistical software (SPSS Inc., Chicago, USA). Results were analyzed with one-way analysis of variance (ANOVA). Data were also evaluated by principal component analysis (PCA) and hierarchical cluster analysis (HCA) using XLSTAT 2017 version (Addinsoft, New York, NY).

Results and discussion

Characteristic properties of safflower seeds from five different Turkish varieties were presented in Table 1. The 1000 seed weight of safflower seeds was between 44.1 g (Remzibey-05) and 48.4 g (Dinçer). In the study of Amini et al. (2007), 1000 seed weight of safflower genotypes from different origins were reported to range between 28.58 and 36.94 g. The water content of safflower seeds was between 6.86% (Olas) and 7.49% (Dinçer) varieties. Işığıgür et al. (1995), reported that the water content of Turkish safflower seeds from different varieties ranged in 7.0 and 8.5%. Similar findings were also reported by Bozan and Temelli (2008).
The oil content of safflower seeds varied between 27.18 and 36.58 % (dry basis). While Dinçer variety had the lowest oil content, the oil percent of Linas and Olas varieties were higher than 35%.

Table 1. Some descriptive characteristics of Turkish safflower varieties

	Dinçer	Linas	Remzibey-05	Balcı	Olas
1000 seed weight (g)	$48.4^{b}\pm2.32$	$46.5^{ab}\pm4.72$	$44.1^{a}\pm2.08$	$44.7^{a}\pm1.88$	$46.8^{ab}\pm4.07$
Water content (%)	$7.49^{c}\pm0.45$	$6.92^{ab}\pm0.18$	$7.47^{c}\pm0.21$	$7.17^{b}\pm0.45$	$6.86^{a}\pm0.24$
Oil content (dry basis, wt%)	$27.18^{a}\pm1.99$	$35.18^{cd}\pm1.55$	$30.28^{b}\pm1.09$	$32.49^{c}\pm4.53$	$36.58^{d}\pm1.81$
Ash (%)	$3.12^{a}\pm0.17$	$3.44^{c}\pm0.09$	$3.21^{ab}\pm0.17$	$3.32^{bc}\pm0.18$	$3.41^{c}\pm0.11$

Different superscript lowercase letters in the same line indicate significant differences ($p < 0.05$) between different varieties

Table 2. Fatty acid composition of safflower oils obtained from different varieties (%)

	Dinçer	Linas	Remzibey-05	Balcı	Olas
C 16:0	$6.05^{d}\pm0.22$	$5.79^{c}\pm0.19$	$5.32^{b}\pm0.12$	$6.04^{d}\pm0.15$	$3.63^{a}\pm0.00$
C 16:1	$0.11^{a}\pm0.08$	$0.31^{b}\pm0.12$	$0.33^{b}\pm0.07$	$0.27^{b}\pm0.11$	$0.28^{b}\pm0.11$
C 17:0	$0.02^{a}\pm0.01$	$0.01^{a}\pm0.00$	$0.02^{a}\pm0.01$	$0.02^{a}\pm0.01$	$0.02^{a}\pm0.00$
C 17:1	-	-	$0.01^{a}\pm0.01$	$0.01^{a}\pm0.00$	$0.02^{a}\pm0.00$
C 18:0	$1.80^{b}\pm0.09$	$1.86^{bc}\pm0.06$	$1.78^{b}\pm0.10$	$1.91^{c}\pm0.12$	$1.46^{a}\pm0.04$
C 18:1	$12.66^{a}\pm0.30$	$14.70^{a}\pm1.36$	$36.63^{b}\pm2.05$	$14.60^{a}\pm0.73$	$47.47^{c}\pm3.64$
C 18:2	$78.95^{d}\pm0.23$	$76.86^{c}\pm1.45$	$55.27^{b}\pm2.16$	$76.68^{c}\pm0.52$	$45.05^{a}\pm3.40$
C 18:3	$0.06^{a}\pm0.02$	$0.13^{ab}\pm0.06$	$0.15^{b}\pm0.04$	$0.12^{ab}\pm0.02$	$0.17^{b}\pm0.14$
C 20:0	$0.20^{ab}\pm0.06$	$0.20^{a}\pm0.03$	$0.28^{c}\pm0.03$	$0.23^{ab}\pm0.02$	$0.25^{bc}\pm0.02$
C 20:1	$0.09^{a}\pm0.06$	$0.10^{a}\pm0.03$	$0.18^{b}\pm0.04$	$0.13^{a}\pm0.03$	$0.21^{b}\pm0.08$

Different superscript lowercase letters in the same line indicate significant *differences* ($p < 0.05$) between different varieties

Işığügür et al. (1995) determined oil seed contents ranging in 18.5 and 34.3% for different safflower cultivars; whereas Geçgel et al. (2007) reported 13.51-32.99 % and 4.72-38.25 % for Montola-2001 and Centennial varieties, respectively. The differences in oil content levels is attributed to the diversity in genes and environment (Yeilaghi et al., 2012). The ash content of safflower seeds ranged from 3.12 (Dinçer) to 3.44% (Linas). Lower ash contents were reported for safflower seeds (1.91%) from Central Anatolian region of Turkey and for safflower seeds (1.20-4.50 %) from Nigerian origin (Akintayo, 2004).

Fatty acid composition

The fatty acid composition of safflower oils obtained from different cultivars is presented in Table 2. C18:1, C18:2 and C16:0 were the principal fatty acids in accordance with previous studies (Bozan and Temelli, 2008; Sabzalian et al., 2008; Kizil et al., 2008; Ashrafi and Razmjoo, 2010). The content of major fatty acid, linoleic acid, ranged from 45.05 to 78.95 % similar to the study of Matthaus et al. (2015). The safflower seed oils of five different varieties were rich in unsaturated fatty acids which provide nutritional value as was earlier reported by Sabzalian et al. (2008).

Oleic acid ratios were 12.66, 14.60 and 14.70 % for Dinçer, Balcı and Linas in the same order; while Remzibey-05 (36.63%) and Olas (47.47%) were found to be oleic-rich varieties. The percentage of C18:2, on the other side, were determined 76.68, 76.86 and 78.95 % for Balcı, Linas and Dinçer; 45.05 and 55.27 % for Olas and Remzibey-05 varieties. Palmitic acid ratio of Olas variety was significantly lower than others. Stearic acid percentages were between 1.46 (Olas)- 1.91 (Balcı) %. The remaining fatty acids including C16:1, C17:0, C17:1, C18:3, C20:0 and C20:1 were in trace quantities, and their total concentration were lower than 2% for all varieties.

Sterol composition

The distribution of individual sterols in the sterol fractions of five different safflower seed oils were presented in Table 3. Total sterol content of samples ranged from 2700.46 (Olas) to 3626.03 (Dinçer) mg/kg, higher than the results reported by Ortega-García et al. (2006). The major sterols were β-sitosterol (1292.58 - 1596.97 mg/kg), followed by Δ-7-stigmastenol (476.06 - 818.97 mg/kg) and campesterol (306.47 -375.46 mg/kg), respectively.

24-methylene-cholesterol (12.01 mg/kg), campesterol (375.46 mg/kg), stigmasterol (213.97 mg/kg), Δ-7-campesterol (125.80 mg/kg), β-sitosterol (1569.97 mg/kg), Δ-5,24-stigmastadienol (77.56 mg/kg), Δ-7-avenasterol (169.85 mg/kg), Δ-7-stigmastenol (818.97 mg/kg) were present at higher levels in Dinçer variety; clerosterol (10.83 mg/kg) was higher in Linas; sitostanol (110.00 mg/kg) and Δ-5-avenasterol (146.53 mg/kg) were higher in Remzibey-05 variety when compared to other varieties analysed.

Table 3. Sterol content of safflower oils obtained from different varieties (mg/kg)

	Dinçer	Linas	Remzibey-05	Balcı	Olas
Brassicasterol	0.86^{a}±0.36	0.67^{a}±0.20	0.74^{a}±0.27	0.98^{a}±0.60	1.27^{a}±0.85
24-methylene-cholesterol	12.01^{b}±4.17	5.57^{a}±1.67	10.75^{b}±4.06	2.37^{a}±1.37	2.24^{a}±0.64
Campesterol	375.46^{b}±41.73	346.37^{ab}±40.24	346.96^{ab}±38.07	325.43^{a}±29.61	306.47^{a}±30.65
Campestanol	4.19^{a}±0.92	4.10^{a}±0.77	4.75^{a}±1.20	3.74^{a}±1.01	4.53^{a}±1.61
Stigmasterol	213.97^{b}±27.23	198.83^{ab}±18.84	201.61^{ab}±18.06	178.06^{a}±26.55	180.73^{a}±19.81
Δ-7-campesterol	125.80^{c}±12.07	78.75^{b}±11.31	81.06^{b}±9.69	81.30^{b}±10.22	64.64^{a}±16.44
Clerosterol	10.49^{a}±1.46	10.83^{a}±1.61	9.65^{a}±1.47	9.45^{a}±1.45	9.31^{a}±1.36
β-sitosterol	1569.97^{b}±186.25	1512.46^{b}±191.45	1488.73^{b}±150.80	1512.31^{b}±87.93	1292.58^{a}±167.87
Sitostanol	108.09^{b}±11.02	86.77^{a}±8.37	110.00^{b}±10.05	108.51^{b}±18.33	87.58^{a}±6.19
Δ-5-avenasterol	138.74^{b}±12.59	140.72^{b}±22.77	146.53^{b}±9.81	112.10^{a}±25.53	111.32^{a}±18.46
Δ-5,24-stigmastadienol	77.56^{c}±6.47	66.35^{b}±8.40	70.06^{bc}±3.86	56.18^{a}±8.58	51.36^{a}±8.80
Δ-7-stigmastenol	818.97^{c}±83.91	603.34^{b}±102.95	604.49^{b}±39.58	633.27^{b}±68.98	476.06^{a}±137.93
Δ-7-avenasterol	169.85^{b}±17.78	147.96^{b}±13.67	156.04^{b}±9.41	114.33^{a}±32.02	109.71^{a}±26.69
Total sterols	3626.03^{c}±386.59	3203.53^{b}±345.55	3230.13^{b}±266.89	3139.03^{b}±275.49	2700.46^{a}±409.68

Different superscript lowercase letters in the same line indicate significant differences ($p < 0.05$) between different varieties

The widespread occurrence of Δ-7-stigmastenol, Δ-5-avenasterol and Δ-7-avenasterol in safflower oil was hitherto reported by Itoh et al. (1973).

The stigmasterol and sitosterol contents of all safflower oils analyzed within the present study were higher than the results obtained by Ortega-García et al. (2006) who demonstrated that stigmasterol content of a high oleic safflower oil produced in Mexico was found as 112.89 mg/kg, while sitosterol contents was 1083.82 mg/kg.

The amount of Δ-7 sterols (Δ-7-campesterol, Δ-7-stigmastenol and Δ-7-avenasterol) ranged from 715.05 (Olas) to 1114.62 (Dinçer) mg/kg, compatible with the study of Itoh et al. (1973) who reported that the safflower oil belongs to the family of Compositae oils, whose characteristic feature was to contain high amounts of Δ-7 sterols. Δ-7-stigmastenol was reported to be a suitable identifier sterol to determine the botanical origin of safflower oil (Aparicio and Aparicio-Ruíz, 2000).

Multivariate analysis

Principal component analysis (PCA) is a multivariate method that lowers the number of variables into small number of factors with maximum variation. The analysis is commonly utilized for classification issues. In this study, PCA was performed to examine the similarities of safflower oils from various cultivars. The first PCA was carried out with fatty acid and individual sterols of safflower oils. The first (F1), second (F2) and third (F3) principal components had eigen values of 9.79, 4.10 and 1.98 and accounted for 40.79, 17.09 and 8.26 of the variance, in the same order. F1 showed positive correlations with campesterol, Δ-7-campesterol, β-sitosterol, Δ-5-24-stigmastadienol, Δ-7-stigmastenol, Δ-7-avenasterol and total sterols. Factor score plot is given in Figure 1. A second PCA was carried out with only fatty acids. The first (F1), second (F2) and third (F3) principal components had eigen values of 4.92, 1.59 and 1.18 and accounted for 49.22, 15.98 and 11.79 of the variance, respectively. F1 had positive scores on C16:0 and C 18:2; negative on C18:1. Factor score plot is shown in Figure 2. The third PCA was applied to only sterols. The first (F1), second (F2) and third (F3) principal components had eigen values of 8.23, 1.51 and 1.13 and accounted for 58.80, 10.84 and 8.12 of the variance. All three PCAs revealed that Olas and Remzibey-05; Balcı, Linas and Dinçer were clustered together.

Hierarchical cluster analysis (HCA) is an unsupervised technique utilized to cluster data. HCA was applied to cluster oils according to varieties based on sterol and fatty acid, only sterols and only fatty acids. Clearer clusters were obtained using fatty acid data. A dendrogram obtained from HCA is given in Figure 3, in which three main groups are observed. The first group consisted of Dinçer, Linas, Olas and Balcı; the second group was formed by Olas and the third cluster included Remzibey-05 variety. HCA results indicated the similarity between Balcı, Linas and Dinçer as in PCA.

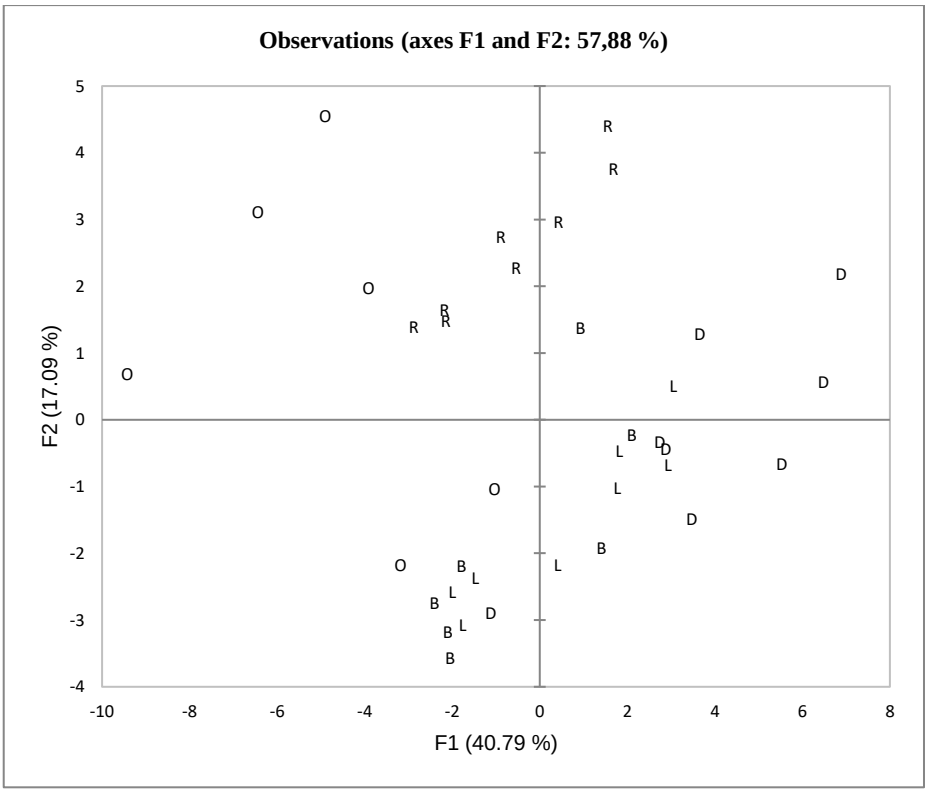

Fig. 1. Factor score plot of Turkish safflower oils obtained from PCA of fatty acids and sterols (D: Dinçer, L: Linas, O: Olas, R: Remzibey-05, B: Balcı)

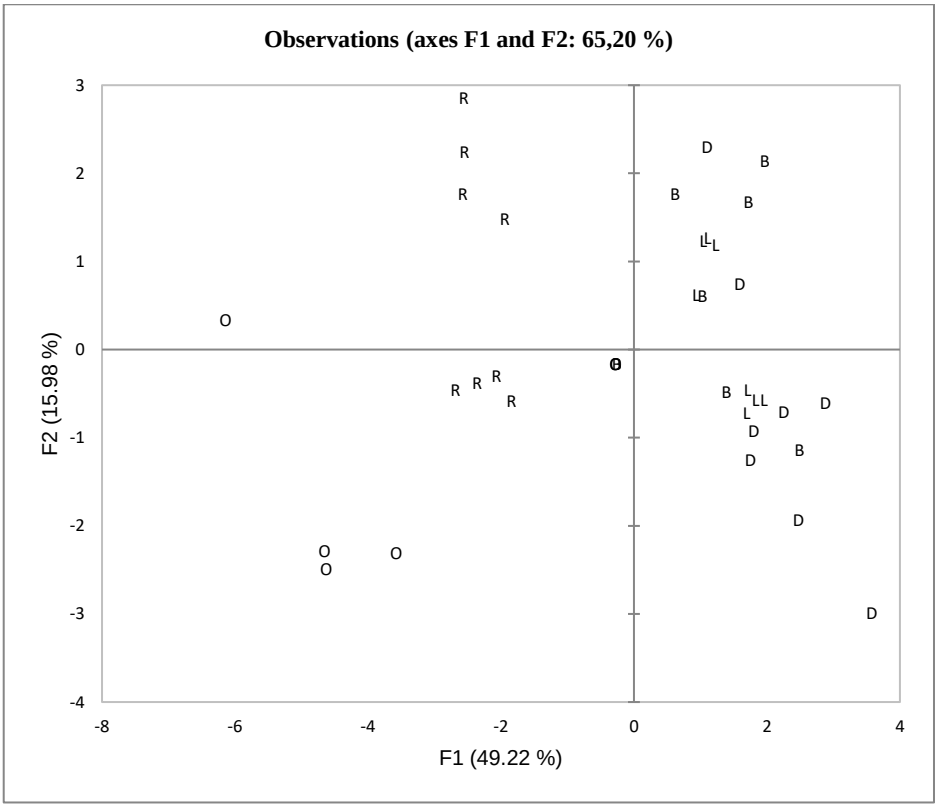

Fig. 2. Factor score plot of Turkish safflower oils obtained from PCA of fatty acids on the plane (D: Dinçer, L: Linas, O: Olas, R: Remzibey-05, B: Balcı).

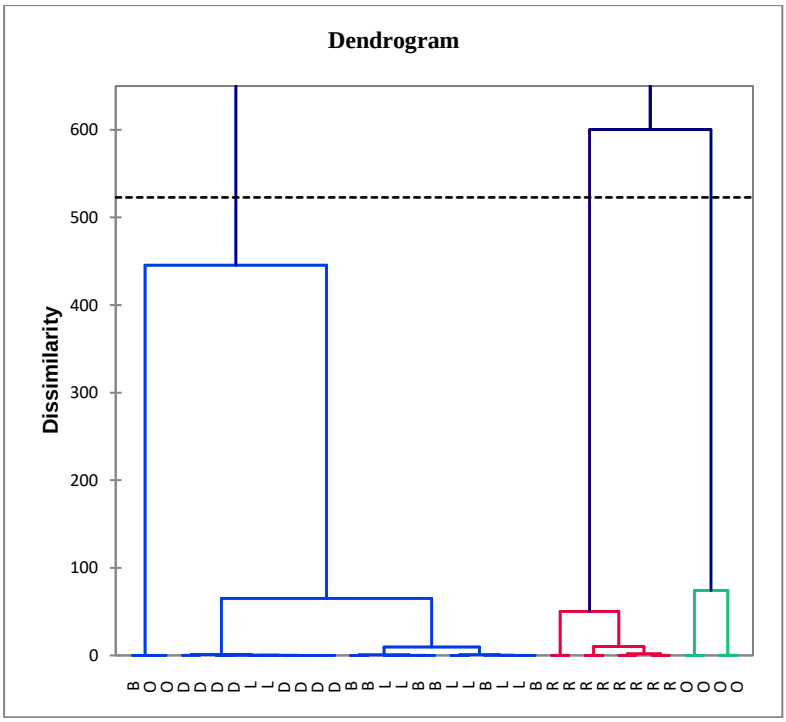

Fig. 3. Hierarchical cluster analysis dendrogram of Turkish safflower oils from various cultivars obtained using fatty acids (D: Dinçer, L: Linas, O: Olas, R: Remzibey-05, B: Balcı)

Conclusion

The present study covers the basics of the varietal differences between safflower oils, belonging to different safflower cultivars of Turkish origin, according to their fatty acid and sterol profiles. The results show that Remzibey-05 and Olas varieties are different from each other due to their oleic acid ratios. However the sterol composition of different varieties were generally similar to each other. The data reported herein may be useful for the orientation of cultivation of oleic-rich safflower cultivars and also for the forthcoming regulatory studies about safflower oils, since compositional similarity of safflower and sunflower oils is an important concern in adulteration issues. Since safflower is an underresearched oily crop, the results may also encourage future researches in order to develop safflower cultivars with desired agronomic performances.

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