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INVESTIGATION OF THERMAL OXIDATION OF COLD PRESSED POPPY (*Papaver somniferum* L.) SEED OIL WITH SOME EDIBLE OILS

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ABSTRACT
The thermal oxidative stability of poppy seed oil (PSO) and refined corn oil (CO), hazelnut oil (HO), walnut oil (WO) were examined. The samples were heated 12 times at 180°C for 5 minutes. The change in total polar matter amount (TPM) and antioxidative capacity level (DPPH), along with some parameters indicating the degree of oxidation, were examined during the study. A significant increase was observed in free fatty acid (FFA), peroxide value (PV), p-Anisidine value (p-AV), conjugated diene (CD), conjugated triene (CT) and TPM of the samples with the applied heating processes (P<0.05). However, antioxidative capacity levels decreased significantly (P<0.05). Generally, according to initial values, changes in p-AV (66.89), TPM (16.17%) and antioxidative capacity level (46.08%) were observed to be lower in PSO than in other oils. This may be related to the phenolics and tocopherol content. The study was concluded that poppy seed oil could be alternative edible oil.
Keywords: Vegetable oils, antioxidative capacity, hazelnut, walnut

SOĞUK PRESLENMİŞ HAŞHAŞ (*Papaver somniferum* L.) TOHUMU YAĞININ BAZI TİCARİ YAĞLARLA BİRLİKTE TERMAL OKSİDASYONUNUN ARAŞTIRILMASI

ÖZ
Bu çalışmada, haşhaş tohumu yağı (PSO), rafine mısır yağı (CO), fındık yağı (HO) ve ceviz yağı (WO)'nın termal oksidatif stabilitesi incelendi. Yağ örnekleri 180°C'de, 5 dakika boyunca, 12 kez ısıtıldı. Çalışma sırasında, toplam polar madde miktarı (TPM) ve antioksidan aktivite kapasitesindeki (DPPH) değişim, oksidasyon derecesini gösteren diğer parametreler ile birlikte incelendi. Uygulanan ısıtma işlemleri ile birlikte yağ örneklerinin serbest yağ asidi içeriği (FFA), peroksit değeri (PV), p-Anisidin değeri (p-AV), TPM, konjuge dien (CD) ve konjuge trien (CT) değerlerinde önemli bir artış gözlendi (P<0.05). Bunun yanında, antioksidan aktivite kapasiteleri ise önemli oranda azaldı (P<0.05). Genellikle, başlangıç değerlerine göre, PSO örneklerinde p-AV (66.89), TPM (%16.17) ve antioksidan aktivite kapasitesinde (%46.08) ortaya çıkan değişim diğer yağlara göre daha düşüktü. Bu durum fenolik maddeler ve tokoferol içeriği ile ilişkili olabilir. Çalışma sonunda, soğuk preslenmiş haşhaş tohumu yağının alternatif yemelik bir yağ olabileceği sonucuna varılmıştır.
Anahtar kelimeler: Bitkisel yağlar, antioksidan kapasite, fındık, ceviz

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INTRODUCTION

The poppy (*Papaver somniferum* L.) plant, which belongs to the *Papaveraceae* family, is grown for its capsules and seeds. The plant is mostly cultivated worldwide in countries such as Türkiye, the Czech Republic and Spain (Dabrowski et al., 2020; Şengün et al., 2020). Although the main purpose of poppy cultivation is opium production, edible oil is obtained from its seeds of different colors such as yellow, white and blue (Ghafoor et al., 2019). Poppy seeds generally have an oil content ranging from 28% to 53%, depending on environmental factors (Dabrowski et al., 2020). The main purpose of the oil obtained is to be edible, but it is also widely used in the production of paint, varnish, and soap (Özbek and Ergönül, 2020). The oil consumed for edibles is obtained by cold pressing without refining (Bozan and Temelli, 2003). In this way, it preserves its characteristic taste, special aroma and intense color. Naturally, it is more preferred by consumers (Çakaloğlu et al., 2018). In addition, in terms of nutritional value, cold pressed oils are superior to refined oils (Ayyıldız et al., 2021). Poppy seed oil is very important in nutrition with its polyunsaturated fatty acid profile. Linoleic acid is the dominant fatty acid in its structure, and its ratio varies between 53-74%. This is followed by oleic acid (13-24%) and palmitic acid (8-19%). It contains less stearic acid and linolenic acid (1-2%) (Dabrowski et al., 2020).

Although vegetable oils are vital in human nutrition, they are foodstuffs that can easily undergo oxidation. The biggest factors in oxidation are oxygen, heat, light, enzymes and metals. Oils are especially exposed to high temperatures during the frying process. This causes many reactions, such as oxidation, conjugation and polymerization. The main factor in these reactions is unsaturated fatty acids (Çelebi et al., 2021). As a result of these reactions, compounds such as aldehydes and ketones are released, and the taste, smell and nutritional quality of the oils change. There is a close relationship between the fatty acid composition of vegetable oils and the number of double bonds in their chemical structures with oxidation. In addition, free fatty acids released as a result of hydrolysis are another factor that negatively affects sensory quality (Yanishlieva and Marinova, 2001).

In this study, the stability of poppy seed oil against oxidation at high temperatures was examined. The same temperature level was applied to refined corn, hazelnut and walnut oil. Other studies have focused mostly on the fatty acid composition and bioactive compounds of oils obtained from poppy seeds of different varieties. In short, investigation of poppy seed oil with different edible oil varieties is quite limited in the literature. This study aims to make poppy seed oil, which is consumed locally and in limited quantities, an alternative commercial option and to increase its consumption rate.

MATERIALS AND METHODS

Materials

Yellow poppy seeds were used in this study. Seeds harvested in July 2023 were obtained from a local farmer (Şuhut, Afyonkarahisar, Türkiye). Oil extraction was carried out by pressing method (Omabeta, Mirandalong-EU) without applying heat treatment and the oil temperature was 25°C. The oil obtained was filtered, and no refining process was used. Corn, hazelnut and walnut oil were obtained from the local market. The batch numbers of the oils were the same and they were all produced and packaged in 2023. The oils were refined and contained no additives. p-Anisidine and 2.2-diphenyl-1-picrylhydrazyl (DPPH) used in the analyses were obtained from Sigma (St. Louis, MO). Acetic acid (glacial), diethyl ether, ethanol (96% v/v), ethyl acetate, phenolphthalein, n-hexane, isooctane, chloroform, methanol, potassium hydroxide, potassium iodide and starch were obtained from Isolab (Germany).

Sample preparation and thermal oxidation of oils

The thermal oxidation method was selected based on similar studies in the literature. For this purpose, 2 liters of oil samples were filled into a fryer (Tefal, Uno M, Rumilly, France) and heated at 180°C for 5 minutes. After each heating, the sample was cooled to room temperature and the next heating was started after waiting for

approximately 1 hour. This process was repeated 12 times for each sample. Analyses were performed after every 4 heating periods. All heat treatments were carried out in triplicate (n=3).

Determination of fatty acids composition

For fatty acid composition, samples were converted to fatty acid methyl esters using the ISO 5509 (1978) method. After the esterification process, analysis was performed with an Agilent 6890N Gas Chromatograph (GC) equipped with a flame ionization detector (FID) and HP-88 column (i.d. = 0.25 mm, length = 60 m, film thickness = 0.2 μm, California, United States). The injector and FID temperatures were set at 270°C and 290°C, respectively. The oven temperature was kept at 165°C for 30 minutes, then increased by 10°C per minute and held at 190°C for 20 minutes. Helium (16.4 psi) was used as the carrier gas. Injection volume, split flow and split ratio are 0.2 μL, 0.4 mL/min, and 1/70, respectively. At the end of the analysis, fatty acid ratios were determined as percentages.

Determination of free fatty acid content (FFA) and peroxide value (PV)

FFA content (%) and PV value (meq O_2/kg oil) of oil samples were determined using IUPAC (1987) and AOCS Cd8b-90 (1997) methods, respectively. All analyses were performed in triplicate (n=3).

Determination of p-Anisidine value (p-AV)

AOCS Cd18-90 (1998) method was used for the p-Anisidine value (p-AV), which provides information about the second oxidation products (aldehydes and ketones) of peroxides in oils. This method dissolved 0.1-0.4 g of oil sample in 10 mL isooctane. The absorbance value of the resulting solution was measured at 350 nm against isooctane. Then, 5 mL of the oil solution was taken and mixed with 1 mL of p-Anisidine solution (2.5 g/L glacial acetic acid). In addition, 5 mL isooctane and 1 mL p-Anisidine solution were mixed in another test tube. After approximately 10 minutes, the absorbance value of the prepared solution was measured at 350 nm against the solution containing isooctane. The p-Anisidine value of the oil samples was calculated using the following formula (Eq. 1). All analyses were performed in triplicate (n=3).

$$p\text{-}AV = 10x[(1.2x(A_2\text{-}A_1))/w] \quad \text{(Eq. 1)}$$

In the formula, w, A_1 and A_2 represent the amount of oil weighed, the absorbance value of the oil solution and the absorbance value of the mixture of oil and p-Anisidine solution, respectively.

Determination of conjugated diene (CD) and conjugated triene (CT) values

The IUPAC (1987) method was used for conjugated diene (CD) and conjugated triene (CT) values in oil samples. Accordingly, 0.1 g of the sample was weighed and dissolved in 100 mL isooctane. Then, absorbance values for CD and CT were measured with a spectrophotometer (Shimadzu UV-1240, Japan) at wavelengths of 232 nm and 268 nm, respectively. CD and CT values of oil samples were calculated using the following formula (Eq. 2). All analyses were performed in triplicate (n=3).

$$E\ \% = A_A/(cLxl) \quad \text{(Eq. 2)}$$

In the formula, E, A_A, cL and l represent the extinction value, the absorbance value of the sample, the concentration of the oil solution (g/100 mL) and the path length in the 1.00 cm quartz cuvette, respectively.

Determination of total polar matter (TPM)

The total polar matter measuring device Testo 270 (Testo) was used to determine the amount of TPM in oil samples. This device detects total polar matter as a function of electrical conductivity in relation to temperature. Since the amount of TPM varies depending on temperature, the measurement was made within a certain temperature range (40-45°C). In the study, the device was calibrated with calibration oil before the measurement process. All analyses were performed in triplicate (n=3).

Antioxidant capacity assay

In the study, DPPH (2.2-diphenyl-1-picrylhydrazyl) free radical scavenging method was used to determine the amount of antioxidant

capacity (Brand-Williams et al., 1995). Accordingly, 200 µL of oil sample and 3 mL of 0.051 mM DPPH solution were mixed and then incubated at room temperature for 30 minutes. Afterward, the change in absorbance values was measured at 517 nm wavelength. Antioxidant capacities of oil samples were calculated using the following formula (Eq. 3). All analyses were performed in triplicate (n=3).

DPPH scavenging activity (%) = $[(A_0-A_1)/A_0]x100$ (Eq. 3)

In the formula, A_0 and A_1 represent the absorbance values of the blank sample and the oil sample, respectively.

Statistical analysis

Analyses were performed in triplicate and results were expressed as mean ± standard deviation (SD). SPSS (version 22, SPSS Inc., USA) program was used to analyze the results. The obtained data were examined using a one-way analysis of variance. Duncan test was used to determine the significance of differences between mean values (significance level of 0.05).

RESULT AND DISCUSSION

Fatty acids composition of samples

Generally, vegetable oils have a high rate of unsaturated fatty acids. This accelerates the oxidation that occurs with heat treatment. The composition rates of fatty acids in oil samples without thermal oxidation are given in Table 1. Total saturated fatty acid (SFA) was determined mostly in CO and PSO samples, 13.9% and 11.4%, respectively. Among these, palmitic and stearic acids were the dominant fatty acids. PSO and WO samples contained the most total polyunsaturated fatty acids (PUFA), 74.1% and 62.7%, respectively. The dominant fatty acid in these oil samples was linoleic acid. Rahimi et al. (2011) examined the fatty acid content of 18 types of poppy seed oil. The dominant fatty acid in the samples was linoleic acid (72.55%). In addition, oleic and palmitic acid rates were between 13.30-17.80% and 7.96-10.19%, respectively. At the end of the study, they reported that poppy seed oil can be used as cooking oil. Similarly, Dabrowski et al. (2020) also obtained similar fatty acid rates in their study.

Table 1. The major fatty acid compositions of oil samples (%).

	PSO	CO	HO	WO
Palmitic (C16:0)	9.06	10.90	5.59	6.40
Palmitoleic (C16:1)	0.15	0.16	0.23	0.12
Stearic (C18:0)	2.05	2.03	2.55	2.48
Oleic (C18:1)	13.70	30.70	75.70	26.20
Linoleic (C18:2)	74.00	53.80	14.60	53.70
γ Linolenic (C18:3)	0.05	0.05	0.05	4.45
α Linolenic (C18:3)	0.05	0.05	0.05	4.05
Arachidic (C20:0)	0.12	0.41	0.21	0.05
Eicosenoic (C20:1)	0.59	0.34	0.05	0.88
Heneikosanoic (C21:0)	0.07	0.05	0.23	0.69
SFA	11.40	13.90	8.78	9.84
MUFA	14.50	31.30	76.10	27.40
PUFA	74.10	53.90	14.70	62.70

PSO: Poppy Seed Oil, CO: Corn Oil, HO: Hazelnut Oil, WO: Walnut Oil

Free acidity content of samples

FFA represents the amount of unesterified fatty acids released as a result of the hydrolysis of triglycerides (Woo et al., 2018). Different compounds are formed as a result of oxidation from unesterified fatty acids. This creates undesirable changes in the sensory and nutritional values of the oils. Therefore, the amount of FFA should not exceed the limits specified in the standards. This parameter is a suitable indicator for investigating the rancidity rate in oils (Iqbal and Bhanger, 2007). The study determined a significant increase in the FFA amount of all oil samples depending on the heating period (P<0.05). The resulting increase is given in Table 2. After heat treatments PSO, CO, HO and WO samples of changes were measured as 0.42%, 0.07%, 0.26% and 0.1%, respectively. CO and WO exhibited the best stability against the increase in acidity caused by thermal oxidation. The low increase in acidity may be due to the refining process applied. Similar to this study, Woo et al. (2018) applied thermal oxidation to corn, soybean and palm oil at 150℃ for different periods of time. FFA values of oil samples increased at different rates with heat treatments.

Peroxide values of samples

PV indicates the amount of peroxide and hydroperoxide resulting from the oxidation of oils. Therefore, it is an important quality criterion showing the oxidation degree (Zhang et al., 2010). The PV change in oil samples is given in Table 2. Generally, it was seen that the number of heating cycles had a significant effect on the regular increase in PV (P<0.05). However, a slightly irregular increase was determined in CO and WO samples. This may be due to the decomposition of hydroperoxides at high temperatures. Hydroperoxides formed in the first stage of oxidation are unstable products. As the reaction progresses, they transform into secondary products such as volatile carbonyl compounds and non-volatile dimers, trimers or polymers (Solak et al., 2018). At the end of the study, the lowest changes were measured in CO and HO samples, 11.42 and 11.74 meq O_2/kg oil, respectively. The reason for the low peroxide change in these samples may be due to their lower PUFA contents. The change in PV was similar to the findings of Solak et al. (2018) in their study on hazelnut oil.

p-Anisidine values of samples

p-AV is considered the index of aldehyde compounds occurring in oils. It is an important parameter indicating the degree of oxidation in research (Kalantzakis and Blekas, 2006). This value is calculated using the absorbance of the color formed as a result of the reaction of aldehydes and ketones, which are the products of hydroperoxides as a result of oxidation (Çelebi et al., 2021). The changes in oil samples due to heat treatments are given in Table 2. According to the results of the analysis, it was seen that the number of heating cycles had a significant effect on the increase in the p-AV value (P<0.05). At the end of the study, WO was most affected by heat treatments, while HO and PSO exhibited the best oxidative stability. These results were consistent with studies using different oils. Wang et al. (2019) applied heat treatments to soybeans, peanuts, perilla and olive oil at 150, 180 and 210℃ for 3 days. The study reported that p-AV values increased depending on time at all temperatures, and the most significant change was in perilla oil.

Conjugated diene and conjugated triene values of samples

CD and CT values are parameters expressing the formation of primary and secondary oxidation products in oils. The structures of these products are unstable and lead to the formation of compounds such as aldehydes and ketones in the later stages of oxidation. This situation causes rancidity and a decrease of sensory quality in oils. The amount of conjugated fatty acids resulting from the displacement of double bonds increases with heat treatment. This also increases the sensitivity of oils to oxidation (Bhattacharya et al., 2008). The study showed a significant increase in the CD and CT values of the oil samples with the effect of heat treatments (P<0.05). When Table 3 was examined, PSO and WO samples exhibited the highest changes during the heating cycles. However, the lowest changes were determined in HO samples. Zhang et al. (2022) applied thermal oxidation to linseed oil at 180℃ for 2 and 4 hours.

CD and CT values increased in oil samples with heat treatments. They reported that the rate of thermal oxidation increase of the samples was related to the applied temperature level and the oil-oxygen contact area.

Table 2. Free fatty acid (FFA), peroxide value (PV) and p-Anisidine value (p-AV) of oil samples.

	Samples	Number of heating cycles			
		0	4	8	12
FFA (%)	PSO	4.16±0.01[a]	4.28±0.01[b]	4.48±0.01[c]	4.58±0.01[d]
	CO	0.11±0.00[a]	0.12±0.01[b]	0.16±0.00[c]	0.18±0.00[d]
	HO	0.22±0.00[a]	0.26±0.01[b]	0.35±0.00[c]	0.48±0.00[d]
	WO	0.18±0.00[a]	0.22±0.01[b]	0.25±0.00[c]	0.28±0.00[d]
PV (meq O_2/kg)	PSO	2.63±0.31[a]	4.96±0.04[b]	11.76±0.25[c]	16.59±0.45[d]
	CO	6.61±0.29[a]	13.58±0.04[c]	12.08±0.27[b]	18.03±0.17[d]
	HO	3.97±0.01[a]	12.35±0.61[b]	14.36±0.06[c]	15.71±0.14[d]
	WO	6.93±0.05[a]	9.19±0.04[c]	8.45±0.04[b]	20.56±0.30[d]
p-AV	PSO	0.00±0.31[a]	24.64±0.12[b]	58.18±0.25[c]	66.89±0.57[d]
	CO	10.83±0.09[a]	43.52±0.62[b]	76.71±1.21[c]	96.18±1.55[d]
	HO	1.47±0.07[a]	24.54±0.22[b]	39.69±0.25[c]	52.15±0.30[d]
	WO	1.89±0.10[a]	56.01±1.73[b]	87.69±1.46[c]	104.47±1.43[d]

Values followed by different lowercase (a–d) letters show significant difference for each row (P<0.05).
PSO: Poppy Seed Oil, CO: Corn Oil, HO: Hazelnut Oil, WO: Walnut Oil

Table 3. Conjugated diene (CD) and conjugated triene (CT) values of oil samples.

	Samples	Number of heating cycles			
		0	4	8	12
CD (%)	PSO	2.08±0.17[a]	4.32±0.07[b]	10.72±0.21[c]	15.92±0.06[d]
	CO	3.37±0.05[a]	6.23±0.27[b]	9.00±0.05[c]	10.96±1.39[d]
	HO	6.00±0.10[a]	5.87±0.16[a]	9.05±0.64[b]	12.78±0.17[c]
	WO	19.60±0.54[a]	24.68±0.39[b]	27.60±0.06[c]	29.23±0.76[d]
CT (%)	PSO	0.24±0.01[a]	1.06±0.02[b]	2.35±0.02[c]	3.18±0.03[d]
	CO	1.92±0.08[a]	3.01±0.02[b]	4.09±0.00[c]	4.61±0.37[d]
	HO	1.08±0.01[a]	1.74±0.00[b]	2.22±0.01[c]	2.67±0.00[d]
	WO	1.84±0.00[a]	4.20±0.07[b]	5.26±0.03[c]	6.01±0.12[d]

Values followed by different lowercase (a–d) letters show significant difference for each row (P<0.05).
PSO: Poppy Seed Oil, CO: Corn Oil, HO: Hazelnut Oil, WO: Walnut Oil

The amount of total polar matter in the samples

The amount of TPM indicates the degradation of oils caused by thermal oxidation. Polar matters contain non-triglyceride components. TPM is a parameter related to the primary and secondary oxidation products formed in oils with heat treatment. It increases with the period and degree of heat treatment applied. The amount of TPM for frying oils is limited to 25% (Ceylan and Basturk, 2022). When Figure 1 was examined, it could be seen that the TPM amounts increased significantly with the effect of heating cycles (P<0.05). In the fourth heating cycle, the TPM amounts of all samples were measured at an acceptable level. But, at the end of the study, PSO, HO and WO samples exceeded this level. In a similar study, frying with palm olein oil was applied 12 times at 180°C. It was reported that the TPM amounts of all samples increased

significantly depending on the number of heat treatments (Ceylan and Basturk, 2022).

Antioxidant activity capacity of samples

The DPPH free radical scavenging method is frequently used when examining the radical scavenging effects of foods because it is simple and fast. In this method, the change in color of the DPPH radical due to its reduction by the antioxidant substance is measured spectrophotometrically (Magalhaes et al. 2008; Albayrak et al. 2010). The antioxidative capacity change of oil samples is given in Figure 1. There was a significant decrease in all samples with the effect of thermal oxidation ($P<0.05$). Especially in the fourth heating cycle, the decrease in HO and WO samples was more evident. CO (29.11%) and PSO (46.08%) samples exhibited the lowest changes when the heating cycles were completed. Freitas et al. (2017) who investigated the effect of thermal oxidation on antioxidative capacity, applied heat treatment to soybean oil at 180°C for 20 hours. They reported that the antioxidative capacities of oil samples decreased significantly depending on the heat treatment time.

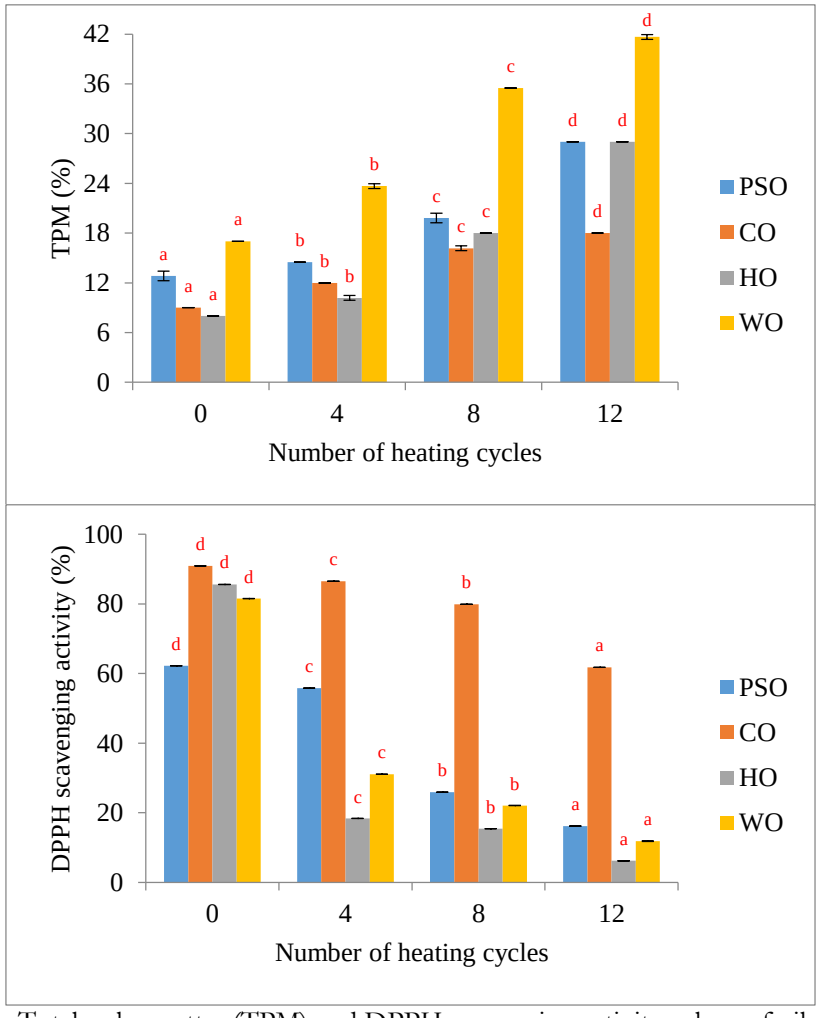

Figure 1. Total polar matter (TPM) and DPPH scavenging activity values of oil samples.
Values followed by different lowercase (a–d) letters show significant difference between heating cycles ($P<0.05$).
PSO: Poppy Seed Oil, CO: Corn Oil, HO: Hazelnut Oil, WO: Walnut Oil

CONCLUSION

There are many studies on the thermal oxidative stability of different types of oils in the literature. However, the stability of poppy seed oil was investigated with other oils for the first time in this study. During heating cycles, poppy seed oil exhibited high free fatty acid, hydroperoxide amount and conjugation. This may be because poppy seed oil was unrefined and had unsaturated fatty acid content. On the other hand, according to initial values, changes in p-AV (66.89), TPM (16.17%) and antioxidative capacity level (46.08%) were observed to be lower than other oils. This may be due to the fact that poppy seeds and oil contain some phenolic compounds and tocopherols (α, β, γ, δ) (Ghafoor et al., 2019). This study showed that unrefined poppy seed oil could be an alternative to commercial edible oils.

Nowadays, interest in natural and unprocessed foods is increasing. This situation is also valid for vegetable oil varieties. For example, the processing steps applied in the refining or hydrogenation process change the cis-trans structure of the oil and increase the amount of saturated fatty acids. Therefore, research should be conducted on oil varieties obtained from different seeds or fruits that are resistant to changing climatic conditions during the cultivation process and have high oxidative stability.

CONFLICTS OF INTEREST

The author has declared no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Kamil ÇELEBİ: Investigation, methodology and analysis, writing, original draft, review and editing, visualization, validation.

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