



# Improving the quality of vegetable oils treated with phytochemicals: a comparative study

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**Abstract** In this study, sunflower, corn, peanut and hazelnut oils were investigated according to their oxidative stability and antioxidant activity parameters. The related vegetable oils were treated with gallic acid, rutin and carotenoid. Olive leaf extract having a large variety of phytochemical was also valorized. After the leaf samples were extracted through a homogenizer, they were added into the vegetable oils, respectively. Moreover, synthetic antioxidants were also dissolved into the oils for control reasons. Stability of the vegetable oils against the oxidation was evaluated via Rancimat by measuring induction time. The quality parameters of treated and untreated oil samples were compared depending on phenolic and carotenoid contents, antioxidant activity and induction time.

**Keywords** Fats and oils · Functional food · Oxidative stability · Natural additives · Rancimat

## Introduction

Vegetable oil consumption has risen worldwide due to its health benefits over the other types of edible oils. However, these fat containing food products have a tendency to be oxidized by temperature, light and oxygen resulting in unpleasantness and formation of toxic materials (Frega et al. 1999; Kochhar and Henry 2009; Şahin et al. 2017). Therefore, the quality of the oil must be increased in order to meet the growing needs of consumers. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and TBHQ (tertbutyl hydroquinone) are the most used synthetic antioxidants even though there are doubts about their health effects. Natural additives such as biophenols, carotenoids, tocopherols and plant extracts have been attractive for prolonging the shelf-life of fat-containing foods due to the oxidation problems of fats (Bodoira et al. 2017). Additionally, this treated products with high-added value compounds might be approved as functional foods due to the increase in their quality and nutritional value (Koprivnjak et al. 2008).

This study gives the findings on stabilisation of the most consumed and the least investigated (according to their stability properties) vegetable oils having relatively short shelf-life.

When the studies on this issue are examined, it is seen that most of the studies have focused on individual phytochemicals such as tocopherols, carotenoids and ascorbic acid in addition to on plant extracts including diverse phytochemicals such as polyphenols, phenolic acids and flavonoids (Yanishlieva and Marinova 2001). Therefore, a phenolic acid (gallic acid), a flavonoid (rutin), a lipophilic compound ( $\beta$ -carotene) and a plant extract containing various phytochemicals have been used as antioxidant agent in this study. Olive leaf has been selected for both

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economical and ecological reasons since it is a biomass of a waste derived from olive and olive oil industry. Olive leaf extract has been valorized as natural food additive due to its phytochemical content which shows activity against oxidation, microbial effects with antiinflammatory, anti-cancer, antiatherogenic, hypoglycemic properties (Visioli and Galli 2002; Cicerale et al. 2008, 2010).

The present work focuses on the three main points concerning the improvement of vegetable oil quality, which are stabilisation of the oils with individual phytochemicals, interaction of antioxidants with synergists and stabilisation of the oils with the extract derived from olive leaf.

## Materials and methods

### Materials

Vegetable oil samples were purchased from a local market. Fully expanded olive leaf samples were collected from *Gemlik* type cultivated in Northwestern Turkey (November). Ethanol (> 99.5%), methanol (> 99.8%) and hexane (> 99%) were from Merck (Darmstadt, Germany), while ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), sodium carbonate, Folin-Ciocalteu, gallic acid, oleuropein, rutin, verbascoside, BHA, BHT and TBHQ were from Sigma-Aldrich (St. Louis, MO, USA).

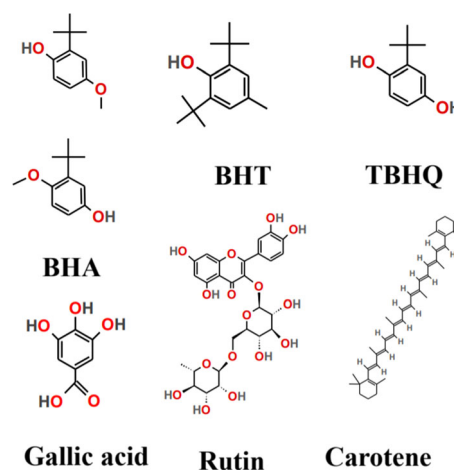
### Olive leaf extraction

Certain amount of olive leaf was extracted of 50% ethanol (v/v) by means of a homogeniser (IKA T25-ULTRA-TURRAX) under 10,000 rpm for 60 s. The extraction was repeated three times. After the heterogeneous extracts were gathered, a centrifuge (Nüve, CN 180) was used to separate the mixture under 5000 rpm for 25 min.

### Treatment of vegetable oils with additives

Considering the restrictions for the level of food additives, selected vegetable oils were treated with 200 mg additive (Fig. 1) per gram of oil (Taghvaei and Jafari 2015). Regarding effects such as toxicity, the related level of additives was also confident (De Leonardis et al. 2007).

The mixture consisted of equal amounts of rutin, carotene and gallic acid was also applied into the selected vegetable oils. The phytochemicals and synthetic antioxidants were blended with the oils under 7000 rpm for 115 s.



**Fig. 1** Molecular structures of synthetic and natural additives used in several vegetable oils

### Extraction of hydrophilic ingredients from the vegetable oils

First, oil samples were treated by hexane. Then, methanol was used as solvent to extract the polar phytochemicals at 7000 rpm for 60 s. Two replications were performed twice. After gathering the liquid extracts, the mixture was washed with hexane. Before maintaining the samples in dark at  $-20\text{ }^{\circ}\text{C}$ , a  $0.45\text{ }\mu\text{m}$  syringe filter was used.

### Spectrophotometric analyzes

The procedure developed by Malik and Bradford (2006) was exploited for the quantification of total biophenol (*TB*) content of the samples (Malik and Bradford 2006). UV-spectrophotometry (PG Instruments, T60/Leicestershire, England) was used as expressed by Şahin and Samli (2013). For the antioxidant activity (AA) of the oil samples, ABTS assay was followed at 734 nm (Re et al. 1999). Scavenging activity of the samples against the ABTS radical was stated as written below:

$$\text{AA (inhibition \%)} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100 \quad (1)$$

$A_{\text{control}}$  = Absorbance of control,  $A_{\text{sample}}$  = Absorbance of sample.

The procedure of Chuang and Brunner was applied for the determination of total carotenoid (*TC*) of the oils at 450 nm (Chuang and Brunner 2006).

### Chromatographic analysis

Analyses of individual phytochemicals were carried out through an HPLC equipment as reported previously (Şahin et al. 2017).

## Rancimat method

As an accelerated method, Rancimat test was used for the measurement of the oils' stability against oxidation. The equipment (Rancimat 892, Metrohm) was set to 140 °C by providing the conditions under 20 L of air per hour to 3 g of vegetable oil (Kurtulbaş et al. 2018).

Induction time (*IT*) is utilized as an index for oxidative stability of the fat-containing foods. Stabilization factor (*F*) was also exploited to evaluate the differences of the effects of applied antioxidants in each oil:

$$F = \frac{IT_{TREATED}}{IT_{UNTREATED}} \quad (2)$$

*IT*<sub>TREATED</sub> Induction time of treated oil with antioxidant,  
*IT*<sub>UNTREATED</sub> Induction time of untreated oil.

## Diffuse reflectance Fourier transform spectroscopy (DRIFT)

The chemical characterization of the oils were made using a Bruker Alpha-T DRIFT spectrometer with a 528/D model through OPUS 6.5 software (Bruker Optics Inc.).

## Statistical analysis

Analysis of variance (ANOVA) statistical test was utilized through Tukey's test of InStat<sup>®</sup> software (GraphPad, San Diego, CA, USA) to analyse the average outputs of three replicates. As an indicator for the correlation between AA, TB and IT, Pearson correlation coefficient (*R*<sup>2</sup>) was also calculated via InStat<sup>®</sup> software.

## Results and discussions

### Olive leaf composition

Table 1 shows the total and individual phenolic profile, and AA of the olive leaf extract. Oleuropein is strikingly the most prominent component in the leaf. It is also known to be the reason for many beneficial effects of the olive leaf. Moreover, the leaf showed a remarkable antioxidant activity.

**Table 1** Phenolic profile of olive leaf extract

Oleuropein (mg/g DS)	Rutin (mg/g DS)	Verbascoside (mg/g DS)	TB (mg GAE/g DS)	AA (%inhibition)
325.81 ± 0.00	43.27 ± 0.00	17.19 ± 0.00	61.576 ± 0.03	91.12 ± 0.00

± indicates the standart deviation of the mean (n = 3)

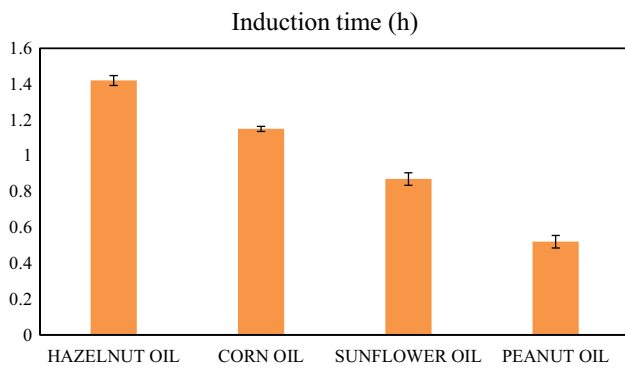
## Stability of the vegetable oils against oxidation

Figure 2 indicates the comparison of each vegetable oil as a function of induction time. *IT* is a kind of description of the oils's resistance to oxidation reaction. Hazelnut oil was found to be the most stable oil followed by corn, sunflower and peanut oils according to the findings of accelerated conditions such as 140 °C for less than one day. It would be much longer if the samples kept at 25 °C (Franco et al. 2016).

## Comparison of the additives in various vegetable oils regarding oxidative stability

Table 2 shows that natural additives surpassed the synthetic ones to enhance the oxidative stability. Actually, BHA and BHT were generally insufficient to improve the oxidative stability of the selected oils. This might be caused by the thermal stability of the relevant synthetic antioxidant. Heat resistance of commercial synthetic antioxidants was shown to be in TBHQ > BHA > BHT order (Santos et al. 2012). Therefore, our result is consistent with this report, where TBHQ was generally sufficient to increase the stability of all oil types.

As seen in Table 2, gallic acid showed the best performance to increase the stability of the sunflower oil (twice). This finding was followed by both TBHQ and rutin, showing statistically the same results (*p* > 0.05). Concerning β-carotene, the stability of the enriched oil was increased by almost 24% comparing to pure one. It was followed by the mixture of phytochemicals and the olive leaf extract, which were found statistically similar at *p* > 0.05. Regarding corn oil, rutin showed the greatest effect by increasing the induction time almost 2.5 times over the untreated sample. The findings of rutin and TBHQ were statistically the same (*p* > 0.05) to improve the quality of the corn oil as a funtion of stabilisation factor. β-carotene, the mixture and the extract had similar effect of less than 10% in increasing the quality with respect to oxidative stability. Oxidative stability of the peanut oil against the oxidation changed remarkably (4 times over that of pure oil) when gallic acid added into the oil. The mixture of gallic acid, rutin and carotene were also very good at favouring the oxidative stability by showing 3 times better performance comparing to the untreated



**Fig. 2** Rancimat analysis of the untreated vegetable oils at 140 °C

peanut oil. TBHQ, plant extract and carotene demonstrated statistically the same outcomes ( $p > 0.05$ ) by more than 60% increase in induction time. With respect to the effects of several additives on the stabilization factor of the hazelnut oil (Table 2), the synergism between individual phytochemicals demonstrated the strongest influence to increase the resistance of oil to oxidation with three times over the pure hazelnut oil. The effect of gallic acid was also similar at  $p > 0.05$ , followed by TBHQ and the remaining additives, which had statistically the same effect ( $p > 0.05$ ).

**Comparison of the additives in various vegetable oils regarding other quality parameters**

Table 3 summarizes the variations in the parameters of the selected vegetable oils enriched by several natural and synthetic phytochemicals.

Even though corn oil had the greatest total phenols and antioxidant capacity (Table 3), the highest induction time was achieved by hazelnut oil. Because, oxidative stability of the oil is correlated not only with total biophenols, but also with the presence of selected major compounds or

other substances such as vitamins, minerals, acids, sugars and their synergistic effects. Actually, Folin assay generally identifies the total molecules with aromatic rings in the sample (Corrales et al. 2008).

To explore the the relationships between antioxidant activity and total biophenols, and oxidative stability and AA/TB in the oils, correlation must be taken into account. Except for the hazelnut oil ( $R^2 = 0.0054$ ), relatively strong correlation between TB and AA of the vegetable oils ( $R^2 = 0.5780$  for sunflower; 0.7959 for peanut and 0.8716 for corn) indicates that mostly phenolic compounds subscribe to the antioxidant capacity of the vegetable oils.

When we consider the sunflower oil, the greatest increase was achieved by gallic acid for all parameters.

Statistical analysis showed that each additive changed the quality of the sunflower oil differently (at  $p < 0.001$ ). Generally, there was a relatively poor correlation between the TB/AA and IT ( $R^2 = 0.3395$  and 0.1051).

Regarding corn oil, rutin showed the best performance for all parameters. Additionally, the relationships between the phenolics/antioxidant activity and oxidative stability were stronger comparing to others ( $R^2 = 0.5813$  and 0.6665).

Concerning peanut oil, gallic acid surpassed the other additives to favour the quality parameters of the oil. The relationships between the antioxidant activity and oxidative stability, and phenolics and oxidative stability were relatively low ( $R^2 = 0.1140$  and 0.3384).

Although the highest increase in the hazelnut oil was attained by gallic acid, the mixture of individual phytochemicals showed the best efficiency to enhance the oxidative stability of the oil. This phenomenon was also verified by the weakest relationship between the related parameters. The values of the correlation parameter were found under 0.25.

Accordingly, gallic acid surpassed the other phytochemicals and synthetic antioxidants with a remarkable

**Table 2** Comparative results of synthetic and natural additives in different vegetable oils regarding stabilization factor

Additive	Stabilization factor, F			
	Sunflower oil	Corn oil	Peanut oil	Hazelnut oil
Gallic acid	2.25 ± 0.092	1.65 ± 0.007a	3.89 ± 0.078	2.39 ± 0.141a
Rutin	1.46 ± 0.014a	2.40 ± 0.000	2.88 ± 0.028a	0.88 ± 0.014b
β-carotene	1.18 ± 0.000ad	1.02 ± 0.000c	2.49 ± 0.007bd	1.08 ± 0.021b
Mixture	1.24 ± 0.000ae	1.08 ± 0.000cd	2.94 ± 0.000a	2.65 ± 0.000a
Olive leaf extract	1.22 ± 0.000abc	1.08 ± 0.000bc	2.32 ± 0.021b	1.00 ± 0.007b
BHA	1.08 ± 0.000bde	0.96 ± 0.057bd	1.56 ± 0.028c	1.03 ± 0.0141b
BHT	0.99 ± 0.000cd	1.01 ± 0.000c	1.63 ± 0.000c	1.09 ± 0.000b
TBHQ	1.73 ± 0.050a	1.53 ± 0.014a	2.61 ± 0.007d	1.86 ± 0.035

± indicates the standart deviation of the mean (n = 3). A different letter in columns shows statistically differences at  $p < 0.05$

**Table 3** Changes in qualitative parameters of different vegetable oils treated with several additives

Vegetable oil	Additive	TB (ppm)	TC (ppm)	AA (inhibition %)
Sunflower oil	Pure	8.00 ± 0.000	N.D	1.83 ± 0.000
	Gallic acid	66.00 ± 0.001	N.D	10.87 ± 0.004
	Rutin	37.00 ± 0.001	N.D	6.93 ± 0.001
	β-carotene	20.65 ± 0.000	83.95 ± 0.003	3.50 ± 0.002
	Mixture	29.00 ± 0.001	28.00 ± 0.001	4.29 ± 0.001
	Olive leaf extract	20.00 ± 0.000	6.10 ± 0.002	1.36 ± 0.001
	BHA	21.00 ± 0.002	N.D	7.60 ± 0.002
	BHT	13.00 ± 0.001	N.D	2.57 ± 0.001
	TBHQ	53.00 ± 0.001	N.D	10.30 ± 0.006
Corn oil	Pure	47.00 ± 0.000	N.D	6.12 ± 0.001
	Gallic acid	86.00 ± 0.000	N.D	29.19 ± 0.001
	Rutin	74.00 ± 0.001	N.D	21.14 ± 0.001
	β-carotene	51.75 ± 0.000	81.92 ± 0.004	12.85 ± 0.000
	Mixture	57.00 ± 0.001	25.00 ± 0.001	14.29 ± 0.001
	Olive leaf extract	55.00 ± 0.002	5.10 ± 0.003	10.29 ± 0.003
	BHA	52.00 ± 0.000	N.D	17.61 ± 0.013
	BHT	44.00 ± 0.001	N.D	8.29 ± 0.001
	TBHQ	82.00 ± 0.000	N.D	28.91 ± 0.013
Peanut oil	Pure	38.00 ± 0.000	N.D	5.30 ± 0.001
	Gallic acid	70.00 ± 0.001	N.D	18.39 ± 0.002
	Rutin	61.00 ± 0.001 a	N.D	16.20 ± 0.023
	β-carotene	49.75 ± 0.000	21.21 ± 0.001	10.39 ± 0.001
	Mixture	51.00 ± 0.001	6.09 ± 0.001	11.07 ± 0.001
	Olive leaf extract	52.00 ± 0.001	1.10 ± 0.001	7.60 ± 0.001
	BHA	42.00 ± 0.000	N.D	8.59 ± 0.015
	BHT	35.00 ± 0.001	N.D	6.50 ± 0.005
	TBHQ	61.00 ± 0.002 a	N.D	15.70 ± 0.002
Hazelnut oil	Pure	25.00 ± 0.000	N.D	0.72 ± 0.000
	Gallic acid	64.00 ± 0.000	N.D	29.47 ± 0.000
	Rutin	58.00 ± 0.000 a	N.D	5.57 ± 0.003
	β-carotene	20.45 ± 0.000	84.05 ± 0.003	5.01 ± 0.002
	Mixture	104.00 ± 0.001	30.01 ± 0.001	5.14 ± 0.001
	Olive leaf extract	27.00 ± 0.002	7.10 ± 0.001	2.69 ± 0.006
	BHA	45.00 ± 0.001	N.D	8.64 ± 0.001
	BHT	18.00 ± 0.000	N.D	1.86 ± 0.003
	TBHQ	58.00 ± 0.003 a	N.D	28.96 ± 0.001

± indicates the standard deviation of the mean (n = 3). A common letter in columns shows statistically the same data at  $p > 0.05$

N.D Not detected

difference. The same finding was also observed when incorporating diverse phytochemicals into olive oil (Artajo et al. 2006). The success of gallic acid is related to the 3,4,5-trihydroxy structure in its ring (Fig. 1), where a comparatively easier hydrogen dislocation can occur.

Potential synergism between the phytochemicals was also investigated depending on the output of the individual ingredients. However, gallic acid was not so successful when synergism with the other antioxidants was

considered. This outcome might be ascribed to the fact that higher concentrations of each ingredient are necessary to obtain a severe stabilisation as for induction time. On the other hand, the success of TBHQ also depends on its molecular structure having two para-hydroxyl groups.

This structure results in giving the hydrogens more rapidly for the inhibition of the oxidation. Previous studies also demonstrated parallel findings as that of ours (Ai-li and Chang-hai 2006; Zhang et al. 2010).

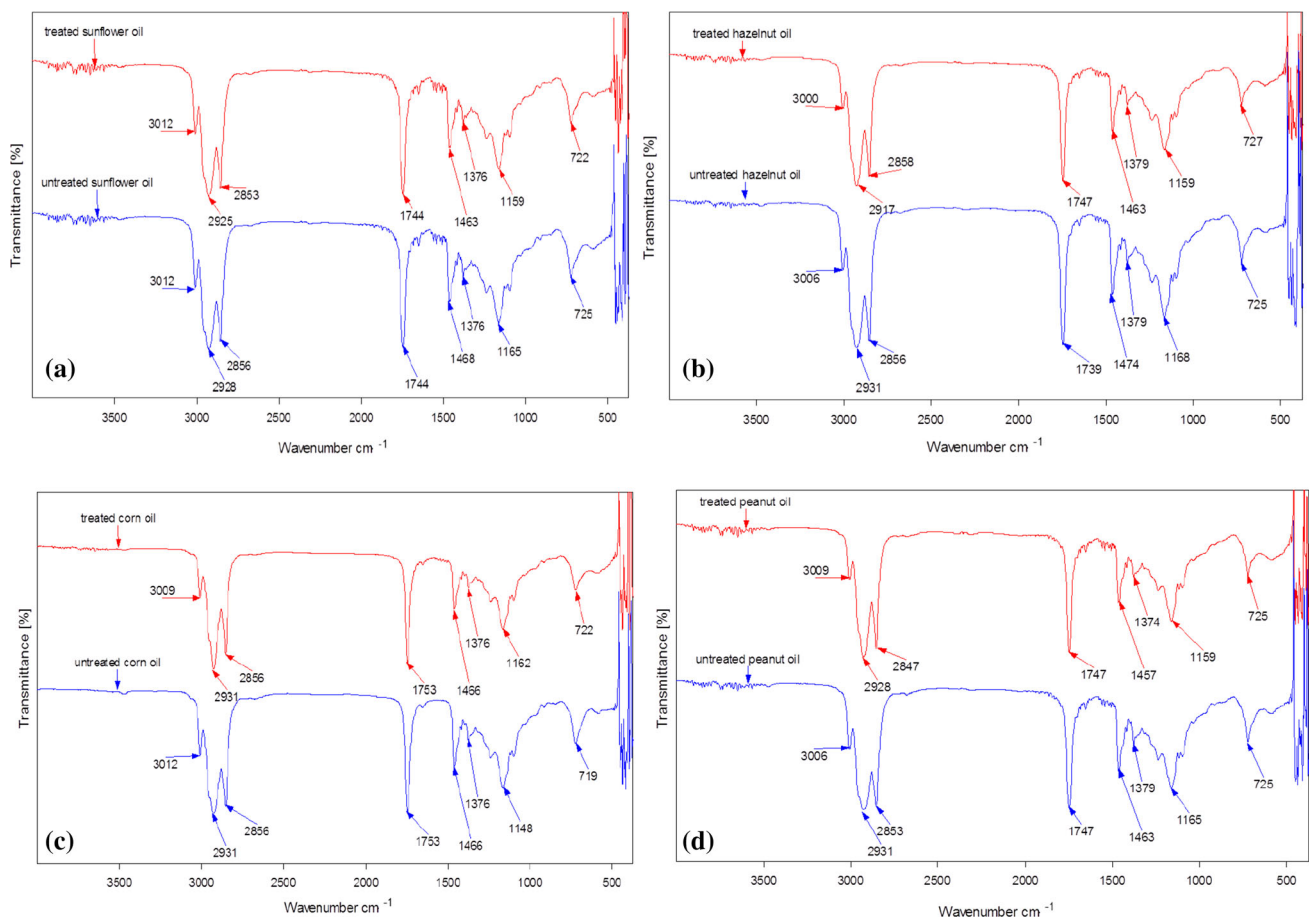
In this study, carotene (Fig. 1) did not show a success due to the fact that only 200 mg was applied into per liter samples. Otherwise, IT would be longer, leading to more stability in the oils (Flora 2009). Besides, more carotene addition would also cause to change of the oil color, which is bad for the physical quality of the oil (Warner and Frankel 1987).

Even though olive leaf extract has a very high antioxidant activity (91%), its performance to increase the quality parameters of the selected has been found to be moderate. It could not exceed the individual phytochemicals. It might be a situation related to the limited solubility of the bio-phenols of the extract in the concerned oils. 3, 13, 20 and 23% TB of the olive leaf extract were dissolved in the hazelnut, corn, sunflower and peanut oils, respectively.

### DRIFT results for vegetable oils

Figure 3 show the Diffuse Reflectance Fourier Transform Spectroscopy of treated and untreated oils measured in the 4000–400  $\text{cm}^{-1}$  region at the room temperature. As seen in Fig. 3a, the strong bands of treated sunflower oil in 2853

and 3012  $\text{cm}^{-1}$  correspondence to methylene group ( $-\text{CH}_2$ ) (Alexa 2005). The peak at 1744  $\text{cm}^{-1}$  is due to vibration of the ester ( $\text{C}=\text{O}$ ) stretching, while the band at 1463  $\text{cm}^{-1}$  is due to  $\text{C}-\text{H}$  bonding. The peak at 1159  $\text{cm}^{-1}$  shows  $\text{C}-\text{O}$  stretching vibration. Figure 3b demonstrates DRIFT spectrum of the hazelnut oil. The bands at 3000 and 2858  $\text{cm}^{-1}$  correspond to asymmetrical and symmetrical stretching of  $-\text{CH}_2$  groups. The treated hazelnut oil showed a unique peak at 1747  $\text{cm}^{-1}$  owing to the carbonyl of the triglyceride ester group. The peaks at 1463 and 1159  $\text{cm}^{-1}$  can be assigned to  $\text{C}-\text{H}$  bending and stretching vibrations of  $\text{C}-\text{O}$ , respectively (Uzun et al. 2018). As seen in Fig. 2c, d, the most intense signal of corn oil and peanut oil were observed at 2931 and 2928  $\text{cm}^{-1}$ , respectively. Those bands are characteristic to the symmetrical and asymmetrical vibrations  $\text{C}-\text{H}$  of the  $\text{CH}_2$  and  $\text{CH}_3$  aliphatic groups from the alkyl rest of the triglycerides in vegetable oils (Alexa 2005). Additionally, 1753 and 1747  $\text{cm}^{-1}$  bands correspond to the ester carbonyl of triglycerides for corn oil and peanut oil, respectively. The samples gave strong peaks in these spectra that relates to bending of  $\text{C}-\text{H}$  at  $\sim 1466$  and stretching of  $\text{C}-\text{O}$ , at  $\sim 1169$   $\text{cm}^{-1}$  (Zahir



**Fig. 3** Comparative results of diffuse reflectance fourier transform spectroscopy for treated and untreated vegetable oils

et al. 2017). Besides these bands, the band at  $720\text{ cm}^{-1}$  represents  $-(\text{CH}_2)_n-$  rocking of the fatty acids (Kurtulbaş et al. 2018). The enriched and pure oils indicate similar peaks due to the fact that DRIFT spectroscopy cannot detect very low concentrations such as 200 ppm. Hence, the peaks at  $3500\text{ cm}^{-1}$ , corresponding to  $-\text{OH}$  stretching region was not observed, although compounds with  $-\text{OH}$  functional groups were added into the oils.

## Conclusion

The findings of this study indicate that addition of 200 mg of natural antioxidant into one liter of the selected vegetable oils subscribes to the oil stability along with the remarkable improvement in their quality indicators. Generally, stabilization of the oils with natural additives was found to be superior to the synthetic ones, especially to BHA and BHT. The best improvement has been observed in hazelnut oil when treated by gallic acid and the mixture of phytochemicals (rutin, carotene and gallic acid). Induction time of the oil was enhanced statistically the same by both treatments. Furthermore, gallic acid increased the antioxidant activity of the hazelnut oil almost 41 times. Consequently, these natural food additives might act as an advanced agent in prolonging the shelf-life of the vegetable oils in addition to providing extra-nutritional properties.

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## Compliance with ethical standards

**Conflict of interest** The authors have no conflict of interest to declare.

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