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Antioxidant efficiency of citrus peels on oxidative stability during repetitive deep-fat frying: Evaluation with EPR and conventional methods

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Abstract

Deep-fat frying process is a commonly used procedure for food preparation, and the oxidative stability is an important quality issue for frying oils. In this study, the effect of citrus peel extracts (orange, lemon, mandarin) and BHT (butylated hydroxytoluene; a synthetic antioxidant) on oxidative stability of sunflower oil during the deep-fat frying process was investigated. For this purpose; classic chemical wet methods (free fatty acid, peroxide, *p*-anisidine, conjugated dienes) and a spectroscopic method (Electron Paramagnetic Resonance [EPR] spectroscopy, spin trapping technique) were used. According to both chemical data and EPR spin trapping results, the antioxidant effects of citrus peel extracts were found to be comparable to BHT. Moreover, the antioxidant effect of lemon peel extract was higher than other citrus peel extracts. EPR spin trapping technique can be used as an earlier and more accurate detection method in determining lipid oxidation during repetitive deep-fat frying.

Practical applications

Citrus peels, which are among the by-products of the fruit juice industry, have antioxidant properties. For this reason, citrus peels have the potential to improve the safety, quality, and nutritional value of industrial edible oils. In addition, waste can be reduced and value-added products can be produced by the utilization of citrus peels. EPR spectroscopy is the only technique in which lipid radicals, which are the most important indicators of lipid oxidation in industrial edible oils, can be directly and precisely defined. However, today mostly classical methods are used to measure oil quality. The more sensitive detection of oxidation in industrial edible oils may give us a clearer knowledge about the efficiency of the antioxidant source and the conditions under which they can be used.

1 | INTRODUCTION

Antioxidants are one of the most widely used groups of food additives and play a key role in preventing the development of off-flavors arising from the oxidation of unsaturated fatty acids (Houlihan & Ho, 1985). However, the commercial use of synthetic antioxidants is strictly controlled, growing consumer awareness of food additives, and safety have prompted increased interest in the use of natural antioxidants as alternatives to synthetic compounds. Extracts of many plants have been reported to have varying degrees of antioxidant activities, which give rise the oxidative stability of fats and oils (Kim et al., 1994).

Oxidative stability of oils is an important quality issue especially during deep-fat frying process. Deep-fat frying is a commonly used

procedure for the manufacture and preparation of foods throughout the world. Nearly one-half of all dinner and lunch food orders include one or more deep-fried products in restaurants. During deep-fat frying, the oil is exposed to elevated temperatures in the presence of air and moisture. A number of chemical reactions (including oxidation and hydrolysis) occur during this time, as do changes due to thermal decomposition. It is well known that lipid oxidation can lead to changes in functional, sensory, nutritive values, and even the safety of fried foods. Generally, these changes reduce the consumer acceptance of oxidized products (Lalas & Dourtoglou, 2003). In addition, reactions between peroxidized lipids and proteins have been shown to cause loss of enzyme activities, polymerization, accelerated formation of brown pigments, and some diseases such as aging, carcinogenesis, DNA damage, parkinsonism, tumor formation, and coronary heart diseases (Gutierrez et al., 1999).

Although peels and by-products are thought to be underestimated generally as agricultural waste, they have been found as potential sources of natural antioxidants in vegetable oils throughout the several studies that examined the efficiency of extracts such as pomegranate peel, potato peel, sugar beet pulp, etc. (Iqbal et al., 2008; Mohdaly et al., 2010).

Citrus processing by-products represent a rich source of naturally occurring flavonoids. The peels which represent roughly half of the fruit mass contain the highest amounts of flavonoids in the citrus fruits. As far as the peels are concerned, extracts from this part of the fruit were found to have a good potential as an antioxidant source in many previous studies (Dahmoune et al., 2013; de Moraes Barros et al., 2012; Khan et al., 2010; Londoño-londoño et al., 2010).

The main conventional methods for the assessment of antioxidant activity are DPPH, FRAP, and β-carotene bleaching assays. These methods determine the free radicals scavenging activity, the iron ions binding ability, and the inhibiting effect on the oxidation of fat-containing food, respectively (Nsimba et al., 2008). Electron Paramagnetic Resonance (EPR) spectroscopy is a potential method that can be used for the assessment of the antioxidant activity of food-based extracts. EPR is used in many interdisciplinary studies, and it is a powerful, reliable, and direct method for the identification and investigation of free radicals and other species with unpaired electrons in any molecular structure. The EPR spectral parameters, that is, the hyperfine splitting constants, g-factor, and signal intensity are the most important values for the identity of the formed radicals and provide structural, quantitative, and mechanistic information about the radicals and their surroundings (Brustolon & Giamello, 2009). Due to its advantages, EPR spectroscopy is a popular technique used for the investigation of lipid oxidation in food science recently (Chen, Cao, et al., 2017; Venkataraman et al., 2004). Compared to the conventional methods, EPR spectroscopy provides the possibility of studying the very early stages of lipid oxidation and determines the inhibitory effect of antioxidants against thermal oxidation (Quiles et al., 2002; Thomsen et al., 2000; Velasco et al., 2004, 2021). The short-lived lipid free radicals that occurred during lipid oxidation can only be detected by the EPR spin trapping technique, which is based on the interaction between the free radicals and diamagnetic spin traps to form more stable and measurable spin adducts (Andersen et al., 2005) and reliably applied since 1960 with many advantages such as convenience, fastness, simplicity, and objectiveness (Brustolon & Giamello, 2009). In lipid oxidation studies, *N-tert*-butyl-α-phenylnitrone (PBN) is a commonly used spin trap due to its high lipophilic and reactivity characteristics (Chen, Cao, et al., 2017).

In the present study, it was aimed to analyze the lipid oxidation that occurred by repetitive deep-fat frying and to determine the efficiency of citrus peel extracts on this oxidation, using both conventional methods and EPR spin trapping technique. Especially, the focus was on the direct determination of lipid radicals created by thermal oxidation using the non-time consuming and eco-friendly ESR spin trapping technique.

2 | MATERIALS AND METHODS

2.1 | Materials

Orange, mandarin, and lemon peels were obtained from local commercial juicing factories in the Mersin-Adana region, Turkey. After collection, the peels were stored immediately at −33°C to minimize the oxidation of the bioactive compounds. Sunflower oil and frozen french fries, used in frying experiments, were purchased from a local market in Mersin (Turkey).

2.2 | Chemicals

Acetic acid, ethanol, n-hexane, diethyl ether, phenolphthalein, potassium iodide, sodium hydroxide, butylated hydroxytoluene (BHT), sodium carbonate, gallic acid, Folin–Ciocalteu reagent, isooctane, chloroform, *p*-anisidine reagent, and sodium thiosulphate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). PBN, with the molecular formula $C_{11}H_{15}NO$, was purchased from Fluka Chemicals and stored under −20°C.

2.3 | Extraction

Citrus peels and extracting solvent (aqueous ethanol, 80%, v/v) were mixed using the sample to solvent ratio of (1 g)/(20 ml) and the prepared solution was homogenized using a blender. Ultrasound-assisted extraction was performed in an ultrasonic cleaning bath. Samples were placed into a volumetric flask (100 ml) and sonicated for 1 hr at room temperature. The extracts were filtered through Whatman No. 1 paper under vacuum.

In order to remove oily materials for a better concentration process, the extracts were blended with n-hexane and the samples were kept in an ultrasonic bath for 20 min. After the ultrasonic treatment, the hydro-alcoholic phase was separated from the solution and centrifuged at 9,000 rpm for 10 min. The resulting extract was

concentrated using a rotary evaporator at 50°C and then lyophilized with a freeze drier. Prepared extracts were stored at −20°C for further analysis.

2.4 | Total phenolic content

Total phenolic content of freeze-dried extracts was determined by Folin–Ciocalteu method (Singleton & Rossi, 1965). Briefly, an aliquot (0.2 ml) of the sample was mixed with 1.5 ml of Folin–Ciocalteu reagent. After 5 min, a solution of sodium carbonate (1.5 ml, 60 g/L) was added to the blend. The absorbance measured at 765 nm using a spectrophotometer (Cary 60 UV/VIS, Agilent Technologies, Palo Alto, CA, ABD) after standing at room temperature for 90 min. The results of the samples were expressed as gallic acid equivalents (GAE).

2.5 | Addition of antioxidants to the oil

For the preparation of the frying medium, the citrus peel extracts and synthetic antioxidant BHT were added to sunflower oil samples at the concentration level of (2 mg)/(g) for extracts and 200 ppm for BHT. The sample was then homogenized with an ULTRA-TURRAX T25 homogenizer at 9,000 rpm for 1 min. After the process, ultrasonic treatment was carried out for 5 min at 40°C. The oil samples were stored at 4°C.

2.6 | Frying conditions

Frying was performed in an uncovered stainless steel pan. Ten grams of potato samples were fried each time in 0.1 L of oil sample at 180°C for 3 min using a hot plate equipped with a thermostat. In addition, the oil temperature was also monitored with an external calibrated digital thermometer attached to a steel probe. Frying process was repeated three times using new potato samples in the same oil sample. Oil samples were periodically taken in triplicate from the different locations of the oil pan and all chemical analyses were conducted with these samples.

2.7 | Conjugated dienes

Samples were analyzed for conjugated dienes according to 2006 Ti 1a-64. Oil samples were diluted with isooctane (0.1 g oil/10 ml of isooctane) and the absorbance was measured at 232 nm.

2.8 | Free fatty acids

Oil sample of 0.5 g was weighed into a flask. First, 20 ml of solution that contains diethyl ether, ethyl alcohol, and water (v/v/v, 3/3/2) was added in order to dissolve the sample. Then, phenolphthalein was added as an indicator and the resulting solution was titrated with 0.01 N NaOH for neutralization. Free fatty acid (FFA) content was given in percent oleic acid (Casal et al., 2010).

2.9 | Peroxide value

Oil samples were analyzed for peroxide value using the 2011 Cd 8b-90 method. The samples were weighed in a range of 1–2 g with flasks. Acetic acid (15 ml), chloroform (10 ml), and potassium iodide (1 ml) were added to these flasks. After 5 min, 75 ml of distilled water and starch solution (indicator, 1%) was mixed with the solutions. Finally, the new solutions were titrated with sodium thiosulphate. The volume of titrants was recorded and the peroxide values were calculated and reported as mEq of active oxygen/kg oil.

2.9.1 | *p*-Anisidine value

p-Anisidine values (*p*AV) of the samples were determined according to the IUPAC method (number: 1987). Briefly, the oil samples in the range of 0.5–4 g were dissolved in 25 ml of isooctane and the absorbance of these solutions was measured at 350 nm. Following this treatment, 5 ml of the solutions was mixed with 1 ml of *p*-anisidine solution prepared in glacial acetic acid. After 10 min, the absorbance of these solutions was measured against the blank solution at 350 nm.

The *p*AV of samples was calculated with the equation; $pAV = 25 \times \left[\left(1.2A_s - A_b\right)/m\right]$. Here A_s is the absorbance of the oil solution after reaction, A_b is the absorbance of the oil solution, and m is the mass of the oil sample.

2.9.2 | Sample preparation and spectrometer conditions for EPR analysis

The spin trap PBN was chosen for the EPR spin trapping study because it has been extensively used both to examine lipid oxidation and to determine the antioxidant capacity that inhibits lipid radicals generated in oils due to oxidation (Chen, Cao, et al., 2017; Falch et al., 2005; Ottaviani et al., 2001; Velasco et al., 2004). PBN was dissolved in 25 μL ethanol to get 2.5 M solution and added to 0.5 ml of examined sunflower oils after 3 min of each frying repetition. It is important to add the spin trap to the oil as soon as the frying process is complete. Then, spin trap-oil solution was mixed for 2 min using vortex and 60 μL of solution with a final concentration of 125 mM was placed into the capillary tube at room temperature for EPR analysis. The same sample preparation procedure was applied to both sunflower oils (SFO, control sample) and the oils containing citrus peel extracts at repeated frying processes.

The spin trapping experiments were performed using JEOL JesFa300 X-band CW-EPR Spectrometer located at Selçuk

University Advanced Technology Research and Application Center in Turkey. The EPR instrumental settings were as follows; center field: 327.4 mT, sweep width: 15 mT, microwave frequency: 9.2 GHz, modulation amplitude: 0.1 mT, microwave power: 20 mW, scanning time: 30 s, time constant: 0.03 s, and accumulation: 5. The spectroscopic splitting values (g) of radicals were measured using Mn^{2+} signals of $MgO(Mn^{2+})$ standard sample. EPR Data Processing Program Version 3.3.35.E XB for X-band JesFa spectrometer was used for hyperfine splitting and g value measurements.

In addition, to investigate the inhibitory efficiency of citrus peel extracts on lipid-free radicals, the antioxidant activity (AA) values were calculated using the $AA = (I_o - I_x)/I_o$ equation (Spasojević et al., 2011; Živković et al., 2009). Here, I_o is the EPR signal intensity of the control oil sample (SFO) and I_x is the EPR signal intensity of oils containing synthetic (BHT) and natural (citrus peel extracts) antioxidants.

2.9.3 | Statistical analysis

All analyzes were performed in triplicate. Statistical analysis was performed using the software STATISTICA (Statistica 8.0, StatSoft Inc., 1984–2007) and significant differences between the values of all parameters were determined at $p < .05$ according to the one-way ANOVA.

3 | RESULTS AND DISCUSSION

3.1 | Chemical characteristics

Citrus peels, which can be produced as by-products in the fruit juice industry, contain high amounts of phenolic compounds. However, it is known that environmental factors and genetic structure affect the chemical content of citrus peels. In the present study, total phenolic contents of orange, mandarin, and lemon peels were found as 1.03 mg GAE/g peel (wb), 1.42 mg GAE/g peel (wb), and 0.98 mg GAE/g peel (wb), respectively. The extraction yields of the peels were 1.6 g extract/100 g orange peel (wb), 2 g extract/100 g mandarin peel (wb), and 1.8 g extract/100 g lemon peel. According to previous studies, naringin, neohesperidin, and hesperidin were found as the most abundant flavanones in citrus peels (Molina-Calle et al., 2015). Specifically, for orange peels (from Canada, China, and the United States), hesperidin was determined as the major flavonoid (Chen, Tait, et al., 2017). For lemon peels, hesperidin, hesperetin, and eriocitrin were detected as the major flavonoids (Xi et al., 2017) For mandarin peels, hesperidin, naringin, rutin, and tangeritin were found as the main flavonoids (Ferreira et al., 2018).

Chemical indices of vegetable oils could partly reflect their quality. Table 1 presents the results about some chemical indices (peroxide, FFA, *p*-anisidine, conjugated dienes) obtained from both fresh oils and the oils after frying sessions. In order to make a detailed

TABLE 1 Chemical characteristics of each sample in the frying repetition

Sample no	Frying repetitions	Peroxide value (meq O_2/kg)	FFA (%, oleic acid)	*p*AV	Conjugated dienes
SFO (control sample)	BF	6.28	0.32	7.56	0.01
	1st	37.25	0.44	25.18	0.09
	2nd	52.18	0.53	42.77	0.09
	3rd	66.21	0.87	65.81	0.12
SFO + BHT	BF	5.98	0.30	7.12	0.01
	1st	25.14	0.35	18.10	0.06
	2nd	39.21	0.38	31.14	0.07
	3rd	55.17	0.70	51.15	0.09
SFO + OE	BF	5.28	0.38	7.18	0.01
	1st	33.12	0.40	23.15	0.02
	2nd	48.14	0.52	39.41	0.07
	3rd	62.21	0.92	61.28	0.10
SFO + ME	BF	6.55	0.36	7.01	0.02
	1st	35.12	0.41	29.01	0.04
	2nd	54.21	0.60	39.47	0.11
	3rd	68.19	0.95	64.12	0.12
SFO + LE	BF	5.92	0.32	8.01	0.03
	1st	33.75	0.43	20.92	0.03
	2nd	53.12	0.57	33.79	0.05
	3rd	67.10	0.78	59.15	0.07

Abbbreviations: BF, before frying; BHT, butylated hydroxytoluene; FFA, free fatty acid; LE, lemon extract; ME, mandarin extract; OE, orange extract; *p*AV: *p*-anisidine value; SFO: Sunflower oil.

evaluation of the influence of each additive on oxidative stability in frying repetitions, it is necessary to examine and interpret each chemical indices separately.

Peroxide value is a measure of the amount of peroxides formed in oils through oxidation processes and indirectly indicates the degree of initial oxidation of oils (Jaswir et al., 2000). The peroxide value above 10 meq O_2/kg shows that the oil is at a high oxidation level and known as unhealthy (Sebastian et al., 2014). According to the results given in Table 1, before the frying process, it is seen that all oil samples have close peroxide values. After all deep-frying repetitions, significant impacts on peroxide values were noted. The oxidation stability was assessed in terms of peroxide value and it was observed that the change in peroxide value decreased as follows:

- In 1st frying: (SFO) > (SFO + ME) > (SFO + OE) ≈ (SFO + LE) > (SFO + BHT)
- In 2nd frying: (SFO + LE) > (SFO + ME) > (SFO + OE) > (SFO) > (SFO + BHT)
- In 3rd frying: (SFO + BHT) > (SFO + OE) > (SFO) > (SFO + ME) ≈ (SFO + LE)

According to these results, the samples that have BHT were assessed to have higher oxidative stability values in 1st and 2nd frying repetitions. However, in 3rd frying repetition, an adverse effect was observed in BHT added samples, namely these were more exposed to oxidation than the other samples. Moreover, for the samples containing lemon extract, it was found that the change in the peroxide value in the first and third frying repetitions was lower than the others and therefore it might be said this extract had a considerable effect on oxidative stability. The water released from the food medium generates a cloak of steam (or steam jacket) and the resulting steam jacket decreases the amount of peroxides in the frying medium. Moreover, it is known that oxygen solubility in oil has been inhibited at high temperatures (Romano et al., 2012). BHT may be removed from sunflower oil during frying due to its volatility. According to previous studies, BHT is known to be evaporated with steam at high temperatures (Zhang et al., 2004). Due to these reasons, the concentration of peroxides in oil samples may reduce after all frying repetitions.

FFA content is the most frequently used analysis for the oxidation in oil samples and the increase in FFA content means the decrease of oxidation stability. In the deep frying process, the moisture in food leads to hydrolysis and thermal hydrolysis of oils and these reactions form FFAs, glycerol, mono, and diglycerides (Chatzilazarou et al., 2006; Tyagi & Vasishtha, 1996). Thus, FFA content rises with exposure of oil samples to food moisture as well as air, at high temperatures. The FFA formation cause off-flavor and odor development in fried foods and oils (Bensmira et al., 2007). As seen in Table 1, the FFA content of all samples was relatively close to each other before frying process, while a gradual increase was observed in FFA contents after frying. This could be caused by the increase in the rate of triacylglycerol hydrolysis when water was introduced into the frying system from the potato samples. The decrease of FFA values is as follows:

- In 1st frying: (SFO) > (SFO + LE) > (SFO + BHT) ≈ (SFO + ME) > (SFO + OE)
- In 2nd frying: (SFO + LE) > (SFO + OE) > (SFO +ME) ≈ (SFO) > (SFO + BHT)
- In 3rd frying: (SFO + OE) > (SFO + ME) > (SFO) > (SFO + BHT) > (SFO + LE)

According to these data, the most effective extracts for 1st, 2nd, and 3rd frying are orange peel extract, BHT, and lemon peel extract, respectively. In 1st frying, the control sample (sunflower oil, SFO) was found to have higher oxidation rate compared to other samples. It can, therefore, be assumed that the extracts and BHT in oil samples may reduce the oxidation rates. In 2nd frying, the oil samples that have extracts were mostly seen to increase FFA contents compared to control samples. Accordingly, it was thought that the extracts caused to increase in the oxidation for this frying repetition. In addition, food crumbs that occur in the deep frying process are known to both rising oxidation and accelerating the formation of FFA contents. Furthermore, food crumbs and extracts may have a synergetic effect and cause a sharp growth in oxidation rates. In the 3rd frying, when compared with the control sample, BHT and lemon extracts decreased oxidation, while orange and mandarin peel extracts increased oxidation.

A repetitive deep-fat frying study accomplished with refined sunflower oil has reported growth of FFA contents from 0.14% to 0.60% besides, an increasing trend in the first 4 hr for peroxide values and then reducing gradually of this value. It has also been argued that the peroxide compounds forming after oxidation transformed to secondary products and that the low peroxide values may result from the faster conversion of peroxides to the secondary product in the frying process (Nayak et al., 2016). In a study about deep-frying of sunflower oil that 50 times repeated for 6 min at 170°C, it was stated that the FFA content increased from 0.17% to 0.29% and the peroxide values decreased from 12.7 meq O_2/kg to 4 meq O_2/kg (Maskan & Bağcı, 2003).

The *p*-anisidine test examines the amount of aldehydic components which are formed after the decomposition of hydroperoxides (Bailey & Shahidi, 2005). This test has enhanced sensitivity for unsaturated aldehydes, especially 2,4-dienals, but does not measure the ketonic secondary products of oxidation (Augustin & Berry, 1983). Table 1 provides the experimental data on *p*AVs. For all samples, before the frying process nearly similar values were found and frying repetitions caused a gradual increase in *p*AVs. This trend may be the result of thermal oxidation that occurs at high temperatures. According to Table 1, *p*AVs were declined as follows:

- In 1st frying: (SFO + ME) > (SFO) > (SFO + OE) > (SFO + LE) > (SFO + BHT)
- In 2nd frying: (SFO) > (SFO + OE) > (SFO + BHT) > (SFO + LE) > (SFO + ME)
- In 3rd frying: (SFO + LE) > (SFO + ME) > (SFO) > (SFO + OE) > (SFO + BHT)

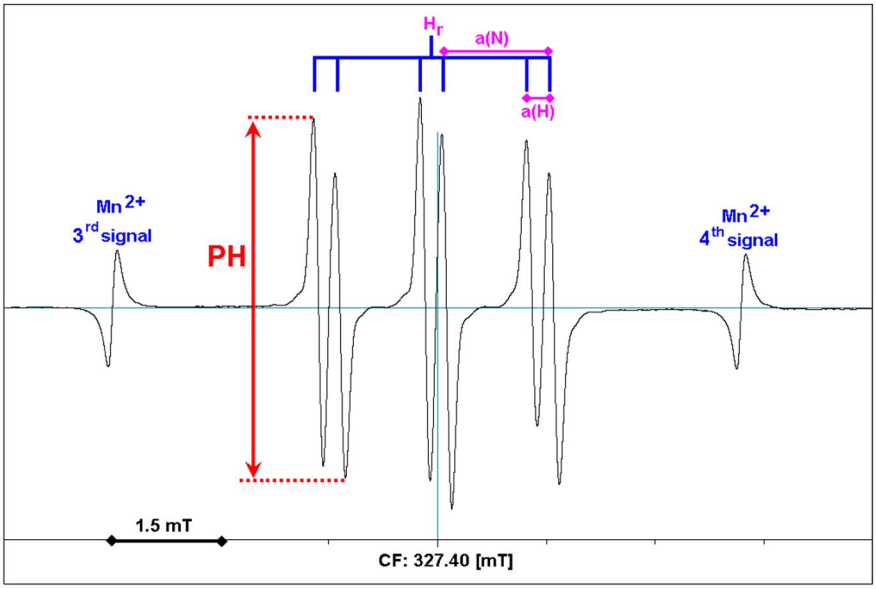


FIGURE 1 The presentation of EPR spectral parameters belong to the spin adduct

On the basis of the data, BHT was found to grow the oxidation stability in 1st and 3rd frying repetitions. In addition, while lemon extract was observed to cause oxidation in the 3rd frying, it increased oxidative stability in both the 1st and 2nd frying repetitions.

Evaluation of conjugated dienes measures the degree of primary oxidation (Maskan & Bağci, 2003). The analysis is based on the formation of conjugated alkene bonds and their measurement at 232 nm. Polyunsaturated fatty acid oxidation in the frying process is accompanied by the rise of ultraviolet absorption (Sumnu & Sahin, 2008). Additionally, the increasing amount of unsaturated fatty acid content is known to cause a rise in conjugated dienes in the frying process (Ghazali et al., 2007; Houhoula et al., 2003). Table 1 provides the experimental data on conjugated dienes. The results are closely related to the peroxide values because both of them are primary oxidation products. A trend of increase was seen for diene content in frying repetitions. Conjugated diene values of oil samples were compared below:

- In 1st frying: (SFO) > (SFO + BHT) > (SFO + ME) ≈ (SFO + LE) > (SFO + OE)
- In 2nd frying: (SFO + ME) > (SFO + OE) > (SFO + LE) ≈ (SFO) > (SFO + BHT)
- In 3rd frying: (SFO) ≈ (SFO + OE) ≈ (SFO + LE) > (SFO + BHT) > (SFO + ME)

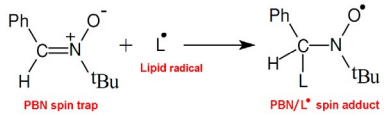
In 1st frying, oil samples containing the additives were determined to have higher oxidation stability compared with the control sample, even extracts were found more effective than BHT added samples. In 2nd frying, extract additives showed an adverse effect on oxidation and increased the oxidation rates. In 3rd frying, mandarin peel extract was evaluated as the most effective additive to have the ability to lower the oxidation rates.

As a result of chemical analysis, the values of all chemical indices showed statistically significant differences for each frying repetitions. According to these results, the frying process gradually increased the oxidation rate of all oil samples. The increase in oils, having BHT and citrus peels, was partly weaker than in the control oil. Thus, it can be thought antioxidant additives have slight impacts on oxidative stability. Accordingly, the antioxidant effect of citrus peel extracts is comparable to that of BHT, and moreover, the effect of lemon extract appears to be more dominant than the others.

3.2 | EPR spin trapping

As discussed in the Introduction section, it is well known that EPR is a unique technique used for the direct detection of free radicals occurred by lipid oxidation. Also, it is possible to take valuable information in understanding the inhibitory effect of antioxidants against temperature-induced free radicals. For this purpose, EPR spin trapping was applied to all investigated sunflower oils (SFO, SFO + BHT, SFO + OE, SFO + ME, SFO + LE) after each repetition of frying. PBN spin trap interacted with lipid radicals to form a long-lived "spin adduct" allowing detection. The general reaction mechanism of PBN and lipid radicals was shown as follows (Falch et al., 2005).

EPR spectra of spin trapping applied samples were recorded given EPR spectrometer conditions in the Experimental section. The presentation of an EPR spectrum belong to spin adduct is given in Figure 1. Six EPR lines appeared due to hyperfine splitting between unpaired electron and the nuclei; N and H atoms which have $I = 1$ and $I = 1/2$ nuclear spins, respectively. In Figure 1, PH is the signal intensity considered as the peak-to-peak amplitude of the low field line ($M_I = +1$) (Falch et al., 2005), H_r is the resonance magnetic field, a_N and a_H are the hyperfine splitting constants that represent the interaction between



unpaired electron and the relevant nucleus. The 3rd and 4th signals of Mn^{2+} concerning MgO (Mn^{2+}) standard sample were used to measure the g values.

In order to make a relative comparison of radical intensity generated by oxidation in all samples, the peak-to-peak amplitude of the low field line proportional to the amount of radical was used (Chen, Cao, et al., 2017; Falch et al., 2005; Thomsen et al., 2000).

The EPR spectra of SFO and SFO + OE samples after first, second, and third frying steps are given in Figure 2. The amount of formed radicals resulting from oxidation can be associated with the signal intensities at the spectral pattern. It was understood from the figure that the lipid radicals began to form with first frying in both SFO and SFO + OE samples. Nevertheless, in the second frying process, the amount of radicals in SFO increased while in SFO + OE decreased. For the control oil, the number of radicals in the third frying process is somewhat lower than that of the second frying, but it is still quite large from the orange extract-containing oil. It was thought that the reduction of radical amount in the third frying for the SFO sample may be due to its own antioxidants. The radical concentration of the SFO + OE sample in each of the three frying repetitions is very low compared to the control sample. Thus, it is interesting to note that orange extract is quite efficient as an antioxidant to inhibit the lipid radicals produced by frying.

For the purpose of determining the radical formation rate at the repetitive deep-frying process, EPR signal intensities of lipid radicals were measured and the change in the parameters was plotted versus frying repetitions for each investigated oil samples.

The calculated EPR parameters and the antioxidant activity (AA) values for all samples are given in Tables 2 and 3, respectively. Considering Table 2, the same EPR parameters (*g* and *a* values) obtained for the samples indicate the formation of the same type of lipid radicals.

The EPR signal intensities and AA values plotted versus frying repetition are given in Figure 3a,b, respectively. In the study of AA determination (Figure 3b), antioxidant supplemented samples were compared with the control one. That is, the EPR signal intensity of the SFO at each frying repetition was taken as the signal intensity of the control sample, so that the antioxidant activity was determined for BHT, ME, OE, and LE contributions.

As seen from Figure 3a, in the first frying, the EPR signal intensities for SFO + OE and SFO + LE were approximately equal to the intensity for SFO; however, the intensities for SFO + BHT and SFO + ME were too big than those, due to the radicals induced by lipid oxidation. So, orange and lemon extracts can be used as an antioxidant in the first frying but BHT and mandarin have increased radical formation. In the second frying, the amount of lipid radicals for SFO + OE, SFO + LE, and SFO + ME samples was less than for the SFO sample; moreover, the least radical formation occurred in the SFO + OE sample. At this stage, considering the SFO + BHT specimen, lipid radical formation was observed at the level of the control specimen. Interesting results

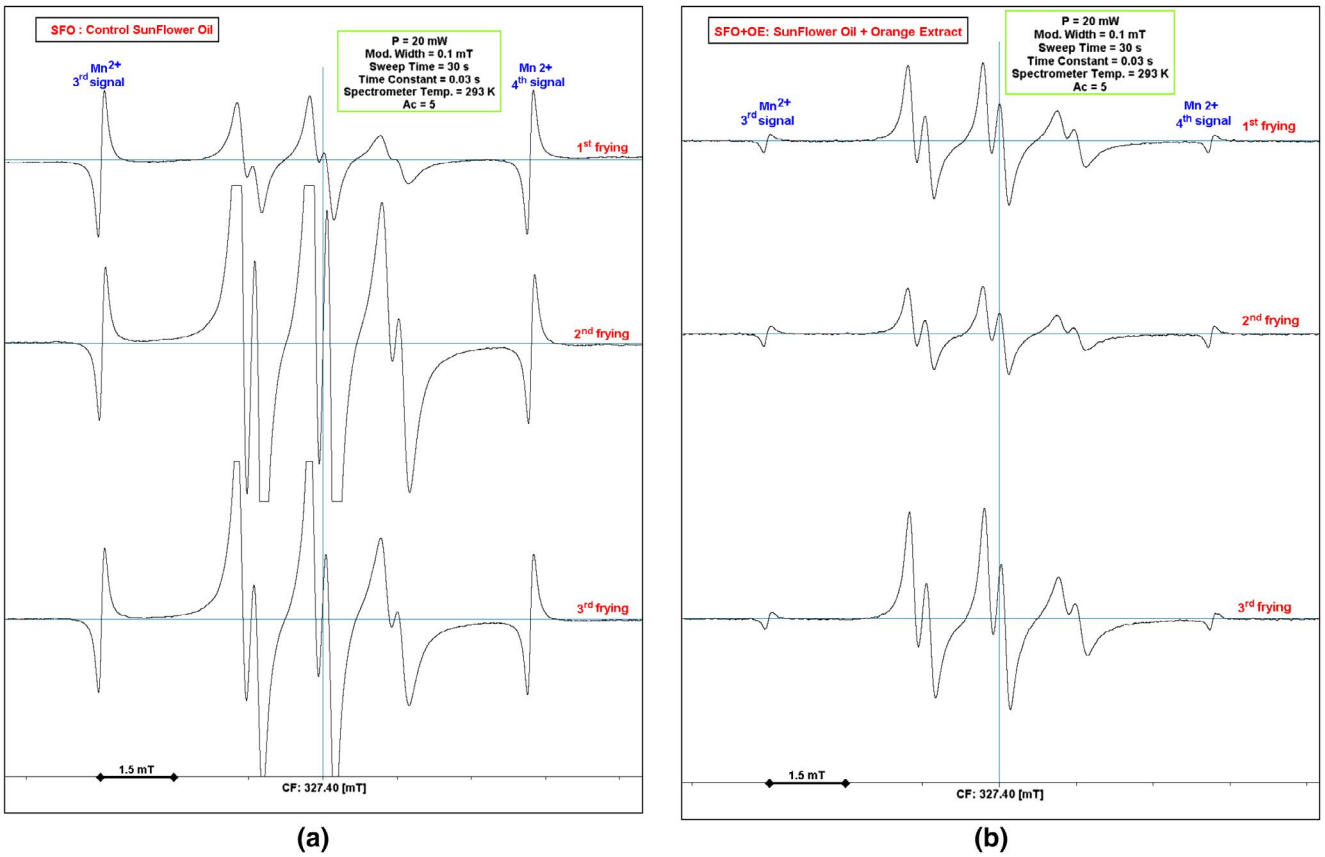


FIGURE 2 (a) EPR spectra of SFO at each frying repetition. (b) EPR spectra of SFO + OE at each frying repetition

have been obtained in the third frying process. For the SFO sample, the intensity of the EPR signal weakened but increased for ME and BHT added samples. In the OE and LE added samples, less radical formation was observed than for the control SFO sample. The graph shown in Figure 3a can be summarized as follows;

- In 1st frying: I(SFO + BHT) > I(SFO + ME) > I(SFO + LE) = I(SFO +OE) = I(SFO)
- In 2nd frying: I(SFO) ≥ I(SFO + BHT) > I(SFO + LE) > I(SFO + ME) > I(SFO + OE)
- In 3rd frying: I(SFO + BHT) > I(SFO + ME) > I(SFO) > I(SFO + OE) > I(SFO + LE)

TABLE 2 EPR parameters of lipid radicals for each sample in the frying repetitions

Sample	Frying repetitions	*I* (a.u.)	*G*	a_N (mT)	a_H (mT)
SFO	1st	191.28	2.0067	1.45	0.30
	2nd	1,065.65	2.0067	1.46	0.34
	3rd	643.88	2.0067	1.46	0.34
SFO + BHT	1st	1,321.93	2.0067	1.46	0.33
	2nd	1,025.53	2.0067	1.46	0.33
	3rd	1,111.70	2.0067	1.46	0.34
SFO + ME	1st	956.23	2.0067	1.46	0.33
	2nd	607.90	2.0067	1.46	0.33
	3rd	1,051.73	2.0067	1.46	0.33
SFO + OE	1st	222.70	2.0067	1.45	0.33
	2nd	135.85	2.0067	1.46	0.33
	3rd	310.35	2.0067	1.46	0.33
SFO + LE	1st	215.33	2.0067	1.47	0.33
	2nd	707.50	2.0067	1.46	0.34
	3rd	151.30	2.0067	1.46	0.33

Here, *I* values represent EPR signal intensities and the more signal intensity means the more radical concentration.

The antioxidant activity values given in Figure 3b support these results. Namely, the BHT and ME did not show the antioxidant property for the 1st frying. Similar comments can also be made for the 3rd frying repetition. While BHT did not show antioxidant properties in all three fries, ME had little activity in the 2nd frying. Unlike these, OE and LE had antioxidant properties in all three fries.

As a result, the EPR spin trapping technique for lipid oxidation at the repetitive deep-fat frying process was first applied and meaningful results were obtained. Accordingly, the following comments can be made, it has been found that BHT synthetic antioxidant and mandarin extract do not have antioxidant property, whereas orange and lemon extracts are good antioxidants for repeated potato fries. Even, the AA values of OE are greater than the values of LE. So, according to EPR results, it can be suggested that OE can be used as an antioxidant for repetitive deep-fat frying for potatoes.

TABLE 3 Antioxidant activity (AA) values for BHT and the citrus peel extracts

	Synthetic antioxidant	Citrus peel extracts		
Frying repetitions	BHT	ME	OE	LE
1st	−5.91087	−3.99904	−0.16424	−0.12573
2nd	0.037645	0.42955	0.872519	0.336086
3rd	−0.72656	−0.63342	0.518003	0.76502

Abbbreviations: ME, mandarin extract; OE, orange extract; LE, lemon extract.

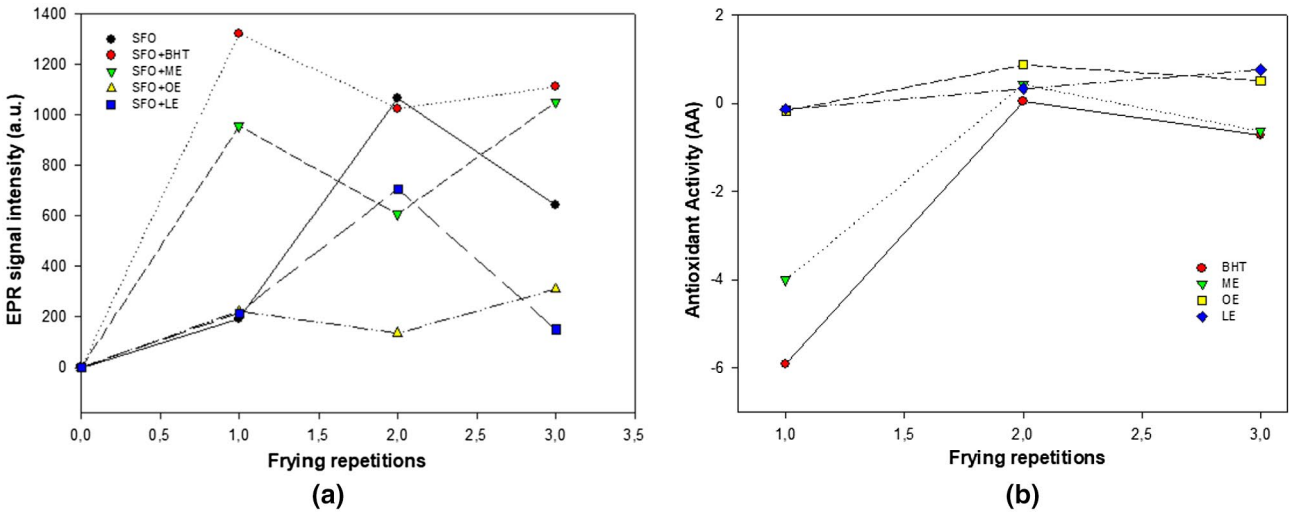


FIGURE 3 (a) Change of EPR signal intensities depending on frying repetitions. (b) Antioxidant activity values against frying repetitions

4 | CONCLUSIONS

It is well known that lipid oxidation is one of the most important deterioration factors observed in oils during frying and is an important quality criterion used to determine frying performance. That is why in the present study, both the effect of repeated frying on lipid oxidation and the effects of citrus peels (orange, lemon, tangerine) sourced antioxidants on the oxidative stability of the sunflower oil during deep frying were investigated using both conventional chemical methods and EPR spectroscopy.

The results can be summarized as follows:

- The data obtained by both chemical methods and EPR showed that repeated deep fat frying increases lipid oxidation considerably.
- Each chemical method evaluates different products associated with different oxidation stages and the values of these products varying due to oxidation may also be influenced by external influences such as potato crumbs. Therefore, it is difficult to say which citrus peel is effective as an antioxidant in repeated frying with the overall evaluation of the results obtained from these methods.
- EPR spectroscopy is the only technique in which lipid radicals, the most important indicator of lipid oxidation, can be identified directly and precisely. It can be said that EPR technique is more sensitive, more controlled, and less affected by environmental conditions than chemical methods. Therefore, this technique is recommended to be preferred instead of chemical methods for the determination of oxidation. Using EPR in the present study, it was found that the concentration of lipid radicals increased when the degree of oxidation rises in each repeated frying. Furthermore, the AA values of orange and lemon extracts were found to be greater than BHT and mandarin extract. In fact, the orange peel extract was more effective than the others in terms of oxidative stability, considering the three frying repetitions. Therefore, due to its natural antioxidant efficiency, it is recommended to be used for this purpose.
- The fact that citrus peel extracts can be used as a natural source of antioxidants will provide added value to these peels in the fruit juice industry as well as supporting sustainability.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Sema Aydın: Investigation; Methodology; Project administration; Writing-original draft; Writing-review & editing. **Ulku Sayin:** Investigation; Methodology; Writing-original draft; Writing-review & editing. **M. Özgür Sezer:** Methodology; Writing-original draft. **Sedat Sayar:** Methodology; Writing-review & editing.

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