Oxidative stabilisation of sunflower oil enriched with olive mill wastewater and olive pomace phenolics-rich extracts with soy lecithin

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Olive oil industry generates huge amounts of by-products that are discarded and can be a serious environmental problem. In this study, the antioxidant activity of olive mill wastewater (OMWW), and olive pomace (OP) extracts (at different concentrations) with soy lecithin, on the thermal oxidative stability of sunflower oil (SO) were determined. The results generally showed that the higher the extract concentration added to SO, the higher the thermal stability of SO. OMWW and OP extracts had similar antioxidant activity in linoleic acid emulsion (87.59% and 97.74%). Trolox equivalent antioxidant capacity (TEAC) of extracts ranged between 6.7-27.1 μM. When extracts with lecithin were added to SO, the induction periods (IP) and protective factors of SO were higher. In addition, the extracts were more effective when added together with lecithin. OMWW extract was more efficient in lowering the conjugated diene (CD) content in SO samples than the OP extract during the thermal oxidation test at 180°C. SO enriched with OMWW extract and lecithin, had lower p-anisidine values, higher tocopherol content and higher IP than SO enriched with butylated hydroxytoluene (BHT) at the end of 40 h.

Keywords: *Olea europaea*, phospholipids, polar lipids, Trolox, thermal stability, antioxidant activity, synergistic effect.

1. INTRODUCTION

The olive oil (OO) (Olea Europea) industry is an important agro-industrial activity in the Mediterranean area, accounting for about 90% of the world quota [1]. The extraction of OO generates huge amounts of agri-waste (10 million ton/year), which might have a great effect on the environments because of their high phytotoxicity [2-4]. Olive products and by-products are a rich source for phenolics that considered as antioxidants with health-promoting traits [5]. Studies mentioned that olive phenolics (i.e., hydroxytyrosol) are effective in retarding and preventing several diseases [6, 7].

Olive oil production is carried out using different extraction systems. Centrifugal systems are commonly used as an extraction tool for OO production [8, 9]. Two main by-products formed in these extraction systems are olive oil wastewater (OMWW) and olive pomace (OP). Although the olive fruit rich in phenolics, about 2% of these phenolics passes through the oil phase, the rest amount is lost in the OMWW (about 53%) and the OP (about 45%) depending on the extraction system [10, 11]. Owing to their high phenolics content, OP and OMWW could be evaluated in various sectors such as pharmaceutical, cosmetic and food industries [12].

OMWW is the main pollutant from extraction systems especially 3-phase systems and traditional olive mills [2]. During OO extraction, olive phenolics are partitioned between the water-phase and the lipid phase. However, the ma-

jor portion is missing in the wastewater from the fact that they are water-soluble and polar compounds. Depending upon the process used, 200-1600 L of OMWW is produced per ton of processed olives [13, 14]. OMWW is generally composed of water (83-96%), organic matters (3.5-15%) and mineral salts (0.5-2%). The concentrations of phenolics in OMWW range between 5 and 25 g/L [15]. The OMWW composition strongly depends on the extraction process, on the type and ripening state of olives, harvest region and climate [14, 16]. For example, the reported amounts of phenolics may vary between 1.3% and 4.0% on the dry-weight basis [14, 17]. As OMWW have high phenolic content, they cause serious environmental problems. The effluent phytotoxicity and its poor biodegradability are normally due to the presence of high levels of phenolics that are toxic to most microorganisms, imparting a great impact on the environment [14]. On the other hand, phenolics exhibited a strong antioxidant potential and could be applied in the pharmaceutical and food industries [18].

Olive pomace (OP) is the other by-product from OO processing. OP is a potentially low-cost, phenolics-rich ingredient for the formulation of novel foods [19]. OP consisted of olive pulp, skin, stones, and oil residues. Even if their production is seasonal, its disposal is potentially harmful to the environment due to its high moisture content (*ca*. 70%) [4, 20]. This by-product is a valuable source of bioactive compounds with well-recognised benefits for human health and well-being [21]. The recovery of antioxidants from OP seems achievable to produce substances industrially exploitable as supplemental food. The composition of OP showed large variability, depending on the harvesting time, cultivar, and oil extraction system [4, 22]. The vitamin E profile of the OP comprised α-tocopherol, β-tocopherol, α-tocotrienol, and γ-tocopherol. α-Tocopherol was the major compound (2.63 mg/100 g), while the other vitamines were present at lower levels. Hydroxytyrosol and comsegoloside represented about 79% of the Total phenolic content (TPC) present in OP. Hydroxytyrosol content was 83.6 mg/100 g, while tyrosol was present in lower (3.4 mg/100 g) levels [21]. Albahari et al. [23] characterised OP extract obtained using cyclodextrin-enhanced pulsed ultrasound-assisted extraction. Extracts contained 887 mg/kg of hydroxytyrosol, 1117 mg/kg of tyrosol, and 1744 mg/kg of oleuropein.

Phospholipids and in particular lecithin have been used as emulsifiers and antioxidant agents in food systems. The synergistic antioxidant potential between lecithin and phenolic compounds was also reported in some investigations [24-27]. Antioxidant traits of phospholipids have been demonstrated and proposed to be due to (i) synergism between phospholipids and tocols, (ii) chelating of pro-oxidant metals by phosphate groups, (iii) formation of Maillard-type products between amino phospholipids and oxidation products, and (iv) action as an oxygen barrier between oil and air interfaces [25-27].

The objective of this work was to investigate the effects of OMWW and OP extracts with/without lecithin on the oxidative stability of refined sunflower oil (SO). SO was chosen to evaluate the antioxidant potential of extracts and lecithin due to its high content of unsaturated fatty acid. Antioxidant activities of extracts were measured using the linoleic acid oxidation system and Trolox Equivalent Antioxidant Capacity (TEAC). Differential Scanning Calorimetry (DSC) and thermal oxidation tests were carried out to determine the effects of extracts and lecithin on oxidative stability of SO at high temperatures (180°C).

2. MATERIALS AND METHODS

2.1. MATERIALS

OMWW and OP used in the study were obtained from a factory operating the two-phase centrifugation system (Taylieli Laleli Olive and Olive Oil Plant, Balıkesir, Turkey) and stored at -18°C until used. The refined SO was purchased from a local market (Bolu, Turkey). All chemicals and reagents were of analytical grade. Linoleic acid (99%), α-tocopherol (99%), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and lecithin (soy lecithin, type II-S, containing 14-23% phosphatidylcholine) were obtained from Sigma-Aldrich (St. Louis, MO, USA). *p*-anisidine reactive, 2,2′-bipyridine (99%) and ferric chloride hexahydrate were obtained from Acros Organics (New Jersey, USA). Other chemicals and reagents were obtained from Merck (Darmstadt, Germany).

2.2 METHODS

2.2.1 Preparation of the extracts, extracts solutions and SO samples

2.2.1.1 Preparation of the extracts

100 g of OMWW and 20 g of OP were weighted in a flask. 100 mL of ethanol or methanol were added. Flasks were shaken at 150 rpm using a shaking water bath for 60 min. After shaking overnight at 20±2°C, the extracts were filtered through a filter paper. The residue was extracted with 100 mL solvent, as described above and the filtrates were combined. In order to remove lipids, which may be present in filtrates, each filtrate was stirred on a magnetic stirrer for 20 min after the addition of *n*-hexane. Methanol: water and hexane phases were separated with a separation funnel. Methanol: water phase was filtered through Whatman 1 filter paper and evaporated under vacuum using a rotary evaporator at 40°C. Extracts were transferred into a coloured bottle and nitrogen gas was given for 20 min in order to remove the alcohol,

then dried using a freeze-dryer. Lyophilized extracts were stored at -18°C.

2.2.1.2 Preparation of OMWW and OP extract solution

OMWW and OP extracts were prepared at 0.5, 1, 2, and 3 mg/mL concentrations in 50% aqueous (v/v) alcohol from lyophilized extracts. Extract solutions were used for antioxidant activity in the linoleic acid system and TEAC analysis.

2.2.1.3 Preparation of SO Samples.

Lyophilised extracts were added to SO samples at different concentrations (1 and 2 mg/g) after dissolving in propane-diol. Lecithin (5 mg/g) was also added to some samples. All samples were vortexed thoroughly and kept at 40°C for 20 min in an ultrasonic water bath to increase the amount of dissolved extract. SO samples were used to analyse the TPC, induction period by DSC and thermal oxidation test.

2.2.2 Antioxidant activity in linoleic acid system (conjugated diene test)

The oxidation degree of linoleic acid is a spectrophotometric method at 234 nm reported by Iqbal et al. [28] and Mau et al. [29]. To prepare the 0.02 M linoleic acid emulsion, linoleic acid (0.2804 g) and Tween 20 (0.2804 g) were weighed and dissolved in potassium phosphate buffer (50 mL, 0.05 M, pH 7.4). The linoleic acid emulsion was held in an ultrasonic water bath and shaken well to stabilise the emulsion. Linoleic acid emulsion (2.5 mL, 0.02 M), extract solution (0.5 mL, at 0.5, 1, 2 and 3 mg/mL) and potassium phosphate buffer (2 mL, 0.2 M, pH 7.0) were mixed well in flasks. Ethanol or methanol (0.5 mL) were used as a control sample instead of the extract solution. Flasks were allowed to incubate for 16 h without a cap in the dark at 37°C. Before and after incubation, 0.1 mL of samples was collected from every bottle and mixed with 6 mL of a methanol solution (60%, v/v). Absorbance differences of each sample and control before and after incubation were calculated. Antioxidant activities of samples were compared with those of BHA, BHT, and α-tocopherol at 0.2 mg/mL concentration. Antioxidant activity (%) was calculated as follow:

$$\text{Antioxidant activity \%} = ((\Delta A_{control} - \Delta A_{sample}) / \Delta A_{control}) \times 100$$

$\Delta A_{control}$: control absorbance difference before and after incubation

ΔA_{sample}: sample absorbance difference before and after incubation

2.2.3 Trolox equivalent antioxidant capacity (TEAC)

TEAC test was carried out according to De Marco et al. [18] with some modifications. $ABTS^{\bullet+}$ (2,2'-Azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) solution was prepared according to the method. $ABTS^{\bullet+}$ solution (990 µL) and extract solution (10 µL, at 0.5, 1, 2, and 3 mg/mL concentration) were mixed and the absorbance of all samples were measured (734 nm) at the end of the 6th min. The control sample was prepared with absolute ethanol (10 µL). Inhibition (%) was calculated as below:

$$\text{inhibition \%} = ((A_{control} - A_{sample}) \times 100) / A_{control}$$

$A_{control}$ and A_{sample}: absorbance at 734 nm for control and sample

Standardised Trolox solutions were prepared at different concentrations from Trolox stock solution (2.5 mM) in methanol and analysed under the same conditions. The equation was obtained by plotting with the absorbance values of Trolox solutions. TEAC values of OMWW and OP extracts were calculated using the same equation.

2.2.4 TPC of SO samples

Absolute methanol (2.5 mL) and SO samples (2.5 g) were vortexed for 2 min. After waiting 10-15 min, 0.5 mL was taken from the upper methanol phase. The TPC of SO samples were determined using the Folin-Ciocalteu reagent according to Iqbal et al. [28]. The TPC was expressed as mg gallic acid equivalents (GAE) per gram of extract. For the calibration curve, absorbance values of standardised gallic acid solution (0.01-0.06 mg/mL) were used and the absorbance was plotted against the concentration. The curve equation was used to calculate TPC as ppm.

2.2.5 Induction periods (IP) analysis using

DSC (Shimadzu, DSC 60, Japan) was used to determine the IP of SO samples at 130°C. SO was used as a control. Samples weighed (1.0±0.1 mg) in an open aluminum pan. The oven was heated from 50°C to 130°C at 10°C/min in the presence of nitrogen (99.999% purity) under a stream of 50 mL/min. When the temperature reached 130°C, the oven was supplied with oxygen (99.99% purity) under a stream of 50 mL/min instead of nitrogen. During the analysis, the temperature was kept constant at 130°C. The time taken until the exothermic oxidation peak observed at 130°C is measured as IP.
Protection factor was calculated by dividing the IP of SO samples by the IP of control.

2.2.6 Thermal oxidation test at 180°C

Thermal oxidation analyses were carried out at 180°C for 40 h and samples were collected at 8 h interval. Collected samples were analysed to determine conjugated diene (CD), *p*-anisidine value, tocopherol content, and IP. All results were compared to control

(SO) and BHT enriched SO. The tocopherol analysis was conducted spectrophotometrically according to Wong et al. [30]. For calibration, absorbance values of solutions containing α-tocopherol at different concentrations (25-200 µg/5 mL) were read under the same conditions. The tocopherol content was calculated as mg/kg (ppm). Conjugated diene (CD) was determined at 232 nm using spectrophotometer (Shimadzu, Japan) according to AOCS [31] Ti 1a-64. The *p*-anisidine value was determined at 350 nm using spectrophotometer (Shimadzu, UV 1700, Japan) according to AOCS [31] Cd 18-90. IP of samples was determined using DSC according to the above method.

2.2.7 Statistical analyses

The statistical analysis was performed with the SPSS package software, version 18.0 (SPSS Inc, Chicago, IL). Results were presented as means ± standard deviation of the two or three replicates of each experiment. The variation analysis was performed (ANOVA). Significant differences among the means ($p<0.05$) were determined by Duncan's multiple tests.

3. RESULTS AND DISCUSSION

3.1 ANTIOXIDANT ACTIVITIES OF OMWW AND OP EXTRACTS

The antioxidant activity of OMWW and OP extracts according to the oxidation of linoleic acid is expressed as percent inhibition (Table I). All extracts showed an antioxidant activity in the range 0.5-3.0 mg/mL. OMWW methanol extracts showed a higher activity than ethanol extracts, while OP methanol and ethanol extracts showed a similar activity ($p<0.05$). Compared to some synthetic antioxidants, the antioxidant activity of OMWW and OP extracts was close to BHA, BHT, and α-tocopherol at 0.2 mg/mL.

The TEAC values of OMWW and OP extracts to inhibit ABTS•⁺ radical are given in Table II. TEAC values of OMWW extracts were between 6.8 and 26.0 µM. It was proved that the OMWW methanol extract exhibited higher antioxidant activity than ethanol extracts. For the OP extracts, TEAC values were ranged between 6.7 and 27.1 µM. Moreover, OP ethanol extracts had higher TEAC values than that of methanol extracts except for the concentration at 2.0 mg/mL. These values are lower than the study done by De Marco et al. [18] with a value of 55.8 mmol Trolox L^{-1} OMWW and higher than Rubio-Senent et al. [32] with a value of 0.22 mg/mL TEAC. These differences could be related to phenolic compounds, which were identified in these extracts. De Marco et al. [18] emphasized that the extracts rich in hydroxytyrosol exhibited a higher effect in radical scavenging activity compared to other extracts.

Table I - Antioxidant activity of OMWW and OP extracts in linoleic acid system

Extract	Concentration (mg/mL)	Antioxidant activity (%)*
	OMWW	
Methanol	0.5	91.70±2.07cA
	1.0	94.68±0.32bA
	2.0	96.98±0.48aA
	3.0	97.74±0.76aA
Ethanol	0.5	87.59±2.11bB
	1.0	91.23±1.68bB
	2.0	95.37±0.53aB
	3.0	95.93±0.5aB
	OP	
Methanol	0.5	91.15±1.36bA
	1.0	92.16±1.27bB
	2.0	93.11±0.15bC
	3.0	95.14±0.81aB
Ethanol	0.5	91.85±1.74cA
	1.0	91.99±0.67cB
	2.0	95.22±0.97bB
	3.0	95.80±0.33aB
BHA	0.2	96.51±1.11
BHT	0.2	95.11±0.13
α-tocopherol	0.2	98.35±0.43

*Analyses were done in triplicate and results are given as mean ± std deviation

[a-c] Small letters show the variation between the different concentrations of the same extract ($p<0.05$)

[A-C] Capital letters show the variation between extracts at the same concentration ($p<0.05$)

3.2 TPC OF SO SAMPLES

Table III shows the TPC of SO and enriched oils. TPC value in control sample (SO without any addition) was 8.6 ppm. TPC increased by adding OMWW and OP extracts at different concentrations. TPC increased even more with a lecithin addition compared to individual OMWW and OP extracts. High TPC (49.3 ppm) was determined in a sample enriched with lecithin (5 mg/g) and OMWW methanol (2mg/g) extract. Besides, in the lecithin-enriched samples, the use of OMWW and OP methanol extracts increased TPC compared to ethanol extracts. Venturi et al. [33] indicated that TPC increased with the addition of OMWW extracts (ethanol and ethanol: diethyl ether) to OO. The other study by Suárez et al. [34] demonstrated that TPC of the OO increased from 172 mg caffeic acid/kg to 562 mg caffeic acid/kg by adding a combination of the olive cake extracts. The results of this study were in agreement with the results obtained by Venturi et al. [33] and Suárez et al. [34]. Lafka et al. [35] examined the effects of different extraction solvents on the recovery of phenolics from OO mill wastes, wherein TPC of these extracts was different

Table II - TEAC of OMWW and OP extracts

Extract	Concentration (mg/mL)	Inhibition (%)	TEAC (μM)
OMWW			
Methanol	0.5	37.0±1.5	9.7±0.5dB
	1.0	57.6±3.3	16.2±1.0cA
	2.0	80.3±1.5	23.3±0.5bA
	3.0	88.8±3.2	26.0±1.0aB
Ethanol	0.5	27.7±2.2	6.8±0.7cC
	1.0	46.0±0.3	12.6±0.1bB
	2.0	77.7±0.2	22.5±0.1aA
	3.0	78.5±0.4	22.7±0.1aB
OP			
Methanol	0.5	27.2±1.0	6.7±0.3dC
	1.0	42.0±0.6	11.3±0.2cB
	2.0	84.7±1.7	24.7±0.5bA
	3.0	89.5±2.0	26.2±0.6aA
Ethanol	0.5	43.8±0.6	11.9±0.2cA
	1.0	54.3±1.8	15.2±0.6cA
	2.0	78.1±8.9	22.6±2.8bA
	3.0	92.3±4.3	27.1±1.3aA

*Analyses were done in duplicate and results are given as mean ± std deviation.
[a-d] Small letters show the variation between the different concentrations of the same extract (p<0.05)
[A-C] Capital letters show the variation between extracts at the same concentration (p<0.05)

from each other. The differences in TPC between the tested oils could be attributed to the phenolic extracts containing different phenolic compounds.

3.3 THERMAL OXIDATIVE STABILITY OF OILS USING DSC

Table IV shows the values for the IP obtained by DSC. The IP of the control sample was 22.98 min, while IP of oil samples containing methanol extracts from OMWW (37.92 min) and OP (34.01 min) was higher than the control sample. There is a greater increase in IP of samples enriched with methanol extracts of OMWW (37.92 min) compared to ethanol extracts (34.01 min). In addition, the extracts added with lecithin increased the IP more than samples containing extracts only. These results are in agreement with results of Günal and Turan [36] who demonstrated that OMWW and OP extracts at 1 mg/g could effectively protect SO. The OMWW and OP extracts exhibited high IP in SO in agreement with the polar paradox theory that stated that polar antioxidants are more effective in bulk lipids than their nonpolar counterparts, whereas nonpolar antioxidants are better antioxidants in oil-in-water media than their polar homologs [37, 38].

BHT was also used to compare IP differences in oil samples containing different extracts and lecithin. As seen in Table IV, both extracts and lecithin improved IP of SO compared to BHT. Zhang et al. [39] explained this situation with the valorisation of BHT and thus removed it from foods at high temperatures. The IP of SO+L was 34.95 min and this value was higher than SO. The similar results are in agreement with Judde et al. [24] who stated that lecithin (1%, w/w) exhibited good antioxidant activity and increased the IP of several oils such as soybean, palm, walnut, fish and pig oils. This literature also assumed that strong antioxidant effect of lecithin could be related to a synergistic effect between amino-alcohol phospholipids and γ-/δ-tocopherols. The synergistic effect of lecithin, when used with antioxidants, is attributed to an increase in antioxidant efficiency by increasing the solubility of antioxidant [40, 41]. Thus, in this study, the use of lecithin together with extract caused higher IP than the extract alone, since lecithin was thought to increase the amount of phenolic substances dissolved in the oil.

Table III - TPC of SO samples

Sample	TPC (ppm)*	Sample	TPC (ppm)*
SO	8.60 ± 1.0	SO+L	9.70 ± 0.4
SO+WWM (1 mg/g)	14.2 ± 1.0	SO+WWM (1 mg/g)+ L (5 mg/g)	21.9 ± 0.3
SO+WWM (2 mg/g)	17.9 ± 2.2	SO+WWM (2 mg/g)+ L (5 mg/g)	49.3 ± 1.5
SO+WWE (1 mg/g)	15.1 ± 0.7	SO+WWE (1 mg/g) + L (5 mg/g)	20.2 ± 1.1
SO+WWE (2 mg/g)	17.7 ± 1.7	SO+WWE (2 mg/g) + L (5 mg/g)	21.2 ± 0.8
SO+PM (1 mg/g)	13.7 ± 1.4	SO+PM (1 mg/g) + L (5 mg/g)	22.5 ± 1.0
SO+PM (2 mg/g)	15.2 ± 2.1	SO+PM (2 mg/g) + L (5 mg/g)	28.3 ± 1.5
SO+PE (1 mg/g)	13.2 ± 1.6	SO+PE (1 mg/g) + L (5 mg/g)	19.8 ± 2.3
SO+PE (2 mg/g)	17.1 ± 1.9	SO+PE (2 mg/g) + L (5 mg/g)	17.3 ± 0.6

*Analyses were done in duplicate and results are given as mean ± std deviation.
SO: Sunflower oil, L: Lecithin, WWM: Wastewater methanol, PM: Pomace methanol, WWE: Wastewater ethanol, PE: Pomace ethanol.

Table IV - Induction periods of SO samples using DSC

Sample	IP (min)	Protection factor
SO	22.98±0.18g	
SO+L (5 mg/g)	34.95±0.54d	1.52
SO+WWM (1 mg/g)	37.92±0.47c	1.65
SO+WWM (1 mg/g)+L (5 mg/g)	44.76±0.43a	1.95
SO+PM (1 mg/g)	34.01±0.30e	1.48
SO+PM (1 mg/g)+L (5 mg/g)	41.22±0.42b	1.79
SO+BHT (0.2 mg/g)	26.99±0.31f	1.17

*Analyses were done in duplicate and results are given as mean ± std deviation.
The induction periods were determined by DSC at 130ºC under a stream of oxygen at 50 mL/min.
SO: Sunflower oil, L: Lecithin, WWM: Wastewater methanol, PM: Pomace methanol.

3.4 THERMAL OXIDATIVE STABILITY OF OILS AT 180°C

Table V shows the mean of changes in the CD, *p*-anisidine value, tocopherol content and IP of oil samples during the heating at 180°C. The CD values significantly increased from 0.28% to 2.29% after 40 h of heating. However, oils enriched with extracts and lecithin exhibited low CD values during heating. The enriched oils had CD content in the range from 1.06% to 2.1%. OMWW extract was more effective than OP extract according to CD levels. CD content of SO+WWM was close to that of SO+BHT. As compared to extracts, the CD content decreased dramatically in samples containing both extracts and lecithin. These results are similar to those reported by Lee et al. [42] who showed that lower CD values in soybean oil mixed with some extracts from olive leaves than the control sample.

The *p*-anisidine value of the control sample reached 276.99 from an initial value of 7.56 after 40 h of heating. The *p*-anisidine values of all treatments except for one sample (SO with PM) were significantly lower than that of the control ($p<0.05$, Table V). The combined addition of lecithin and extracts produced an increment in the oxidative stability of all enriched SO samples compared to the control in all studied combinations. According to *p*-anisidine values, BHT was more effective against oxidation than extracts except for one sample (SO+WWM+L).

A steady decrease in the tocopherol content was recorded for all oils (Table V), with final values between 177.42 ppm and 312.65 ppm at the end of heating. After 40 h of heating, higher levels of tocopherols remained in oil samples mixed with OMWW extract or lecithin. In our study, thermal oxidation caused a significant decrease of tocopherols in all experiments. The lowest tocopherol values in the first 16 h of oxidation were determined in SO. Addition of lecithin to SO provided slowly degradation of tocopherols and there could be a synergistic effect of lecithin on tocopherols. Similar results were obtained in several studies on the synergistic effect of lecithin on tocopherols [25, 27, 41, 43, 44].

The synergistic or antioxidant effect of lecithin or phospholipids when used with antioxidants is based on several reasons in literature; (1) lecithin increases antioxidant efficiency by increasing the solubility of antioxidant [40, 41], (2) phospholipids located at the oil/water or air interface and acted like an oxygen barrier to protect the oil/fat from oxidation [24, 25, 45], (3) amino-carbonyl reactions between amino groups of phospholipids and oxidation products cause the formation of compounds that have antioxidant properties [25-27, 46, 47].

IP decreased like in the case of tocopherol content during thermal oxidation (Table V). Results demonstrated that all enriched oils showed higher IP compared to SO. At the end of heating, IP value decreased from 22.98 min to 1.73 min. Among the extracts, the highest value for the IP was observed in OMWW extract with the value of 7.31 min at the end of heating. When lecithin was added in combination with extracts, the IP of SO was better than when individual extracts were added.

4. CONCLUSION

OMWW and OP extracts significantly inhibited the formation of hydroperoxides in SO and had an antioxidant activity close to BHA. The antioxidant activities of OMWW extracts determined by CD method in the linoleic acid emulsion were higher than those of OP extracts. TEAC values of OMWW extracts were between 6.8 and 26.0 μM, while TEAC values of OP were between 6.7 and 27.1 μM. The study showed that the amount of phenolic substances dissolved in SO were related to the antioxidant capacities of samples. When extract and lecithin added together into SO samples, TPC of samples was higher than SO enriched only with extracts.

The addition of extracts ensured an increase in the IP of SO. In our study, OMWW and OP extracts had a considerable amount of polar-structured phenolic compounds. Thus, these polar phenolic compounds protected SO from oxygen at oil-air interface according to polar paradox hypothesis. The use of lecithin combined with the extracts was more effective and higher protection factors were achieved. The IP of those samples was higher than SO+BHT. In brief, the addition of lecithin combined with extracts increased

Table V - Properties of SO at 180°C

Period (h)	SO	SO+L	SO+WWM	SO+WWM+L	SO+PM	SO+PM+L	SO+BHT
Conjugated Diene Content (%)							
Zero	0.28±0.01eA	0.24±0.00eB	0.23±0.00fB	0.23±0.00eB	0.27±0.01fA	0.25±0.00eB	0.24±0.01fB
8	0.63±0.01dA	0.51±0.01dC	0.60±0.03eAB	0.49±0.04dCD	0.57±0.00eB	0.45±0.02dD	0.49±0.01eCD
16	1.28±0.01cA	0.87±0.01cC	0.69±0.03dE	0.69±0.00cE	0.78±0.03dD	1.11±0.00cB	0.78±0.02dD
24	1.71±0.01bA	1.31±0.06bB	0.95±0.04cD	0.91±0.00bD	1.66±0.05cA	1.09±0.04cC	0.96±0.03cD
32	2.06±0.01bA	1.31±0.00bC	1.04±0.01bE	0.93±0.00bF	1.94±0.03bB	1.25±0.02bD	1.06±0.05bE
40	2.29±0.00aA	1.46±0.06aC	1.17±0.01aD	1.06±0.00aE	2.1±0.02aB	1.42±0.05aC	1.21±0.00aD
p-anisidine value							
Zero	7.56±0.62A	7.82±020fA	5.22±0.06fD	4.93±0.18fD	6.70±0.04eB	5.91±0.14fC	7.24±0.13fAB
8	100.58±1.33dB	74.83±0.13eC	70.57±0.37eE	61.72±0.28eF	107.47±0.73dA	73.22±0.28eD	60.38±0.19eF
16	178.99±1.85cB	124.06±3.41dC	120.87±0.63dC	88.57±1.75dE	231.71±2.08cA	101.18±2.00dD	89.99±0.27dE
24	256.79±7.96bB	173.74±3.13cC	163.24±0.68cD	118.06±0.79cF	309.01±4.05bA	150.40±1.84cE	122.74±0.47cF
32	268.17±0.84bB	181.52±1.09bC	174.15±1.89bD	122.47±0.61bF	325.05±5.19aA	181.59±0.29bC	129.93±4.69bE
40	276.99±8.74aB	191.88±5.48aC	178.52±0.11aD	127.75±1.79aF	322.82±3.98aA	194.18±6.05aC	141.52±2.86aE
Tocopherol content (ppm)							
Zero	521.56±1.46aAB	527.72±4.12aA	502.14±8.98aB	533.1±10.80aA	501.38±14.82aB	532.73±5.42aA	536.41±5.32aA
8	238.65±12.39bF	340.75±0.18bD	515.62±5.24aA	437.07±10.18bB	297.52±4.92bE	375.88±9.01bC	423.56±6.89bB
16	118.57±3.78eF	226.86±1.91cE	444.85±2.34bA	352.49±12.46cC	127.59±3.32eF	319.49±0.81cD	367.19±8.44cB
24	157.08±8.77dE	180.77±5.31dD	383.5±2.31cA	320.41±1.39dB	144.34±21.25eE	205.49±7.98dC	312.17±6.80dB
32	206.87±9.08cD	156.84±1.35eG	345.36±11.22dA	296.19±7.43eB	188.11±0.07dE	173.11±1.49eF	245.83±1.03eC
40	245.52±0.77bB	177.42±1.55dE	312.65±2.68eA	301.33±4.98deA	221.89±0.88cC	177.64±5.89eE	208.75±11.70fD
IP (min) at 130°C							
Zero	22.98±0.18aG	34.95±0.47aD	37.92±0.47aC	44.76±0.43aA	34.01±0.30aE	41.22±0.42aB	26.99±0.31aF
8	18.28±0.93bD	16.86±0.40bE	32.90±0.36bA	33.37±0.07bA	21.55±0.11bC	28.92±0.08bB	16.63±0.04bcE
16	9.00±0.08cE	15.15±0.12cD	22.01±0.04cB	26.92±0.37cA	6.83±0.51F	21.32±0.26cB	16.10±0.46cC
24	2.85±0.20dF	7.97±0.36dE	10.60±0.15dC	27.50±0.60cA	2.05±0.56dF	9.39±0.22dD	13.54±0.04dB
32	2.25±0.16deF	4.26±0.25eE	8.79±0.50eC	24.05±0.33dA	0.80±0.07eG	7.93±0.08eD	14.02±0.35dB
40	1.73±0.02eD	4.07±0.23eC	7.31±0.14fB	16.94±0.26eA	1.80±0.38dD	6.97±0.49fB	17.27±0.71bA

*Analyses were done in duplicate and results are given as mean ± std deviation.
a-f Small letters show the variation between the different heating times (p<0.05).
A-G Capital letters show the variation between extracts at the same heating times (p<0.05).
SO: Sunflower oil, L: Lecithin (5 mg/g), WWM: Wastewater methanol (1 mg/g), PM: Pomace methanol (1 mg/g)

the TPC, antioxidant activity and IP of SO samples. These results could be due to that lecithin is thought to increase the amount of phenolic compounds dissolved in the oil and there is a synergist effect of lecithin with phenolic compounds in the extract.
During the thermal oxidation test at 180°C, OMWW extract was more effective than OP extract in reducing the CD content. Again, lecithin increased the efficiency of OMWW or OP extracts. OMWW extract was effective in lowering *p*-anisidine value, while OP extract was pro-oxidant. In addition, in the presence of lecithin, OMWW extract had better *p*-anisidine, tocopherol content and IP values than in SO containing BHT. The study had shown that OMWW extract and lecithin had a protective effect against thermal oxidation of oils and had increased the effect of phenolic compounds during thermal oxidation.

Acknowledgements
Authors would like to thank Bolu Abant Izzet Baysal University Scientific Research Projects for funding this research (Project No: 2012.09.01.505) and Bolu Abant Izzet Baysal University, Innovative Food Technologies Development Application and Research Center (YENIGIDAM) for the supports in DSC analyses.

Compliance with ethical standards

Conflict of interest
The authors declare that they have no conflict of interest

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Received: February 13, 2019
Accepted: May 24, 2019