Combined use of some natural antioxidants in sunflower oil

Betül Bozyokuş
Derya Arslan✉

Division of Food Sciences
Department of Food Engineering
Faculty of Engineering
Necmettin Erbakan University
Konya, Turkey

Combined use of antioxidants is highly effective in preventing oxidation. In this study, combined effects of ethanolic extracts of turmeric (T) and potato peel (PP), α-tocopherol (α), β-carotene (β), ascorbyl palmitate (AP), citric acid (C) and lecithin (L) were evaluated in sunflower oil. The antioxidant activities of these substances were evaluated individually and with binary and ternary combinations with different concentrations. The combined effect of AP (1000 ppm) and T (5000 ppm) was strong when all oxidative evaluation analysis were considered: lowering peroxide value by 3.5-fold, almost doubling the induction time and having the highest protection factor percentage (94.95%). AP in blends was found efficient in terms of protecting sunflower oil against oxidation and was not present in non-efficient mixtures. L followed it regarding efficiency. In the mixtures consisted of AP-L-PP (83.7% protection factor (PF)) and AP-L-T (19.6% PF), namely in the presence of identical concentrations of PP and T, superior PF for the mixture containing PP was determined, which was thought to be based on the higher hydrophilicity of PP. C and α in mixtures were not effective enough to retard the oxidation. Combinations of PP-L-β (-23.8%) and T-L-α (-19.5%) showed negative relationships. The results also demonstrated concentration-dependency of the antioxidants in terms of antioxidant efficiency. Our findings about the efficient interactions between different natural compounds could provide information to develop natural plant-based antioxidant mixtures with greater effectiveness than their single use in retarding oil oxidation.
Keywords: Ethanolic extracts; Natural antioxidants; Potato peel; Sunflower oil; Antioxidant efficiency; Turmeric

1. INTRODUCTION

Antioxidants used in oils increase quality by inhibiting oxidation and assistance to extend shelf life. The use of synthetic antioxidants as food additives is common in the food industry. However, despite their high stability, synthetic antioxidants are being questioned due to their possible negative effects on human health [1]. In addition, the high volatility of synthetic antioxidants causes their quantity to decrease during heat treatment. Most of natural additives have been reported to have more antioxidant activity and thermal stability in edible oils than synthetic ones [2, 3].

The oxidation process is a complex mechanism and sometimes more than one antioxidant may be required to provide complete protection. Therefore, more successful results can be obtained by using combinations of antioxidants with synergistic effect [4-6]. The affirmative effect caused by the fortification of each other during the oxidation of antioxidants is called “synergism”. When the two antioxidants are combined, the effect of synergism happened is statistically greater than the sum of the individual effects of the two.

α-Tocopherol is known to increase the antioxidant effect by sheltering carotene and ascorbic acid. Phenolic compounds act as stabilizers of tocopher-

✉ CORRESPONDING AUTHOR:
Derya Arslan
NEÜ Köyceğiz Yerleşkesi
42080 Meram/Konya Turkey
Email: dears@erbakan.edu.tr
Tel: +905355640994
ORCID ID:
https://orcid.org/0000-0002-6655-9312

Received: March 6, 2022
Accepted: May 11, 2022

ols. Stabilised tocopherols keep the carotenoids and make them reusable [7].
β-carotene as a remarkable chain-breaker antioxidant [8] increases shelf life of foods rich in unsaturated fatty acids depending on concentration [9]. Tocopherols have been divulged to be more effective when used with other antioxidants [5]. Pedrielli and Skibsted [10] reported that the regeneration of tocopheroxyl radicals to tocopherols by phenolic groups in the synergy mechanism depends on the flavonoid concentration. Furthermore, the rate of penetration of non-covalently linked polyphenols into the lipid phase depends on the regeneration rate of carotene. The O - H bond in phenolic compound demonstrates the importance of kinetic effects for antioxidant synergism of carotenoids and polyphenols [11].
Turmeric, one of the most studied spices, has a polyphenolic compound named as curcumin with bitter taste [12]. Water-soluble peptide turmerin and lipid-soluble curcumin have been isolated in turmeric and it has been reported that these compounds show strong antioxidant effects which is even more successful than vitamin E and as effective as butylated hydroxy toluene (BHT) in preventing oxidation [13]. Although there are many studies indicating that turmeric has high antioxidant effect, there are not enough studies examining its effectiveness in oil particularly combined use with other antioxidants. For this reason, in this study, it was aimed to get an idea about the efficacy of curcumin alone and together with other antioxidants in sunflower oil.
Potato peel is a valueless waste that occurs in large quantities after processing and recently there is an increasing interest in the use of this waste to produce food additives or nutritional supplements [14]. The overpowering phenolic compounds of PP are chlorogenic, gallic and caffeic acids [15]. These are strong sources of natural antioxidants that prohibit oxidation of vegetable oils [16-18]. Potato peel is known to improve nutritional quality while reducing lipid peroxidation of lamb meat [19], minced mackerel [20], biscuits [21] and soybean oil [22, 23]. In addition, potato peel extract has been reported to be six times more effective than BHA and BHT [24].
Lecithin contains both hydrophilic and hydrophobic groups in its molecular structure and is an additive with antioxidant activity used in industry due to its emulsifying feature. This antioxidant effect is due to the phospholipids that make up lecithins. Phospholipids, being a synergistic antioxidant, have the potential to improve the antioxidant properties of phenolic compounds. The capacity of phospholipids to convert tocopheryl radicals to tocopherols in the presence of L-ascorbic acid depends on the reducing potential of the surrounding phenolic compounds in the medium [25].
Ascorbyl palmitate is accepted as a natural antioxidant as it can be hydrolysed to palmitic acid and ascorbic acid in the body. The use of ascorbyl palmitate with other antioxidants stabilises vegetable frying oils [26].
Citric acid, when used with antioxidants, is a common and effective chelating agent that enhances its effects and has a wide range of applications. Citric acid and rosemary extract (especially when combined with ascorbyl palmitate) are known to show extra antioxidant effect, but antagonism in mixtures including α-tocopherol [27].
Hence, in this study all aforenamed ingredients [natural antioxidants (α-tocopherol (α), β-carotene (β), plant-based extracts (turmeric (T) and potato peel (PP)) and some compounds of natural origin (ascorbyl palmitate (AP), citric acid (C) and lecithin (L))] were added into sunflower oil (SO) with different concentrations and combinations. SO was preferred due to its very common usage worldwide. The effectiveness of these compounds was evaluated by the peroxide (PV), *para*-anisidine (*p*-AV), TOTOX and oxidative stability index values.

2. MATERIALS AND METHODS

2.1. MATERIALS

A fresh lot of refined SO with no additives was obtained directly from a vegetable oil refinery company located in Konya (Turkey). Turmeric powder was purchased from a local market. Sunflower lechitin (E 322) was procured from Alfasol (Turkey). The potato peels used in this study were the wastes of a catering company located in the city of Konya, Turkiye. α-Tocopherol (E 307), anhydrous granular citric acid and β-carotene (E 160) were purchased from Alfasol (Turkey). Ascorbyl palmitate (E 304) obtained from Arkem Chemistry (Turkey). All the chemicals and solvents (analytical grade) were purchased from Merck, USA. Water was purified with a Mili-Q-system (Milipore, Bedford, MA, USA) for Rancimat test.

2.2 METHODS

2.2.1 Preparation of turmeric and potato peel extracts

Turmeric powder was mixed with ethanol (≥ 97%, v/v; at 1:10 (g/mL) ratio and kept in ultrasonic water bath (Bandelin RK 100H, 35 kHz, Germany) for 1 hour. The mixture was filtered through Whatman No 1 filter paper. The filtrate was evaporated under vacuum in a rotary evaporator (Heidolph, Hei-Vap Core, Germany) at 50°C until the alcohol was removed [28]. The extraction yield was 40 g/kg turmeric powder.
Potato tubers were washed in a local catering company and peeled manually by using a peeler knife, then transferred to laboratory immediately. The potato peels were washed and then dried in an oven (Nuve, Turkey) dried at 70°C for 24 h. The dried peels were ground into powder in a cof-

fee grinder (Premier, PRG 259, South Korea). Potato peel powder was mixed with ethanol (≥ 97%, v/v; at a ratio of 1:10 (g/mL) and stirred in a water bath at 50°C for 6 hours. The supernatant was collected by filtering through Whatman No 1 filter paper. The ethanol contained in the filtrate was removed on a rotary evaporator at 45°C [29]. The extraction yield was 1.2 g/kg potato peel.

2.2.2 Sample Preparation

The mixtures selected according to preliminary PVs were added to 120 g of oil sample. Additives and extracts were homogenously dissolved in oil by stirring at 700 rpm for 30 second with the homogenizer (Witeg, WiseTis HG15D, Germany) and incubated for 20 s in the ultrasonic water bath (Bandelin, RK100H, Germany) to eliminate air bubbles. All samples containing antioxidant mixtures were stored in tightly closed containers at room temperature and protected against light and air. Concentrations of natural additives and extracts were determined according to the values reported in literature, and concentrations not previously used in literature were also included in the study [7, 25, 27, 30-33]. The abbreviations of additives and their concentrations are shown in Table I.

In the beginning of the study, for pre-elimination, antioxidants were added into oil single or binary and ternary combinations. The Schaal oven test was applied to all samples to expedite the formation of hydroperoxide [34]. 12 g of oil sample was weighed into each petri dish and the analysis took 6 days at 65±1°C. (Nuve, Turkey). PVs were measured on the day 0,3 and 6. The samples with the lowest PVs were selected for further experiments which were carried out with different concentrations of the compounds. The single addition of all the antioxidant compounds used in the study were not as much effective as their binary and ternary mixtures. We continued to work with combinations of these compounds that provide superior oxidative stability detected in terms of PVs. The PVs of the samples were determined also initially before subjected to the oven test (presented in Figure 1 as day 0). The research continued with examples having PVs below 20 (Table II).

2.2.3 Determination of oxidative stability

PV was measured in 3-days intervals by using the method of AOCS [35]. As a result of the peroxide analysis, 28 samples (details are given below the figures) with the lowest PVs were determined and the study continued with these samples. Formation of secondary oxidation products was determined by *p*-AV according to AOCS [34]. The total oxidation (TOTOX) capacity was acquired by a calculation using the PV determined on the first day of oven test and *p*-AV results as in Eq 1[36]:

$$TOTOX = 2PV + p\text{-}AV \qquad \text{(Eq 1)}$$

Table I - Concentrations of antioxidants used at the first stage of the study

Additives (abbreviations)	Concentrations (ppm)
Turmeric extract (T)	7500, 6500, 5000, 3500, 2166.6, 1750
Potato peel extract (PP)	7500, 6500, 5000, 3500, 2166.6
Ascorbyl palmitate (AP)	2000, 1500, 1000, 666.6, 500, 166.6
Lecithin (L)	750, 650, 400, 325, 216.6, 150, 133.3
α-Tocopherol (α)	1500, 1200, 800, 400, 133.3
β-Carotene (β)	1000, 750, 450, 225, 200, 150, 100, 66.6
Citric acid (C)	1000, 750, 450, 225, 200, 66.6

Table II - Twenty seven mixtures selected to be used in subsequent analysis

Mixtures	Concentrations (ppm)
T-C	T(6500) C(225)
PP-L	PP(3500) L(650)
PP-C	PP(6500) C(200)
L-α	L(650) α(400)
L-β/1	L(650) β(200)
L-β/2	L(325) β(100)
T-L	T(1750) L(325)
β-C	β(225) C(225)
L-C	L(1750) C(325)
T-AP/1	T(6500) AP(500)
T-AP/2	T(5000) AP(1000)
PP-AP/1	PP(6500) AP(500)
PP-AP/2	PP(5000) AP(1000)
AP-L	AP(1000) L(400)
AP-L-T	AP(666.6) L(133.3) T(2166.6)
AP-L-PP/1	AP(666.6) L(133.3) PP(2166.6)
AP- L- PP /2	AP(666.6) L(216.6) PP(2166.6)
T-AP-PP	T(2166.6) AP(166.6) P(2166.6)
AP-C-L	AP(666.6) C(66.6) L(216.6)
AP-C-β	AP(666.6) C(66.6) β(150)
AP-L-α	AP(666.5) L(216.6) α(133.3)
AP-L-β	AP(666.5) L(216.6) β(150)
T-C-L	T(2166.6) C(66.6) L(216.6)
T-PP-L	T(2166.6) PP(2166.6) L(216.6)
T-L-α	T(2166.6) L(216.6) α(133.3)
PP-L-β	PP(2166.6) L(216.6) β(150)
PP-C-β	PP(2166.6) C(66.6) β(150)

T: Turmeric extract, PP: Potato peel extract, AP: Ascorbyl palmitate, L: Lecithin, α: α-tocopherol, β: β-carotene, C: Citric acid.
The numbers in parentheses specify concentrations in "ppm".

2.2.4. Determination of the Induction period (IP by Rancimat test)

The induction period of the samples stored at room temperature protected from light and air for three months was analysed by Metrohm Rancimat 892 (Herisau, Switzerland) in accordance with the ISO 6886 procedure. Samples (3 g ± 0.1 g in each reaction tube) were oxidised at a temperature of 110°C under a 20 L/h air flow. During the analysis, the conductivity of the water was continuously measured in a measuring apparatus containing 60 mL of ultra-distilled water (Merck Millipore, Direct-Q3UV, Germany). Volatile oxidative products were collected in this apparatus [37, 38].

2.2.5 Calculation of protection factor (PF, %)

The induction period results were used to calculate the PF as percentage. The equation is shown in Eq 2:

$$\% \text{ PF} = \frac{(IP_b - IP_c)}{IP_c} \times 100 \qquad \text{(Eq 2)}$$

where IP_b and IP_c represent the IP (h) at 110°C of oil with antioxidant blends and control sample, sequentially. A positive value denotes a protective effect between the used antioxidants, while a negative value stands for negative interaction among them [39].

2.2.6 Statistical analysis

Each analysis was done in triplicate and data reported as mean±standard deviation (SD). The results were analysed using one-way analysis of variance ANOVA. Statistical analysis for functional properties were performed using SPSS (Statistical Programme for Social Sciences, SPSS Corporation, Chicago, IL, USA). The confidence limit used in this study was 95% (p<0.05).

3. RESULTS AND DISCUSSION

3.1. EVALUATION OF PV, P-AV, TOTOX AND INDUCTION TIME

At the beginning of the study on day 1st, PP-L-β, PP-C-β, β-C, T-PP-L mixtures, showed clearly higher PVs compared to other mixtures (Fig. 1). The binary mixtures of AP with T exhibited low PVs. Although L (650 ppm) – β (200 ppm) (L-β/1) and L (650 ppm) – β (400 ppm) (L-α) mixtures initially showed low PVs, looking at the 3rd and 6th days of analysis results they couldn't stop the rapid progress of PVs and exhibited higher values compared to other mixtures. Eventually, T-AP/1, T-AP/2, PP-AP/1, PP-AP/2, T-AP-PP were the mixtures which exhibited the greatest stability in terms of PVs on the 6th day of analysis. Upadhyay and Mishra [32] demonstrated the synergistic relationship between rosemary and sage extracts and AP which provided better stabilization than TBHQ. Similarly, the powerful antioxidant effect of AP containing mixtures was observed in the present study. C showed a slight antioxidative effect against the oxidative stability of SO, while α showed prooxidative effect (results not reported) as reported in the study of Hras et al. [27]. In fact, only three of the selected mixtures contain α.

Similarly, from the mixtures containing α, three mixtures were eliminated in terms of preventing oxidation in the first stage, L-α showed prooxidative effects in the later stages, the AP-L-α mixture exhibited a modest antioxidant effect. Results agree with the findings of Hras et al. [27]; as they reported a considerable and a slight antioxidative effects of AP and CA, respectively. The authors also reported about the prooxidative effect of α. Pop [40] showed efficient stabilisation of fish oil microencapsuls by a ternary use of tocopherols derivatives, lecithin and ascorbyl palmitate. Bodoira et al. [33] determined the antioxidant activity of tocopherols in chia oil (in the range of 50-800 ppm) and reported that when the α was used at 800 ppm level, it possessed the lowest antioxidant activity. Similarly, according to our findings, α in concentrations ranging from 133.3-1500 ppm was not effective enough to retard the oxidation.

As a result of the peroxide analysis carried out on the 3rd and 6th days, T-AP, PP-AP, T-AP-PP mixtures were clearly differentiated from others by exhibiting strong antioxidant activity. T-L-α, T-PP-L, T-C-L, PP-L-β samples showed high PVs (above 37 meq O2/kg). However, PVs were calculated as 31 meq O2/kg in SO with no antioxidants. It was clear that L (216.6 ppm) and the combination of T and PP (2166 ppm each), when used only at certain concentrations, showed antagonist effect. In addition, the absence of AP in these mixtures was also noticeable. Hras et al. [27] debated that merely C decreased PV and *p*-AV, even though CA alone has antioxidant activity, this effect was greater when combined with another antioxidant. However, in this study the antioxidative efficiency of C was not evident among the other additives.

TOTOX results were consistent with the PV results mentioned above (Fig. 3). Samples with low PVs showed low TOTOX values, too. In terms of p-AV, the combined use of AP and natural extracts each (PP-T) resulted in low TOTOX values just like the PVs. However, some mixtures that did not stand out in terms of PVs that were found to give affirmative *p*-AV results (Fig. 2). The T-L-α mixture, which gave undesirable results in terms of PVs, contradictorily provided low *p*-AVs. While this mixture could not prevent the formation of peroxyl radicals that appeared in the initial phase of oxidation, it was able to prevent secondary oxidation products that could have toxic effects. Doert et al. [25] explained that the combination of α and L enabled the regeneration of tocopherols. Although samples containing a mixture of L-β and L-T had low PV on day 0 of the study, the formation of hydroperoxides increased rapidly on the day 3 and 6 measurements. However, it was observed that these samples started to maintain their stability during the propagation phase of oxidation and did not allow further rancidity. In a study examining the synergistic effect of L and α's in various oils, it was reported that L delayed the oxidation of monounsaturated fatty acids in the absence of α's. In addition, for linoleic acid rich oils such as sunflower, soybean and rapeseed, while the IP increased with the L concentration, the PV decreased [5].

The mixtures which gave positive results for both PV and *p*-AVs consisted of AP (500 ppm) – T (6500 ppm) and AP (1000 ppm) – T (5000 ppm). AP-L-β added oil sample also followed a stable course and showed lower PVs than that of control, and this sample also showed low *p*-AVs.

AP (1000 ppm) – T (5000 ppm) (T-AP/2) and AP

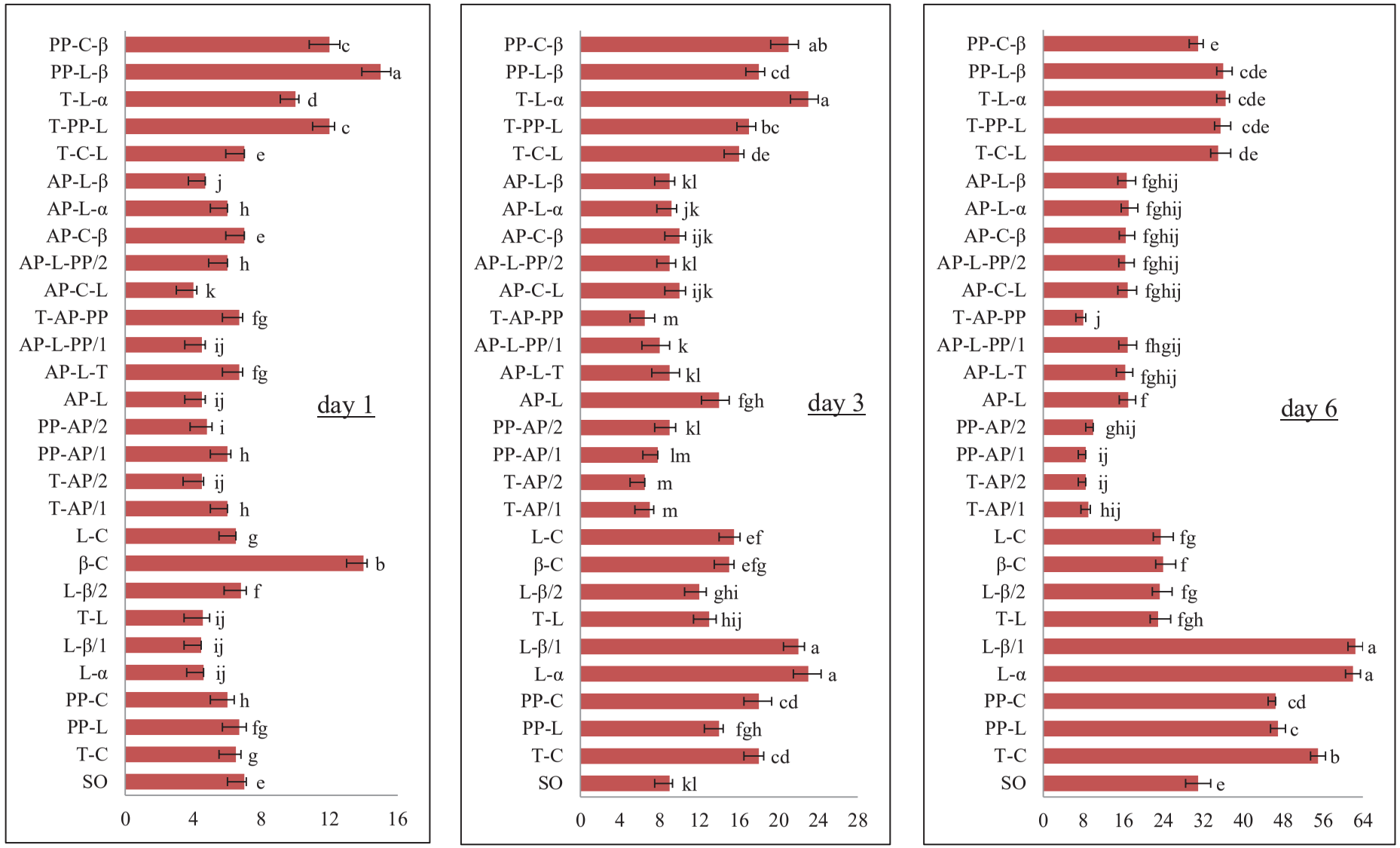

Figure 1 - Effect of specified additives on the peroxide value (meq O_2/kg) of sunflower oil stored at 65°C for days 0, 3 and 6. Bars represent standard deviations, n = 3. Small case letters show significant differences between values of different oil samples.
SO: Sunflower oil without antioxidant, T: Turmeric extract, PP: Potato peel extract, AP: Ascorbyl palmitate, L: Lecithin, α: α-tocopherol, β: β-carotene, C: Citric acid.

Figure 2 - Effect of antioxidants on the *p*-anisidine value (g^{-1}) of sunflower oil stored at room temperature for 90 days. Bars represent standard deviations, n = 3. Small case letters show significant differences between values of different oil samples.
SO: Sunflower oil without antioxidant, T: Turmeric extract, PP: Potato peel extract, AP: Ascorbyl palmitate, L: Lecithin, α: α-tocopherol, β: β-carotene, C: Citric acid.

Figure 3 - Total oxidation (TOTOX) results of the oils in the presence of antioxidant mixtures. Bars represent standard deviations, n = 3. Small case letters show significant differences between values of different oil samples.
SO: Sunflower oil without antioxidant, T: Turmeric extract, PP: Potato peel extract, AP: Ascorbyl palmitate, L: Lecithin, α: α-tocopherol, β: β-carotene, C: Citric acid.

(1000 ppm) - PP (5000 ppm) (PP-AP/2) which led to one of the lowest PV on the 6th day, confirmed this positive effect with longer IPs. Combination of AP (666.6 ppm) – L (133.3 ppm) - PP (2166.6 ppm) (AP-L-PP/1), which was one of the mixtures providing the lowest PVs at the beginning (on day 0), demonstrated its effectiveness again with high IP values. In the absence of δ- and γ-tocopherols, L was shown to delay the oxidation of monounsaturated acids but not of polyunsaturated fatty acids. So, the antioxidant activity of L was not effective for SO as a linoleic rich oil and contains α predominantly [5].

The induction time of SO in the presence of antioxidants were between 2.97 to 7.60 h at 110°C. Generally, the results for the TOTOX and Rancimat IP values (Fig. 4) were compatible, and both showed that AP combined with T or PP with given concentrations (T-AP/2, PP-AP/2 and AP-L-PP/1) resulted in greater stability in the SO. Oxidative protection resulting from the addition of AP together with plant extracts in vegetable oils has been reported in literature [41-43]. Negative interactions between the compounds in mixtures PP-C-β, PP-L-β, T-L-α, T-C-L and PP-C were evident, as they produced a lower induction time compared to that of the control sample which contained no antioxidant. Definite concentrations of the compounds were remarkable in these antagonistic mixtures.

3.2 ANTIOXIDANT EFFICIENCY AS PROTECTION FACTOR (PF) OF THE MIXTURES

The percentages of the PF are given in Table III. T-AP/2, PP-AP/2 and AP-L-PP/2 showed the highest percentage which were the mixtures showing best antioxidant effect in terms of induction time results. The antioxidative effect in improving the oxidative stability of SO for the combination of rosemary extract and AP were reported before [5]. This effect was explained by the help of AP in regeneration of oxidised antioxidant compounds in the extract [44]. It was also noteworthy that AP was not present in the antagonistic mixtures. Regarding the addition of single antioxidant, the lowest PV value was found in the oil sample containing AP (25 meq O_2/kg), followed by the sample containing PP (40.9 meq O_2/kg). T showed the highest PV value (127 meq O_2/kg) (values not shown). The highest antioxidant efficiency of AP was observed when used with T. The fact that PP was more effective than T can be explained by polar paradox. Polar antioxidants are more effective than non-polar homologues in bulk oil, while non-polar antioxidants are more effective in oil-in-water emulsions and liposomes [45]. Polar antioxidants have a higher affinity capacity at the oil-air interface where oxidation is vigorous in bulk oils. However, the polarity of oil is higher than air. The high efficacy of hydrophilic components in oils is thought to be due to the minor components in the oil (such as phospholipids, free fatty acids, monoglycerides) affecting the micelle structures formed by the minor components [46]. These minor compounds in oils have a water phase effect and can easily have a prooxidant character. Thus, the medium acts as a water-in-oil emulsion and hydrophilic antioxidants can conduct on these minor compounds to stabilise the oil. Phenolic compounds (especially caffeic acid) present in potato peels have water soluble characteristics [24]. With the known powerful antioxidant activity of curcumin, a lipophilic component, turmeric has become very important. The antioxidant mechanism of curcumin is on account of the separation of an H atom from the phenolic group [13]. Becker et al. [30] synergy of hydrophilic antioxidants in the phenolic structure had a higher performance in bulk lipid systems. The antioxidant activity observed in oil depends on the polarity of antioxidants. Solubility was also cited as an important factor on the activity of antioxidant compounds.

The compounds of the five given mixtures (PP-C-β, PP-L-β, T-L-α, T-C-L and PP-C), which provided the lowest induction times, exhibited negative effects. A low protection but no negative effect was detected for the combination T-PP-L. However, there was a negative interaction between T-L-α. This confirms the negative effect of α. Prooxidant effects were observed as a result of the increase of free radicals in vegetable oils containing tocopherol as an antioxidant. Tocopherol used in various concentrations in the range of 100-1000 ppm showed prooxidant activity in soybean oil [47]. Huang et al. [48] reported maximum antioxidant activity of α at concentrations less than 100 ppm and higher concentrations show prooxidant activity. Banias et al. [49] reported that α and plant extracts showed antagonism. In the study of Hras et al. [27], when rosemary extract used with C and AP antioxidative effect increased, antagonism occurred in combinations containing α. In the literature, it is also

Table III - Protection factor (%) calculated for the binary and ternary mixtures of the antioxidants used in the assay

Mixtures	Protection factor (%)	Mixtures	Protection factor (%)
T-C	16.42	AP-L-T	19.59
PP-L	68.43	AP-L-PP/1	83.66
PP-C	-7.10	AP- L- PP /2	62.45
L-α	54.75	T-AP-PP	55.52
L-β/1	12.75	AP-C-L	32.68
L-β/2	11.12	AP-C-β	12.23
T-L	42.26	AP-L-α	27.89
β-C	1.71	AP-L-β	34.47
L-C	13.26	T-C-L	-3.25
T-AP/1	24.38	T-PP-L	16.51
T-AP/2	94.95	T-L-α	-19.50
PP-AP/1	57.40	PP-L-β	-23.78
PP-AP/2	79.64	PP-C-β	-4.62
AP-L	41.32		

SO: Sunflower oil without antioxidant, T: Turmeric extract, PP: Potato peel extract, AP: Ascorbyl palmitate, L: Lecithin, α: α-tocopherol, β: β-carotene, C: Citric acid. See Table II for the concentrations.

Figure - 4 Induction periods (h) of oil samples determined under 20 m/L air flow at 110°C. Bars represent standard deviations, n = 3. Small case letters show significant differences between values of different oil samples.
Abbreviations: SO: Sunflower oil without antioxidant, T: Turmeric extract, PP: Potato peel extract, AP: Ascorbyl palmitate, L: Lecithin, α: α tocopherol, β: β-carotene, C: Citric acid. See Table II for the concentrations.

possible to come across examples in which the positive antioxidative effects of tocopherols. The direct interactions between α and β in addition to the possible regeneration of α by *Centella asiatica* extract in the emulsion systems was demonstrated by Thoo et al. [50]. Sacha inchi oil showed a synergistic antioxidant effect with polyphenols, α, AP and C [51]. Murakami et al. [52] also observed the synergistic antioxidant effects of α and ascorbic acid with polyphenolic compounds. An antioxidant interaction was also reported by Marinova et al. [53] for the use of combinations of α and myricetin, which was concentration dependent for α but not for myricetin. The significant antioxidative contribution of AP:α combination (50:50) in chia oil was observed by Bodoira et al. [33] with a synergy percentage of 141%. Protective effect of combined use of AP and α was observed in our study only in the presence of L (216.6 ppm, AP-L-α) though high percentage of protection was not obtained (27.9%). Judde et al. [5] suggested that the high linoleic acid content of SO was also effective in the failure of lecithin to provide a high antioxidant effect. On the other hand, they proposed that the partial antioxidant properties of L were correlated not only to their synergism with tocopherols but also to an antiradical effect which was based on the amino-alcohol groups found in some phospholipids.

The antagonism observed in bulk oils, was also explained by Becker et al. [30] that antioxidants formed intermediates which may be chain-carrying. Metals found in extracts can also be effective in antagonism. The antagonism determined between ascorbic acid - lettuce extract [54] and ascorbic acid - malt rootles extract [55] was partially attributed to the iron in the extracts. Additionally, the prooxidant effect of 150 ppm β (PP-L-β, -23.8%) can be seen from the protection percentages of the ternary mixtures containing PP and L (T-PP-L 16.5% and AP-L-PP/2 62.45%).

When the mixtures AP-L-PP/1 (83.7% PF) and AP-L-T (19.6% PF) compared, which means in the presence of identical concentrations of PP and T, different percentages of protection with a prominently higher ratio in favour of PP in blends was determined. The hydrophilic nature of PP could play a role in here.

Mixtures containing several different amounts of the same substance used at the beginning of the study showed different antioxidant activity. In addition, (PP-AP/1, PP-AP/2), (T-AP/1, T-AP/2) and (L-β/1, L-β/2) mixtures are the mixtures consisting of the same component but containing these components in different amounts. Significant differences were found between their antioxidant effects. This showed us that the effects of the mixtures were dose dependent. Thoo et al. [50] correlated the interactions between antioxidant compounds to the bioactive compounds involved and their ratios in the mixture. Dose dependency of synergism was shown for combinations of almond skin polyphenols and antioxidant vitamins [56].

4. CONCLUSION

It was found that the binary and ternary mixtures of the antioxidants were more effective than their use alone. Based on low peroxide and TOTOX values along with long induction periods, AP-T, AP-PP, AP-T-PP mixtures were clearly differentiated from the others. Additionally, combination of AP (666.6 ppm)-L (133.3 ppm)-PP (2166.6 ppm), which was one of the mixtures providing the lowest PVs at the beginning and demonstrated its effectiveness again with high IP values. These samples also showed the highest protection percentages (94.95% for T and AP combination). AP (1000 ppm) and T (5000 ppm) was best combination when all oxidative evaluation analysis were considered, lowering PV by 3.5 fold and almost doubling the induction time. AP was the most effective in terms of antioxidant efficiency in blends and was not present in the negatively interacted mixtures. C did not exhibit satisfactory efficiency in blends. α when included in mixtures was also not effective enough to retard the oxidation. Negative relationships between PP-L-β and T-L-α were evident. Definite concentrations of the compounds were remarkable in these mixtures. The observed results are indicative of concentration dependency on antioxidative efficiency in combined use. In the mixtures consisted of AP-L-PP (83.7% synergy) and AP-L-T (19.6% synergy), namely in the presence of identical concentrations of PP and T, superior percentages of protection for PP were determined, which was thought to be based on the higher hydrophilicity of PP. Given the results described above, the use of AP with natural ethanolic extracts of PP and T was found promising in providing oxidative stability of bulk edible oils. In the future, further investigations should be undertaken to determine optimum rates and agitation conditions for industrial applications.

Acknowledgement

This paper was drawn up from the master thesis of Betül BOZYOKUŞ and was financially supported by the Office of Scientific Research Projects in Necmettin Erbakan University (NEÜ-BAP, Project number: 191319004).

Compliance with ethical standards

Conflict of interest

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

[1] S. Iqbal, M.I. Bhanger, Stabilization of sunflower oil by galic extract during accelerated storage. Food Chemistry 100, 246-254, (2007).

[2] M.Taghvaei, S. Jafari, Application and stability

of natural antioxidants in edible oils in order to substitute synthetic additives. Journal of Food Science and Technology *52*(3), 1272-1282, (2015).

[3] M. Cisneros-Yupanqui, V.I. Chalova, H.R. Kalaydzhiev, D. Mihaylova, A.I. Krastanov, A. Lante, Preliminary characterisation of wastes generated from the rapeseed and sunflower protein ısolation process and their valorisation in delaying oil oxidation. Food and Bioprocess Technology *14*, 1962–1971, (2021).

[4] J. Yin, E.M. Becker, M.L. Andersen, L.H. Skibsted, Green tea extract as food antioxidant. Synergism and antagonism with α-tocopherol in vegetable oils and their colloidal systems. Food Chemistry *135*, 2195–2202, (2012).

[5] A. Judde, P. Villeneuve, A. Rossignol-Castera, A. Le Guillou, Antioxidant effect of soy lecithins on vegetable oil stability and their synergism with tocopherols. Journal of The American Oil Chemists' Society *80*, 1209-1215, (2003).

[6] J.Y. Kim, S. Oh, B. Yi, M-J. Kim, J.H. Lee, Synergism of phosphatidylcholine on the antioxidant properties of α-tocopherol in corn oils under different relative humidity. International Journal of Food Science and Technology *50*, 1421–1428, (2015).

[7] K. Hasiewicz-Derkacz, A. Kulma, T. Czuj, A. Prescha, M. Zuk, M. Grajzer, M. Lukaszewicz, J. Szopa, Natural phenolics greatly increase flax (*Linum usitatissimum*) oil stability, BMC Biotechnology *15*, 62, (2015).

[8] M. Kumar, V. Pratap, A.K. Nigam, B.K. Sinha, M.K. Singh, J.K. Gour, plants as a source of potential antioxidants and their effective nanoformulations. Journal of Scientific Research *65*, 1-3, (2021).

[9] E. Meroni, V. Raikos, Physicochemical stability, antioxidant properties and bioaccessibility of beta-carotene in orange oil-in-water beverage emulsions: influence of carrier oil types. Food and Function *9*, 320-330, (2018).

[10] P. Pedrielli, L.H. Skibsted, Antioxidant synergy and regeneration effect of quercetin, (–)-epicatechin, and (+)-catechin on α-tocopherol in homogeneous solutions of peroxidating methyl linoleate. Journal of Agricultural and Food Chemistry *50*(24), 7138-7144, (2002).

[11] L.H. Skibsted, Carotenoids in antioxidant networks. Colorants or radical scavengers. Journal of Agricultural and Food Chemistry *60*(10), 2409-2417, (2012).

[12] M.E. Abd El-Hack, M.T. El-Saadony, A.A. Swelum, M. Arif, M.M. A. Ghanima, M. Shukry, A. Noreldin, A.E. Tahah, K.A. El-Tarabily, Curcumin, the active substance of turmeric: its effects on health and ways to improve its bioavailability. Journal of the Science of Food and Agriculture *101*, 5747–5762, (2021).

[13] G.K. Jayaprakasha, L. Jagan, K.K. Sakariah, Chemistry and biological activities of *C. longa*. Trends in Food Science & Technology *16*, 533–548, (2005).

[14] O. Vasso, T. Constantina, Utilization of Plant By-Products for the Recovery of Proteins, Dietary Fibers, Antioxidants, and Colorants. In: Utilization of By-Products and Treatment of Waste in the Food Industry. Ed. By: Vasso Oreopoulou and Winfried Russ. Springer Science Business Media, LLC, pp. 209-232, (2007).

[15] H.H. Wijngaard, M. Ballay, N. Brunton, The optimisation of extraction of antioxidants from potato peel by pressurised liquids. Food Chemistry *133*(4), 1123–1130, (2012).

[16] A.A. Mohdaly, M.A. Sarhan, I. Smetanska, A. Mahmoud, Antioxidants properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. Journal of the Science of Food and Agriculture *90*, 218–226, (2010).

[17] S.F.K. Habeebullah, N.S. Nielsen, C. Jacobsen, Antioxidant activity of potato peel extracts in a fish-rapeseed oil mixture and in oil-in-water emulsions. Journal of the American Oil Chemists' Society *87*(11): 1319-1332, (2010).

[18] I.R. Amado, D. Franco, M. Sánchez, C. Zapata, J. Vázquez, A. Optimisation of antioxidant extraction from Solanum tuberosum potato peel waste by surface response methodology. Food Chemistry *165*, 290-299, (2014).

[19] A. Alaa, A. Abdulhakim, T. Rawaa, Potato and tomato peel extract–A natural antioxidant for retarding lipid peroxidation in lamb meat (Awassi) refrigerator storage. Journal of Food, Nutrition and Agriculture *2(1),* 1–5, (2019).

[20] F.K.H. Sabeena, H.D. Grejsen, C. Jacobsen, Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (*Trachurus trachurus*): effect on lipid and protein oxidation. Food Chemistry *131*, 843–851, (2012).

[21] G. Rowayshed, A.M. Sharaf, S.Y. El-faham, M. Ashour, A.A. Zaky, Utilization of potato peels extract as source of phytochemicals in biscuits. Journal of Basic and Applied Research International *8*(3), 190-201, (2015).

[22] D. Franco, M. Pateiro, I. Rodríguez Amado, M. López Pedrouso, C. Zapata, J.A. Vázquez, J.M. Lorenzo, Antioxidant ability of potato (*Solanum tuberosum*) peel extracts to inhibit soybean oil oxidation. European Journal of Lipid Science and Technology *118*, 1891–1902, (2016).

[23] K. Ben Jeddou, F. Bouaziz, S. Zouari-Ellouzi, F. Chaari, S. Ellouz-Chaabouni, R. Ellouz-Ghorbel, O. Nouri-Ellouz, Improvement of texture and sensory properties of cakes by addition of potato peel powder with high level of dietary fiber and protein. Food Chemistry *217*, 668–677, (2017).

[24] A.M. Samarin, H. Poorazarang, N. Hematyar, A. Elhamirad, Phenolics in potato peels: extraction and utilization as natural antioxidants, World Applied Sciences Journal *18*(2), 191-195, (2012).
[25] M. Doert, K. Jaworska, J. Moersel, L. Kroh, Synergistic effect of lecithins for tocopherols: lecithin-based regeneration of α-tocopherol, European Food Research and Technology *235*(5), 915-928, (2012).
[26] J.D. Dziezak, Antioxidants. Food Technology *40*(9), 94-102.
[27] A.R. Hras, M. Hadolin, Z. Knez, D. Bauman, Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chemistry *71*, 229-233, (2000).
[28] D.S. Sogi, S. Sharma, D.P.S. Oberoi, I.A. Wani, Effect of extraction parameters on curcumin yield from turmeric. Journal of Food Science and Technology *47*(3), 300-304, (2010).
[29] Z. Rehman, F. Habib, W.H. Shah, Utilization of potato peels extract as a natural antioxidant in soy bean oil. Food Chemistry *85*(2), 215-220, (2004).
[30] E. Becker, G. Ntouma, L. Skibsted, Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organisation, Food Chemistry *103*, 1288-1296, (2007).
[31] K. Omar, L. Shan, Y. Wang, X. Wang, Stabilizing flaxseed oil with individual antioxidants and their mixtures. European Journal of Lipid Science and Technology *112*, 1003-1011, (2010).
[32] R. Upadhyay, H.N. Mishra, Multivariate optimization of a synergistic blend of oleoresin sage (*Salvia officinalis* L.) and ascorbyl palmitate to stabilize sunflower oil. Journal of Food Science and Technology *53*(4), 1919–1928, (2015).
[33] R. Bodoira, M. Penci, P. Ribotta, M. Martinez, Chia (*Salvia hispanica* L.) oil stability: Study of the effect of natural antioxidants. Lebensmittel-Wissenschaft & Technologie *75*, 107-113, (2017).
[34] AOCS American Oil Chemists' Society. Official method Cd 18-90, p-Anisidine value and accelerated aging test. Official Methods and Recommended Practices of the AOCS (5th ed.). USE: AOCS; (1998).
[35] AOCS. Method Cd 8–53 and Cg 5-97. In Official Methods and Recommended Practices of the American Oil Chemists' Society, 6th ed.; AOCS Press: Champaign, IL, USA, (2017).
[36] D.P. De Abreu, P.P. Losada, J. Maroto, J.M. Cruz, Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar* L.). Food Research International 43(5), 1277-1282, (2010).
[37] S. Gorjanović, D. Komes, F.T. Pastor, A. Belščak Cvitanović, L. Pezo, I. Hečimović, D. Sužnjević, Antioxidant capacity of teas and herbal infusions: Polarographic assessment. Journal of Agricultural and Food Chemistry *60*, 9573-9580, (2012).
[38] F. Anwar, U. Kalsoom, B. Sultana, M. Mushtaq, T. Mehmood, H.A. Arshad, Effect of drying method and extraction solvent on the total phenolics and antioxidant activity of cauliflower (*Brassica oleracea* L.) extracts. International Food Research Journal *20*(2), 653-659, (2013).
[39] A. Slavova-Kazakova, M. Karama, V. Kancheva, R. Amarowicz, Antioxidant Activity of Flaxseed Extracts in Lipid Systems, Molecules 21, 17 (2016).
[40] F. Pop, Chemical stabilization of oils rich in long-chain polyunsaturated fatty acids during storage. Food Science and Technology International *17*(2), 0111-7, (2011).
[41] S.S.M. Allam, H.M.A. Mohammed, Thermal stability of some commercial natural and synthetic antioxidants and their mixtures. Journal of Food Lipids *9*, 277- 293, (2002).
[42] L.I. Merrill, O.A. Pike, L.V. Ogden, M.L. Dunn, Oxidative stability of conventional and high-oleic vegetable oils with added antioxidants. Journal of the American Oil Chemists' Society *85*(8), 771-776, (2008).
[43] M.E. Abd-ElGhany, M.S. Ammar, A.E. Hegazy, Use of olive waste cake extract as a natural antioxidant for improving the stability of heated sunflower oil. World Applied Sciences Journal *11*(1), 106–113, (2010).
[44] S.V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, M.G Simic, Flavonoids as antioxidants. Flavonoids as antioxidants. Journal of the American Chemical Society *116*(11), 4846-4851, (1994).
[45] F. Shahidi, Y. Zhong, Revisiting the polar paradox theory: a critical overview. Journal of Agricultural and Food Chemistry *59*(8), 3499–3504, (2011).
[46] W. Chaiyasit, R.J. Elias, D.J. McClements, E.A. Decker, Role of association colloids in bulk oils on lipid oxidation. Critical Reviews in Food Science and Nutrition *47*(3), 299–317, (2007).
[47] M.Y. Jung, D.B. Min, Effects of α-, γ-, and δ-tocopherols on the oxidative stability of soybean oil. Journal of Food Science *55*, 1464–1465, (1990).
[48] S.W. Huang, E.N. Frankel, J.B. German, Antioxidant activity of α- and γ-tocopherols in bulk oils and in oil-in-water emulsions. Journal of Agricultural and Food Chemistry *42*, 2108–2114, (1994).
[49] C. Banias, V. Oreopoulou, C.D. Thomopoulos, The effect of primary antioxidants and synergists on the activity of plant extracts in lard. Journal of

the American Oil Chemists' Society *69*(6), 520-524, (1992).
[50] Y.Y. Thoo, F. Abas, O.M. Lai, C.W. Ho, J. Yin, R.V. Hedegaard, L.H. Skibsted, C.P. Tan, Antioxidant synergism between ethanolic *Centella asiatica* extracts and α-tocopherol in model systems. Food Chemistry, *138*(2-3) 1215-1219, (2013).
[51] R. Terán-Hilares, R. Chirinos, R. Pedreschi, D. Campos, Enhanced antioxidant properties of tara (*Caesalpinia spinose*) gallotannins by thermal hydrolysis and its synergistic effects with α-tocopherol, ascorbyl palmitate, and citric acid on sacha inchi (*Plukenetia volubilis*) oil. Journal of Food Process Engineering *41*(1), e12613, (2018).
[52] M. Murakami, T. Yamaguchi, H. Takamura, T. Matoba, Effects of ascorbic acid and α-tocopherol on antioxidant activity of polyphenolic compounds. Journal of Food Science *68*(5), 1622-1625, (2003).
[53] E. Marinova, A. Toneva, N.J.F.C. Yanishlieva, Synergistic antioxidant effect of α-tocopherol and myricetin on the autoxidation of triacylglycerols of sunflower oil. Food Chemistry *106*(2), 628-633, (2008).
[54] A. Altunkaya, V. Gökmen, Effect of various anti-browning agents on phenolic compounds profile of fresh lettuce (*L. sativa*). Food Chemistry *117*(1), 122-126, (2009).
[55] M.N. Peytar-Maillard, M.E. Cuvelier, C. Berset, Antioxidant activity of phenolic componds in 2, 2-azobis (2-amidopropane) dihydrochloride (AAPH)-induced oxidation: synergistic and antagonistic effects. Journal of American Chemical Society *80*(10),1007-1012, (2003).
[56] C.Y.O. Chen, J.B. Blumberg, In vitro activity of almond skin polyphenols for scavenging free radicals and inducing quinone reductase. Journal of Agricultural and Food Chemistry *56*(12), 4427-4434, (2008).