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Antioxidant activity of citron peel (*Citrus medica* L.) essential oil and extract on stabilization of sunflower oil

Somayeh Okhli[1], Habibollah Mirzaei[2,*] and Seyed Ebrahim Hosseini[3]

[1] Department of Food Science and Technology, Sari Branch, Islamic Azad University, Sari, Iran
[2] Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[3] Food Science and Technology, Islamic Azad University, Science and Research Branch, Tehran, Iran

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Abstract – Due to the unfavorable effects of synthetic antioxidants, the use of various sources of plant antioxidants to prevent foods oxidation, especially oil-based or fat-based varieties, has been recently got considerable attention. In this study, the antioxidant effect of essential oil and extract from the citron fruit (*Citrus medica* L.) was investigated on the thermal stability of sunflower oil. Aqueous, ethanolic, and methanolic extracts of citron peel (800 ppm), BHT synthetic antioxidant (200 ppm), and citron peel essential oil (800 ppm) were added to sunflower oil. The oil oxidation stability was evaluated during 5 days through analyzing the values of peroxide, anisidine, thiobarbituric acid, totox, and oxidative stability index (OSI). Results showed that the peroxide, anisidine, and totox value had an increasing trend over time. The effects of storage time, extract, and essential oil were statistically significant in reducing the oxidation rate of sunflower oil during storage. Ultrasonic-assisted ethanolic extract at 30 min showed the highest OSI. The results of this study demonstrated the positive effects of citron peel extract essential oil and on sunflower oil stability and its superiority over synthetic antioxidants.

Keywords: citron peel / sunflower oil / natural antioxidant / essential oil / thermal stability

Résumé – Activité antioxydante de l'huile essentielle et de l'extrait d'écorce de cédrat (*Citrus medica* L.) sur la stabilisation de l'huile de tournesol. En raison des effets défavorables des antioxydants synthétiques, l'utilisation de diverses sources d'antioxydants végétaux pour prévenir l'oxydation des aliments, en particulier les variétés à base d'huile ou de graisse, a récemment fait l'objet d'une attention considérable. Dans cette étude, l'effet antioxydant de l'huile essentielle et de l'extrait du fruit du cédrat (*Citrus medica L.*) a été étudié sur la stabilité thermique de l'huile de tournesol. Des extraits aqueux, éthanoliques et méthanoliques d'écorces de cédrat (800 ppm), un antioxydant synthétique BHT (200 ppm) et de l'huile essentielle d'écorces de cédrat (800 ppm) ont été ajoutés à l'huile de tournesol. La stabilité de l'huile à l'oxydation a été évaluée pendant 5 jours en analysant les indices de peroxyde, d'anisidine, d'acide thiobarbiturique, le paramètre TotOx (*Total Oxydation*) et l'indice de stabilité à l'oxydation (OSI). Les résultats ont montré que les indices de peroxyde, d'anisidine et de TotOx avaient une tendance à la hausse au fil du temps. Les effets du temps de stockage, des différents extraits et de l'huile essentielle étaient statistiquement significatifs dans la réduction du taux d'oxydation de l'huile de tournesol pendant le stockage. L'extrait éthanolique assisté par ultrasons a montré l'OSI le plus élevé à 30 min. Les résultats de cette étude ont démontré les effets positifs de l'huile essentielle et de l'extrait d'écorce de cédrat sur la stabilité de l'huile de tournesol et sa supériorité par rapport aux antioxydants synthétiques.

Mots clés : écorce de cédrat / huile de tournesol / antioxydant naturel / huile essentielle / stabilité thermique

*Correspondence: habibollah.20200@gmail.com

1 Introduction

Citrus medica is a type of citrus fruits consisting of two parts including the outer layer, which is an important part of citron in international trade, and the other layer is the pulp as an edible part of the fruit and a good source of pectin (Abdul, 2014). *C. medica* has long been used for medical purposes, for example, to combat seasickness, lung problems, intestinal diseases, and other disorders. Flavored essential oils of this fruit (located in the outermost, pigmented layer of the peel) are also considered as antibiotics. Citron peel contains citronellal, terpenes, aldehydes, ketones, esters, alcohols, organic acids, etc. Limonene counteracts the effect of pathogenic microorganisms and contains terpenes, sesquiterpene aliphatic terpenes, oxygenated derivatives, bicyclic terpenes, nonterpene aliphatic components, aromatic hydrocarbons, and nitrogen-containing esters. Geraniol and other monoterpenes found in citron fruit peel extract are reported to have anticancer properties (Meena *et al.*, 2011).

Oils are important components of human diets that are used either directly (*e.g.* frying processes) or in combination with other components (such as biscuits). Because of unsaturated fatty acids in many oils, these substances are subject to oxidation and rancidity. If oxidation exceeds a certain level, it renders the oil or the containing material unusable for food consumption. Besides, some compounds produced by oxidation are detrimental to human health. The oxidative stability of oils can be improved by altering the composition of oil fatty acids or by applying antioxidants as supplementing (Chung et al., 2004).

Butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), and tertiary butylhydroquinone (TBHQ) are the most common synthetic antioxidants, which are usually added to the oil after the deodorization process. According to Suja *et al.* (2004), the use of these antioxidants, despite their effective role in prevention of oils oxidation, may be associated with carcinogenic, mutagenic, or other effects on humans under certain conditions. Therefore, the enrichment of oils with substances containing high amounts of natural antioxidant compounds increasing oxidative stability of oils (Mir-Ahmadi *et al.*, 2005). Sunflower oil is one of the most widely used vegetable oils in the industry that can withstand high cooking temperatures and is, therefore, a desirable option for the frying process. Sunflower oil also has advantages than other frying oils, including fewer contents of saturated fatty acids and the uppermost vitamin E content (Johnson *et al.*, 2009).

A considerable number of studies have focused on the antioxidant capacity of extracts and essential oils and replacement of synthetic antioxidants with plant bioactive compounds (Mallet *et al.*, 1994). Popovich (2008) investigated the antioxidant properties of parsley, thyme, and fennel on sunflower oil and found that the natural antioxidants of these plants were effective in sunflower oil stability. Oktaya *et al.* (2003) studied the antioxidant properties of ethanolic and aqueous extracts of fennel seeds through total antioxidant capacity, free radical scavenging capacity, superoxide anion radical scavenging, hydrogen peroxide inhibition, and metal chelating activity tests, and compared them with alpha-tocopherol and synthetic antioxidants (BHT and BHA). They reported that the antioxidant properties of fennel were concentration-dependent and increased with rising concentrations.

Due to the important role of edible fats in diets and adverse effects of synthetic antioxidants on human health, in this study, the antioxidant effect of essential oil and extract from citron fruit peel was evaluated on thermal stability of sunflower oil.

Table 1. Independent variables with corresponding codes.

Treatments code	Solvents	Ultrasonic time (min)
T1	Ethanol	10
T2	Ethanol	20
T3	Ethanol	30
T4	Methanol	10
T5	Methanol	20
T6	Methanol	30
T7	Water	10
T8	Water	20
T9	Water	30
T10		BHT
T11		Essential oil

2 Materials and methods

2.1 Materials

Citron fruit (*Citrus medica* L.) was obtained from a local market in Tehran. The materials used in this study were procured from the Merck Company, Germany.

2.2 Methods

2.2.1 Preparation of the citron peel ethanolic extract

The inner peel (mesocarp) of *C. medica* was separated, dried completely at 45 °C in a dryer (Memmert 800, Germany), powdered by an electric mill (Eka, Germany), and the powder was passed through a sieve (Mesh-80).

Citron peel was extracted using an ultrasound bath (DT 255 H, Bandelin Co., Germany). The peel powder (40 g) with 400 ml of ethanol, methanol, and water were exposed to ultrasound with a frequency of 40 kHz for 10, 20, and 30 minutes (Albu *et al.*, 2004). The extracts were then filtered by Whatman Grade 42 filter papers. The residue was re-extracted under identical conditions and the solvent residue was vacuum-evaporated with a rotary evaporator (Heidolph, Germany). After solvent evaporation, the extract was kept frozen at −18 °C until use (Rehman, 2006). Table 1 represents the independent variables with the corresponding codes.

2.2.2 Preparation of essential oil from citron peel

Citron peel essential oil was prepared using a Clevenger apparatus (Brosilicat, Germany) and steam distillation. To this end, 100 g of the dried plant was poured into the balloon of the apparatus along with adding water, and the essential oil was extracted during 2 h (Hokmollahi, 2010).

2.2.3 Preparation of treatments

Aqueous, ethanolic, and methanolic extracts of citron fruit peel at 800 ppm, BHT synthetic antioxidant at 200 ppm, and citron peel essential oil at 800 ppm were added to sunflower oil. The oil oxidation stability was evaluated every 24 h for 5 days at 65 °C by analyzing the values of peroxide, anisidine, thiobarbituric acid (TBA), totox, and oxidative stability index (OSI).

2.3 Tests

2.3.1 Peroxide index

In this test, the concentrations of peroxide and hydroperoxides formed in the initial stage of reaction were measured and expressed in milli-equivalents peroxide per kg of fat. The value of this index was calculated according to the reference method (AOCS, 2003, cd 8-53) during the storage (Matthaus, 2006).

2.3.2 Anisidine index

The sample (0.5 g) was weighed accurately in a 5 ml volumetric flask, then dissolved in isooctane, reached a volume, and mixed well. The absorption of the fat solution against pure isooctane was read at 350 nm with a spectrophotometer (JENWAY uv/vis, 6305, England) (a). The solution (5 ml) and isooctane (5 ml) were poured into test tubes A and B, respectively. One milliliter of anisidine was added to two tubes, shaken vigorously, and put in a dark place for 10 minutes. The absorbance of tube A content *versus* tube B was measured at 350 nm in a 1 cm glass cell (b). The anisidine index was calculated by the following equation:

$$\text{Anisidine index} = \frac{25(1.2\text{A1} - \text{A2})}{m}, \quad (1)$$

where, A_1 and A_2 represent the absorption of test solutions (b) and (a), respectively, at 350 nm, and m is the mass (g) of the test substance in test solution (a) (Dieffenbacher and Pocklington, 1987).

2.3.3 Totox index

The totox index was calculated using peroxide and anisidine indices according to the following equation (AOCS, 1998):

$$\text{Totox index} = 2(\text{peroxide index}) + \text{anisidine index}. \quad (2)$$

2.3.4 Thiobarbituric acid (TBA) index

The TBA value was determined through the direct method using butanol as a solvent in the presence of TBA reagent by measuring the absorbance of the solution at 350 nm wavelength *versus* a blank (the solvent and reaction solution) with a spectrophotometer (AOCS, 1998, cd 19-90). The results were calculated using the following equation:

$$\text{TBA value} = \frac{50(\text{A} - \text{B})}{m}, \quad (3)$$

where TBA denotes thiobarbituric acid value, A and B are respectively the absorbance of test and blank solutions at 530 nm, and m is sample mass (mg).

2.3.5 Thermal stability

Oxidative stability of oil samples was measured using a Rancimat device (Metrohm Rancimat 743, Switzerland) according to the AOCS method (AOCS, 1998).

2.4 Statistical analysis

All experiments were conducted in triplicate and an analysis of variance was performed. The least significant difference at $p < 0.05$ was calculated using the Duncan Multiple Range Test on SPSS software version 20. The charts were drawn by Excel software (2010).

3 Results and discussion

3.1 Peroxide index of sunflower oil samples

Table 2 shows the changes in the peroxide index of sunflower oil containing the extract and essential oil of citron peel during storage at 65 °C. Statistically significant difference of peroxide values were measured in samples containing ethanolic, methanolic, and aqueous extracts from zero to 5 days, which increased by rising time. On the second day of storage, the ethanolic sample extracted by ultrasound had the lowest peroxide index at 30 min. The highest peroxide value was observed in the aqueous extract with increasing storage time at the end of the fifth day.

The use of water for extraction created a completely polar environment; consequently, some phenolic compounds with low polarity were extracted in minor quantities. In addition, aqueous extracts contain such impurities as organic acids, proteins, and soluble sugars (Chirinos *et al.*, 2007). However, ethanol and methanol with water are more capable of extracting phenolic compounds (Suzuki *et al.*, 2002). The accumulation of phenolic compounds will reduce the peroxide value. The decrease of the oil peroxide index in the samples resulted from the antioxidant activity and phenolic compounds present in the extract of citron fruit peel. Nevertheless, antioxidants compounds remain active for a certain period and gradually lose their effects over the time, which may be related the storage of samples under heat conditions storage. Thus, increasing the storage time of oil samples under oxidation conditions led to an increased peroxide index. Esmaeilzadeh Kenari and Mehdipoor (2012) investigated the antioxidant properties of kiwifruit peel to stabilize sunflower oil and observed increased peroxide value with rising the storage time. Abdalla and Roozen (1999) also confirmed the efficacy of natural essential oils and extracts in preventing the oxidation of edible oils compared to synthetic antioxidants. In another study, it was shown that peroxide values in control oil samples were higher than those of oil samples containing BHT and anemone plant extract, with the lowest peroxide value belonging to extract-containing samples (Agregán *et al.*, 2017), this finding was in agreement with our findings.

Table 2. Comparison of peroxide values (meq O_2/kg) in treatments during 5 days of storage. (uppercase and lowercase letters show significant differences in each row and column, respectively, at $p < 0.05$).

Treatments	First day	Second day	Third day	Fourth day	Fifth day
T1	$2.90 \pm 0.02^{D,d}$	$2.22 \pm 0.06^{E,d}$	$3.30 \pm 0.08^{C,d}$	$3.90 \pm 0.05^{B,b}$	$4.79 \pm 0.08^{A,d}$
T2	$2.30 \pm 0.09^{D,f}$	$2.16 \pm 0.05^{D,d}$	$2.90 \pm 0.04^{C,f}$	$3.10 \pm 0.08^{B,fg}$	$4.61 \pm 0.06^{A,e}$
T3	$0.70 \pm 0.01^{E,k}$	$1.60 \pm 0.03^{D,f}$	$2.51 \pm 0.06^{C,h}$	$3.11 \pm 0.04^{B,f}$	$3.60 \pm 0.07^{A,e}$
T4	$3.20 \pm 0.09^{D,c}$	$3.26 \pm 0.05^{D,b}$	$3.71 \pm 0.02^{C,b}$	$3.76 \pm \pm 0.02^{B,c}$	$5.10 \pm 0.06^{A,c}$
T5	$1.81 \pm 0.07^{E,i}$	$2.10 \pm 0.09^{D,de}$	$2.61 \pm 0.04^{C,gh}$	$3.22 \pm 0.07^{B,f}$	$4.20 \pm 0.07^{A,f}$
T6	$1.12 \pm 0.02^{E,j}$	$2.03 \pm 0.07^{D,e}$	$2.71 \pm 0.10^{C,g}$	$3.00 \pm 0.04^{B,g}$	$3.90 \pm 0.03^{A,g}$
T7	$3.60 \pm 0.06^{D,b}$	$3.26 \pm 0.05^{E,b}$	$4.11 \pm 0.03^{C,a}$	$4.30 \pm 0.06^{B,a}$	$5.20 \pm 0.03^{A,b}$
T8	$4.10 \pm 0.06^{B,a}$	$3.46 \pm 0.04^{D,a}$	$3.61 \pm 0.06^{C,c}$	$4.20 \pm 0.08^{B,a}$	$5.60 \pm 0.07^{A,a}$
T9	$2.60 \pm 0.03^{C,e}$	$2.34 \pm 0.05^{D,c}$	$2.51 \pm 0.06^{C,h}$	$3.40 \pm 0.03^{B,e}$	$4.60 \pm 0.08^{A,e}$
T10	$1/91 \pm 0.02^{E,h}$	$2.15 \pm 0.04^{D,d}$	$3.30 \pm 0.04^{C,d}$	$3.81 \pm 0.06^{B,bc}$	$4.73 \pm 0.03^{A,d}$
T11	$2.10 \pm 0.07^{E,g}$	$2.21 \pm 0.03^{D,d}$	$3.10 \pm 0.09^{C,e}$	$3.60 \pm 0.07^{B,d}$	$4.73 \pm 0.04^{A,d}$

Table 3. Comparison of anisidine values (meq/kg) in treatments during 5 days of storage. (uppercase and lowercase letters show significant differences in each row and column, respectively, at $p < 0.05$).

Treatments	First day	Second day	Third day	Fourth day	Fifth day
T1	$9.70 \pm 0.21^{E,b}$	$31.01 \pm 0.17^{D,c}$	$40.20 \pm 0.15^{C,d}$	$69.00 \pm 0.29^{B,a}$	$75.12 \pm 0.22^{A,c}$
T2	$8.20 \pm 0.27^{D,de}$	$21.11 \pm 0.16^{C,h}$	$30.03 \pm 0.11^{B,g}$	$46.05 \pm 0.12^{A,h}$	$46.08 \pm 0.19^{A,i}$
T3	$7.00 \pm 0.23^{E,g}$	$16.00 \pm 0.14^{D,j}$	$25.01 \pm 0.10^{C,i}$	$34.01 \pm 0.14^{B,k}$	$43.05 \pm 0.15^{A,k}$
T4	$9.23 \pm 0.17^{E,c}$	$26.02 \pm 0.14^{D,d}$	$42.00 \pm 0.09^{C,b}$	$58.99 \pm 0.20^{B,d}$	$72.00 \pm 0.14^{A,d}$
T5	$8.10 \pm 0.15^{E,e}$	$19.01 \pm 0.11^{D,i}$	$32.02 \pm 0.16^{C,f}$	$42.01 \pm 0.10^{B,i}$	$47.34 \pm 0.17^{A,h}$
T6	$7.51 \pm 0.20^{E,f}$	$15.00 \pm 0.15^{D,k}$	$27.01 \pm 0.14^{C,h}$	$35.01 \pm 0.24^{B,j}$	$45.12 \pm 0.24^{A,j}$
T7	$10.41 \pm 0.12^{E,a}$	$33.04 \pm 0.11^{D,a}$	$44.05 \pm 0.15^{C,a}$	$64.00 \pm 0.21^{B,b}$	$76.02 \pm 0.17^{A,b}$
T8	$10.04 \pm 0.24^{E,b}$	$32.00 \pm 0.13^{D,b}$	$44.11 \pm 0.25^{C,a}$	$61.07 \pm 0.12^{B,c}$	$77.01 \pm 0.26^{A,a}$
T9	$8.62 \pm 0.19^{E,d}$	$25.01 \pm 0.24^{D,e}$	$41.01 \pm 0.14^{C,c}$	$56.10 \pm 0.19^{B,e}$	$67.02 \pm 0.27^{A,e}$
T10	$8.10 \pm 0.18^{E,e}$	$22.01 \pm 0.09^{D,g}$	$39.00 \pm 0.23^{C,e}$	$51.03 \pm 0.15^{B,g}$	$57.00 \pm 0.17^{A,g}$
T11	$7.91 \pm 0.12^{E,e}$	$22.99 \pm 0.31^{D,f}$	$41.01 \pm 0.12^{C,c}$	$53.00 \pm 0.17^{B,f}$	$64.01 \pm 0.26^{A,f}$

The peroxide value of BHT synthetic antioxidant sample increased from an initial value of 1.91 to 4.73 meq O_2/kg oil after 5 days of oil storage at 65 °C. Peroxide value was increased by statistically significant differences in oil samples containing ethanolic, methanolic, and aqueous extracts at sonication of 30 min, from an initial value of 0.7 to 3.6, 3.9, and 4.6 meq O_2/kg oil, respectively, after 3 days of storage at 65 °C. Peroxide value resulted from citron peel essential oil added to sunflower oil was similar to BHT addition.

Peroxide value cannot indicate oil oxidation because it is an indicator of the presence of primary oxidation products and does not reveal the production of secondary products. Because of the decomposition of hydroperoxides at high temperatures and the formation of secondary compounds (*e.g.* aldehydes), a test such as an anisidine index seems to be necessary as an indicator of oxidation development (Matthaus, 2006).

3.2 Anisidine values f sunflower oil samples

Based on the results of ANOVA (Tab. 3), changes in the anisidine index of sunflower oil stored at 65 °C were significantly affected by the extract of citron fruit peel ($p < 0.05$). The anisidine values were different in the oil samples and had an increasing trend in all samples by rising storage time at 65 °C. On the first storage day, oil samples containing ethanolic extract had the lowest anisidine index. The highest anisidine value was observed in the oil-containing aqueous extract by increasing storage time at the end of the fifth day. The anisidine index rose from an initial, pre-oven value of 10.04 to 77.01 ($p < 0.05$).

The anisidine values of extracts were significantly different at the end of 5-day storage at 65 °C ($p < 0.05$). The highest (77.01 meq/kg) and the lowest (43.05 meq/kg) anisidine indices were recorded in the oils containing aqueous and methanolic extracts, respectively. The index was low in the early days and rose in the final days.

An increase in anisidine index indicates the expansion of spontaneous oxidation reaction leading to the increase of secondary products resulting from the decomposition of hydroperoxides and carbonyl compounds during the time (Namiki, 1990). Tahami *et al.* (2011) evaluated the antioxidant effect of fennel seed extract on sunflower oil stability value. They reported higher efficacy of fennel seed extract than both

Table 4. Comparison of totox values (meq/kg) in treatments during 5 days of storage. (uppercase and lowercase letters show significant differences in each row and column, respectively, at $p < 0.05$).

Treatments	First day	Second day	Third day	Fourth day	Fifth day
T1	$15.47 \pm 0.19^{E,c}$	$35.44 \pm 0.17^{D,c}$	$46.80 \pm 0.22^{C,d}$	$82.93 \pm 0.28^{B,a}$	$83.58 \pm 0.21^{A,d}$
T2	$12.81 \pm 0.26^{E,e}$	$25.42 \pm 0.18^{D,h}$	$35.81 \pm 0.24^{C,g}$	$52.20 \pm 0.33^{B,h}$	$55.22 \pm 0.31^{A,h}$
T3	$8.40 \pm 0.22^{E,h}$	$19.21 \pm 0.42^{D,j}$	$30.01 \pm 0.36^{C,i}$	$40.20 \pm 0.31^{B,k}$	$50.21 \pm 0.33^{A,j}$
T4	$15.61 \pm 0.24^{E,c}$	$32.51 \pm 0.26^{D,d}$	$49.42 \pm 0.28^{C,c}$	$66.52 \pm 0.37^{B,d}$	$84.31 \pm 0.35^{A,c}$
T5	$11.70 \pm 0.38^{E,f}$	$23.20 \pm 0.45^{D,i}$	$37.23 \pm 0.23^{C,f}$	$48.44 \pm 0.39^{B,i}$	$55.70 \pm 0.23^{A,h}$
T6	$9.71 \pm 0.16^{E,g}$	$19.06 \pm 0.30^{D,j}$	$32.42 \pm 0.32^{C,h}$	$41.01 \pm 0.35^{B,j}$	$52.90 \pm 0.42^{A,i}$
T7	$17.61 \pm 0.31^{E,b}$	$39.53 \pm 0.33^{D,a}$	$52.20 \pm 0.41^{C,a}$	$72.60 \pm 0.11^{B,c}$	$86.41 \pm 0.27^{A,b}$
T8	$18.20 \pm 0.26^{E,a}$	$38.92 \pm 0.19^{D,b}$	$51.22 \pm 0.39^{C,b}$	$77.80 \pm 0.28^{B,b}$	$88.20 \pm 0.17^{A,a}$
T9	$13.80 \pm 0.29^{E,d}$	$29.68 \pm 0.24^{D,e}$	$46.02 \pm 0.27^{C,e}$	$62.91 \pm 0.34^{B,e}$	$76.20 \pm 0.21^{A,e}$
T10	$11.91 \pm 0.14^{E,f}$	$26.31 \pm 0.30^{D,g}$	$45.60 \pm 0.33^{C,e}$	$58.63 \pm 0.22^{B,g}$	$66.47 \pm 0.16^{A,g}$
T11	$12.11 \pm 0.31^{E,f}$	$27.42 \pm 0.28^{D,f}$	$47.20 \pm 0.51^{C,d}$	$60.26 \pm 0.48^{B,f}$	$73.47 \pm 0.46^{A,f}$

Table 5. Comparison of TBA index (mg MA/kg) in treatments during 5 days of storage (uppercase and lowercase letters show significant differences in each row and column, respectively, at $p < 0.05$).

Treatments	First day	Second day	Third day	Fourth day	Fifth day
T1	$0.436 \pm 0.010^{D,c}$	$0.457 \pm 0.008^{C,d}$	$0.475 \pm 0.006^{B,d}$	$0.485 \pm 0.007^{B,d}$	$0.536 \pm 0.013^{A,c}$
T2	$0.348 \pm 0.06^{D,e}$	$0.374 \pm 0.012^{C,fg}$	$0.396 \pm 0.004^{B,g}$	$0.403 \pm 0.003^{B,g}$	$0.411 \pm 0.004^{A,e}$
T3	$0.183 \pm 0.004^{E,i}$	$0.206 \pm 0.007^{D,i}$	$0.228 \pm 0.005^{C,i}$	$0.251 \pm 0.010^{B,i}$	$0.272 \pm 0.008^{A,g}$
T4	$0.456 \pm 0.005^{E,b}$	$0.480 \pm 0.009^{D,c}$	$0.526 \pm 0.004^{C,c}$	$0.540 \pm 0.006^{B,c}$	$0.561 \pm 0.011^{A,b}$
T5	$0.316 \pm 0.004^{E,g}$	$0.380 \pm 0.006^{D,f}$	$0.390 \pm 0.003^{C,g}$	$0.401 \pm 0.003^{B,g}$	$0.610 \pm 0.006^{A,a}$
T6	$0.205 \pm 0.007^{E,h}$	$0.242 \pm 0.009^{D,h}$	$0.263 \pm 0.008^{C,h}$	$0.286 \pm 0.004^{B,h}$	$0.305 \pm 0.011^{A,f}$
T7	$0.421 \pm 0.006^{E,c}$	$0.521 \pm 0.010^{D,b}$	$0.579 \pm 0.007^{C,a}$	$0.596 \pm 0.005^{B,a}$	$0.606 \pm 0.003^{A,a}$
T8	$0.529 \pm 0.008^{E,a}$	$0.561 \pm 0.007^{D,a}$	$0.582 \pm 0.003^{C,a}$	$0.591 \pm 0.004^{B,a}$	$0.610 \pm 0.007^{A,a}$
T9	$0.405 \pm 0.002^{E,d}$	$0.426 \pm 0.007^{D,e}$	$0.446 \pm 0.004^{C,e}$	$0.456 \pm 0.004^{B,e}$	$0.604 \pm 0.008^{A,a}$
T10	$0.334 \pm 0.005^{E,f}$	$0.358 \pm 0.006^{D,g}$	$0.540 \pm 0.007^{C,b}$	$0.561 \pm 0.006^{B,b}$	$0.571 \pm 0.003^{A,b}$
T11	$0.340 \pm 0.09^{E,ef}$	$0.383 \pm 0.006^{D,f}$	$0.406 \pm 0.004^{C,f}$	$0.415 \pm 0.004^{B,f}$	$0.433 \pm 0.008^{A,d}$

synthetic antioxidants and reduced anisidine values and peroxide values in oil samples. According to Gharekhani *et al.* (2009), extracts containing natural antioxidants are capable of reacting with free radicals resulting from lipid oxidation.

3.3 Totox index of sunflower oil samples

The results of this study revealed that the peroxide, anisidine, and totox indices had an increasing trend during time. The ethanolic extract presented the least totox values of 8.40 and 50.21 on the first and fifth days, respectively (Tab. 4). At the end of the fifth storage day, totox values were 50.22, 83.58, and 88.20 for sunflower oil samples containing aqueous, ethanolic, and methanolic extracts, respectively, which were significantly higher than those of the first day ($p < 0.05$). On the fifth day, totox values of 66.47 and 73.47 were obtained in the sample containing synthetic antioxidant and essential oil respectively. Ethanolic extracts were more effective in preventing oil oxidative rancidity than the other treatments, particularly the synthetic antioxidant and the essential oil.

Similarly, Mazaheri Kalahrodi *et al.* (2014) reported that the use of fennel seed extract (at 100, 200, 300, 400, 500, 600, 700, and 800 ppm) in soybean oil reduced totox values in oil samples. In their study, the extract at levels of 300 and 400 ppm had higher antioxidant activity than BHT and BHA.

3.4 Thiobarbituric acid (TBA) index of sunflower oil samples

The TBA index represents the milligram of malondialdehyde in one kg of oil (mg MA/kg), indicating the secondary stage of lipid oxidation and the presence of secondary oxidation products in the samples. Thus, a high TBA index in the oil indicates greater oil oxidation and less stability. The results showed that the TBA index was low in the initial days as it increased due to the decomposition of formed hydroperoxides and their conversion into aldehydes and ketones in the first days (Tab. 5). As a result, this index increased in the final days and was significantly different from the other days of storage ($p < 0.05$). The ethanolic extract treatment had the highest ability to prevent the formation of secondary oxidation products. TBA values were equal to 0.183 and 0.272 mg MA/kg for the ethanolic extract treatment with 30 minutes sonication time in the first and fifth days, respectively. Increased antioxidant activity of the ethanolic extract was related to the amounts of phenolic compounds in this extract; therefore, TBA index was decreased. TBA value was uppermost in the aqueous extract

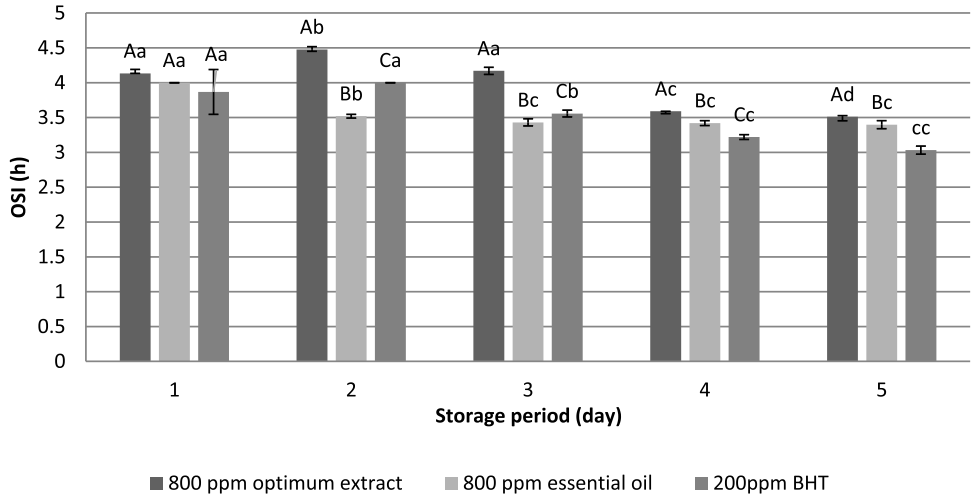

Fig. 1. Changes in oxidative stability index of sunflower oil samples during 5 days of storage at 65 °C (different lowercase letters in the same treatment indicate significant differences ($p < 0.05$) between different time points. Different capital letters at the same time point indicate significant differences ($p < 0.05$) between different treatments).

during all days so that it increased from 0.405, at the pre-oven stage, to 0.604 mg MA/kg after 72 h storage of sunflower oil at 65°C. A comparison between peroxide and TBA values reveals a direct relationship between the production of hydroperoxide and the increased TBA value.

In the ultrasonic treatments, the peroxide and TBA values were significantly lower due to further extraction of phenolic compounds in these samples. In the synthetic antioxidant sample, peroxide began to decompose as it reached a specific level by time, thereby producing aldehyde, ketone, and acid leading to a rise in TBA and a decrease in peroxide content.

In a similar study, Ayoughi *et al.* (2009) evaluated the antioxidant activity of dill (*Anethum grareolens*) essential oil in soybean oil by measuring peroxide and TBA values. They concluded that dill essential oil could prevent the production of primary and secondary oxidation products in crude soybean oil at a concentration of 0.6 mg/ml, which is approximately equal to BHA chemical oxidation at a concentration of 0.1 mg/ml. Sahari *et al.* (2004) reported a high antioxidant activity in tea seed oil that could improve sunflower oil storage. Ozcan and Arslan (2011) presented evidence that hazelnut oil samples containing clove, cinnamon, and rosemary essential oils at 0.25 and 0.5% could effectively delay the formation of primary oxidation products than the control sample. Furthermore, peppermint extract also enhanced the stability of extra virgin olive oil. Hashemi *et al.* (2014) also observed that the essential oil of ajwain (aniseed) reduced the oxidation rate of sunflower oil.

3.5 Oxidative Stability Index (OSI)

OSI is the time to reach a point that one of the oxidative quantities, like the peroxide or carbonyl value, increases abruptly after an increasing trend. Accelerated tests to investigate oxidative stability increase the speed of natural oxidation process and are important quality control tools for determining the shelf life of a product (Abdalla and Roozen, 1999). Changes in the OSI of the sunflower oil affected by the optimum extract (ethanolic extract treatment with 30 min ultrasound), essential oil, and BHT during the storage at 65 °C are shown in Figure 1. A continued decrease in OSI with the increase in storage period was observed in all the samples. According to Figure 1, the lowest (3 h) and the highest (4.47 h) thermal stability were recorded in the fifth day for sunflower oil containing BHT and the second day for sunflower oil containing optimum extract, which were significantly different ($p < 0.05$). In the sunflower oil containing optimum extract, the OSI decreased from baseline value (4.13 h) to 3.51 h after 5 days of storage. For the oil samples containing essential oil, however, the OSI decreased from an initial value of 4 to 3.39 h after 5 days of storage at 65 °C.

Tahami *et al.* (2011) investigated the antioxidant effect of fennel seed extract on sunflower oil stability and found that fennel extract at concentrations of 250 and 300 ppm had a higher resistance to oxidation than synthetic antioxidants (BHT and BHA). Iqbal and Bhanger (2007) investigated the effect of garlic extract on the physicochemical stabilization of sunflower oil during storage (65 °C). They noticed that garlic extract could significantly stabilize sunflower oil compared to synthetic antioxidants. This result was attributed to different active compounds, particularly phenolic components, in garlic extract.

4 Conclusion

According to our observations, the lowest and highest peroxide value was observed in the ethanolic sample extracted with ultrasound at 30 minutes and aqueous extract respectively. Anisidine index rose in all oil samples with increasing storage time. On the first day of storage, the lowest anisidine and totox index was recorded in oil samples containing ethanolic extract. Ethanolic extracts were more effective in preventing oil oxidative rancidity than the other treatments, particularly the synthetic antioxidant and the essential oil. The TBA index was low in the early days of storage. The ethanolic extract treatment had the highest potential to prevent the formation of secondary oxidation products. After 5 days of storage, a decreasing effect was detected on the oxidation rate of sunflower oil containing ethanolic extract treatment with ultrasound at 30 minutes and the essential oil. Since the

ethanolic extract played a more effective role than the essential oil in preventing oil oxidation, it can, therefore, be a good substitute for synthetic antioxidants in frying oils.

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