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Improving the oxidative stability of sunflower oil by using the oil from the Baneh skin in very small amounts

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ABSTRACT

In the present study, the effect of different amounts of Baneh skin oil (0.05% to 0.5%) on the oxidative stability of refined sunflower oil during 8 hours of thermal process at 170 °C was investigated, which was 100 ppm of synthetic antioxidant TBHQ. used for comparison. Evaluating the results of various oxidative stability tests (peroxide value, anisidine value, totox value, Conjugated diene value and acid value) showed that the use of Baneh skin oil improved the oxidative stability of sunflower oil. The best conditions of oxidative stability were observed in sunflower oil containing 0.05% of Baneh skin oil, followed by the oil sample containing 0.1% of Baneh skin oil, both of which had a superior antioxidant effect than TBHQ. In order to better interpret the results of oxidative stability tests, the changes of tocopherol and polyphenolic compounds as two indicator antioxidant compounds were investigated during the thermal process. The results showed that there is no relationship between the changes of these compounds and oxidative stability tests. The sample containing TBHQ had the greatest protective effect on antioxidant compounds, which due to the creation of a peroxidative state caused by the increase of antioxidants, it decreased the oxidative stability of sunflower oil. Also, the investigation of changes in antioxidant activity during the thermal process with the help of two DPPH radical scavenging and Rancimet tests also showed that the sunflower oil sample containing 0.05% of Baneh skin oil had the best conditions, which was consistent with the results of oxidative stability tests. The results of the present research are very important because the oil of the Baneh skin oil was not pure at all compared to TBHQ.

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1- Introduction

Vegetable oils are an important part of human food products. These oils are carriers of fat-soluble vitamins and a source of energy and essential fatty acids for humans, such as linoleic and linolenic acids, and play an important role in human nutrition [1]. One of the problems faced by edible oils is their oxidation. To prevent this reaction, antioxidant compounds are used. Chemical antioxidants are most widely used in edible oils [2]. With the development of people's living standards, they are increasingly interested in using natural antioxidants to prevent the potential toxicity of synthetic antioxidants such as TBHQ [3]. For this purpose, a lot of research has been done to identify natural antioxidants. But using natural antioxidants instead of chemical antioxidants has problems. Most antioxidants do not have proper stability. They may have good antioxidant activity in the initial moments of extraction, but they cannot show good antioxidant power over time, especially during the thermal process. Another problem with the use of these antioxidants is the high cost of their preparation and extraction, which makes them economically unaffordable for industrial use [4]. In this regard, several plant species with different antioxidant properties have been identified in Iran [5-7]. In the past years, various researches have been carried out on the oil of baneh (Pistacia atlantica) skin, all of which indicate the very high antioxidant power of this oil during the thermal process at high temperature [5, 10-8]. Baneh is one of the God-given natural resources of Iran, whose fruit consists of three parts, the outer fatty skin, the inner woody skin, and the kernel. In Iran, baneh is found in mass between Fars and Kurdistan provinces and scattered in the rest of the country. The area of baneh trees in Iran is about one million and 1200000 hectares [11].

The skin of the Baneh makes up about 25% of the fruit, which contains about 35% of oil. Farhosh et al. reported that the oil of Beneh skin, as a new source of very stable and antioxidant vegetable oils, has recently been introduced to the world [9]. Also, based on the results of Sharif et al., it is possible to directly add Baneh skin oil to edible oils as a natural antioxidant. In this research, it was found that Baneh skin oil, compared to very sesame and rice bran oils, caused the greatest improvement in the oxidation stability of sunflower oil, which was used in amounts between 2 and 8 %. Based on the results, it was determined that this oil had the highest antioxidant activity in sunflower oil in amounts of 2% [8]. Of course, during this research, the researchers did not pay attention to the fact that the use of 2 to 8% of crude oil in sunflower oil has problems: seed oil is used unrefined . It has inappropriate impurities such as wax and phospholipids, which reduce the quality of refined sunflower oil by using high amounts of oil. Also, crude oil from the seed pods has a pungent smell, and adding it in high amounts reduces the marketability of sunflower oil. On the other hand, the supremacy of the rind oil in the lowest amount in this research (2%), indicates that this oil may have a better antioxidant effect in lower amounts. On the other hand, it should be mentioned that, despite the abundance of Baneh trees in Iran and the recognition of its very high antioxidant power, Baneh skin oil can be used as an effective natural antioxidant in other edible oils. In terms of extraction cost, because this oil is directly added to other oils and is not converted into different fractions, it is affordable. Therefore, according to the mentioned points, in the present study, it was decided to use the oil of the Baneh skin in an amount less than 0.5% to replace the synthetic antioxidant TBHQ in sunflower oil during the thermal process at 170 degrees Celsius.

2- Materials and methods

2-1- Materials

The fruit of Baneh tree was collected from the forests of Maimand city in Fars province (autumn 2019) and kept in a freezer at 4 ºC until the time of oil extraction. Refined sunflower oil was also obtained from Golestan Dezful Agricultural Oil Factory. All standards, chemicals and solvents were obtained from Sigma and Merck companies.

2-2- Extraction of oil from the Baneh skin

First, the Baneh fruit was dried at ambient temperature in the shade, and then its green skin was separated by grinding with the help of a grinder. In the next step, the oil of the Baneh skin was extracted using hexane solvent (1:4 ratio), then the solvent was separated using a vacuum oven and stored in dark colored

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containers containing nitrogen at a temperature of 4ºC[11].

2-3- thermal process

First, different treatments of sunflower oil were prepared using amounts of 0.05, 0.1, 0.2, 0.35, and 0.5% of Baneh skin oil and 100 ppm of TBHQ. Then 800 grams of each oil sample was poured into the beaker and placed in an oven and kept at 170 ºC for 8 hours. At 2 hour intervals, 50 grams of samples were taken for various tests [7, 12].

2.4. Fatty Acid Composition

The fatty acid composition of the vegetable oils was determined by gas-liquid chromatography according to the method previously established [4].

2-5- Acid value, peroxide value, anisidine value, TOTOX value and Conjugated diene value

Acid value and peroxide value were measured by the method described by Tavakoli et al. [13]. , Anisidine value and conjugated diene value of different oil samples were also calculated according to the method described by Roshanpour et al. [14] and Tavakoli et al. [9], respectively. TOTOX value was also calculated according to the following formula:

TOTOX value = (2×peroxide value) + (anisidine value)

2-6- Unsaponifiable matter, polyphenolic, tocopherol and sterol compunds

The amount of unsaponifiable matter content in the oils studied in this project was determined by the method of Lezano et al. [15]. The amount of total polyphenolic compounds was determined by the method described by Esfahlan et al. based on gallic acid [16]. In this method, the Folin-Ciocalcho reagent was used. The amount of tocopherol compounds was also determined by the method of Tavakoli et al. [13].

2-8- Measurement of DPPH free radical scavenging power

The DPPH free radical scavenging power of the oils studied in this study was determined by the method described by Yim et al. [17].

2-9- Measurement of Oxidative Stability Index with the help of Rancimat device

To determine the oxidative strength of different oil samples, Rancimat device (Metrohm Company, model 743) was used in such a way that 3 grams of oil was tested at a temperature of 110 ° C with an air flow rate of 15 liters per hour. The obtained data were compared based on the oxidative stability index of the oil (hours) [18].

2-10- Statistical analysis

All experiments related to the initial chemical properties were performed in a completely randomized experimental design with three replications. Also, experiments during the thermal process were performed with three replications and 7 treatments in the form of Duncan's split-plot design. The means were compared with MStatC software and based on Duncan's test at the five percent level ($p < 0.05$). The graphs were drawn with Microsoft Excel software.

3- Discussion and Conclusion

3-1- Examination of some basic chemical properties of Baneh skin oil

Some basic properties of Baneh skin and sunflower oils are listed in Table 1. As is clear, oleic (51.8%), palmitic (21.25%) and linoleic (8.71%) fatty acids were the three main fatty acids of Baneh skin oil, while in sunflower oil, linoleic (54.4%), oleic (27.8%) and palmitic (8.6%) fatty acids had these conditions. Also, the amount of unsaponifiable matter in sunflower and Baneh skin oils was determined to be 0.65 and 2.1%, respectively. Considering that in the present study, crude Baneh skin oil was added to sunflower oil as an antioxidant, the high content of unsaponifiable matter as the main source of antioxidant compounds plays an important role in improving the oxidative stability of this oil. The levels of these

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substances in crude rice bran, corn, sesame, crude soybean, canola, virgin olive and cottonseed oils were reported to be 2.4, 2.8 maximum, 0.9 to 2.3, 1.6, 2 maximum, 0 to 1.5 and 0.5 to 0.7 percent, respectively [19]. Therefore, it was found that the level of unsaponifiable matter in the oil of the Baneh skin was in a good condition compared to most common edible oils. Also, the levels of total tocopherol and phenolic compounds in the oil of Baneh skin were determined to be 734 and 86 mg/ml, respectively. The amount of tocopherols in walnut, cottonseed, canola, olive, palm, peanut, soybean, and sunflower oils has been reported to be 1500, 830 to 900, 690 to 695, 30 to 300, 360 to 560, 330 to 480, 900 to 1400, and 630 to 700 mg/kg of oil, respectively [19]. It was found that the amount of these compounds in the Baneh skin oil was in appropriate conditions.

3-2- Studying the effect of Baneh skin oil on various properties of refined sunflower oil during thermal processing

Crude Baneh skin oil was added to sunflower oil in amounts of 0.05, 0.1, 0.2, 0.35 and 0.5% as an antioxidant to determine its effect on oxidative stability, antioxidant activity and some chemical properties of sunflower oil during 8 hours of thermal processing at 170°C. Synthetic antioxidant TBHQ was used at 100 ppm to compare with the antioxidant activity of Baneh skin oil. For this purpose, acid value, peroxide value, anisidine value, TOTOX value, conjugated diene value, total tocopherol compounds, total phenolic compounds, DPPH free radical scavenging power and oxidative stability index (Rancimat) were performed.

3-2-1- Changes in oxidative stability during thermal processing

Figure 1 shows the effect of adding different amounts of Baneh skin oil on changes in the acid number of sunflower oil during 8 hours of thermal processing at 170°C. The acid value is a symbol of the hydrolysis of triglycerides to free fatty acids. As fatty acids are released from the triglyceride structure, their oxidation rate increases exponentially, leading to off-flavors in the oil and fried products [2]. In the present study, the acid value of different Baneh skin oil samples at time zero did not differ significantly. However, after 8 hours of thermal processing, the conditions were different. As is known, the increase in the acid value of pure sunflower oil and sunflower oil samples containing 0.05, 0.1, 0.2, 0.35 and 0.5% of Baneh skin oil and 100 ppm of TBHQ after 8 hours of thermal processing compared to the zero moment was determined to be 281.8, 93.7, 87.5, 83.6, 76.7, 158.5 and 0.12%, respectively. These results showed that the synthetic antioxidant TBHQ created the best conditions in terms of acid value testing in sunflower oil, and after that Baneh skin oil was at levels of 0.35, 0.2, 0.1, 0.05 and 0.5%, respectively. According to Codex Standard No. 210-1999, the acid value of edible oils for consumption should be less than 0.6 [20]. Therefore, at the end of the thermal process, the acid value of all the samples studied in this study, except for pure sunflower oil and the oil sample containing 0.5% of the Baneh skin oil, was less than 0.6, indicating the appropriate conditions for the mentioned oil treatments. In the present study, by increasing the concentration of Baneh skin oil in sunflower oil to 0.35%, the resistance to the formation of free fatty acids increased, but by increasing the level of Baneh skin oil from 0.35% to 0.5%, the situation was reversed, which was due to the creation of a peroxidative state due to the increase in antioxidants in sunflower oil. In a similar study, different amounts of Baneh skin oil were used as an antioxidant in soybean oil. After 8 hours of thermal process at 170 °C, the acid value of soybean oil samples containing 1, 2, 4 and 6% bark oil increased by 282, 100, 236 and 172% compared to the zero moment, respectively [4]. Comparison of the results of this study with the present study showed that the use of Baneh skin oil in amounts less than 0.5% had a superior antioxidant effect in edible oils compared to amounts greater than 0.5%. The use of Baneh skin oil in amounts of 0.5% and above had a negative effect on the acid value of edible oils due to the occurrence of peroxidative activity instead of antioxidant activity. Sharayei et al. used different amounts of Baneh kernel oil as an antioxidant in canola oil in a study. The results of this study showed that the increase in acid value after 8 hours of thermal processing in canola oil containing 0.05, 0.1, 0.2 and 0.4% Banek kernel oil was 126, 82, 120 and 139%, respectively, with the best baneh kernel oil treatment reported to be 0.1% [5], which was

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close to the results of the present study at levels of 0.1, 0.2 and 0.35% Baneh skin oil. Also, in the aforementioned study, after 48 hours of thermal processing, canola oil containing 0.1% of the Baneh kernel oil was the best sample in the acid value test. In another study, 0.5 to 10% of Kolkhoung (*Pistacia khinjuk*) fruit oil was used to improve the oxidative stability of olive oil during 8 hours of thermal processing at 170 °C. The results of the acid value test showed that Kolkhoung fruit oil at the level of 1% was the best antioxidant treatment in olive oil. The increase in this factor after 8 hours of thermal processing was 31% [21], which was more suitable compared to the present study.

Peroxide value is an indicator of the initial oxidation of edible oils. Hydroperoxide compounds are not highly stable and can be decomposed and converted into secondary compounds [2]. The trend of changes in the peroxide value of refined sunflower oil during 8 hours of thermal processing, under the influence of different amounts of Baneh skin oil and 100 ppm TBHQ, is shown in Figure 2. As is clear, there was no significant difference between the peroxide values of different oil samples at time zero. Also, during the thermal processing, the trend of changes in the peroxide value of all oil samples was increasing. The increase in peroxide value of pure sunflower oil and sunflower oil samples containing 0.05, 0.1, 0.2, 0.35 and 0.5% of Baneh skin oil and 100 ppm of TBHQ at the end of the thermal process compared to the zero moment was determined to be 828.4, 291.6, 485.5, 542.6, 488.7, 474.1 and 416.7%, respectively. The results showed that, unlike the acid value test, the oil sample containing 0.05% of Baneh skin oil had the best conditions in the peroxide value test, followed by sunflower oil samples containing 100 ppm of TBHQ, 0.5% of Baneh skin oil, 0.1% of Baneh skin oil, 0.35% of Baneh skin oil and 0.2% of Baneh skin oil and pure sunflower oil. Unlike the acid value test, changes in peroxide value during thermal processing were more extensive. Also, the superiority of 0.05% of Baneh skin oil over TBHQ and the close competition of other levels of Baneh skin oil with this antioxidant were quite valuable results of this study, because crude Baneh skin oil is not pure like TBHQ. Farhoush et al. reported in a study that the use of Baneh skin oil compared to rice bran oil at a level of 0.5% in sunflower oil caused a better effect on changes in peroxide value during 10 days of incubation at 50 °C [10]. This was while in another study with similar conditions, the use of Baneh skin oil at a level of 0.5% compared to rice bran oil in sunflower oil had a less effect on changes in peroxide value [18]. Of course, it should be noted that the conditions of the two studies mentioned were completely different from the present study in terms of temperature and time.

During secondary oxidation, hydroperoxides are broken down and the oil enters the secondary oxidation stage. Carbonyl compounds, especially aldehydes, are the most prominent secondary oxidation compounds and are known as odor-producing compounds. Unlike hydroperoxides, these compounds have good resistance to temperature and their measurement is a suitable criterion for determining the progress and spread of oxidation of edible oils. The anisidine value is used to measure the degree of secondary oxidation [2]. The trend of changes in the anisidine value of refined sunflower oil during 8 hours of thermal processing, under the influence of different amounts of Baneh skin oil and 100 ppm TBHQ is shown in Figure 3. Based on the graph, it was found that the initial value of the anisidine value of different oil samples did not differ significantly from each other, but unlike the peroxide value test, the trend of changes in this index was not increasing in all samples. The increase in the anisidine value of pure sunflower oil and sunflower oil samples containing 0.2, 0.35, and 0.5%t of Baneh skin oil oil and 100 ppm of TBHQ at the end of the thermal process was determined to be 252, 0.04, 14.5, 27.9, and 126.4 percent, respectively, compared to the zero moment, while in sunflower oil containing 0.05 and 0.1% of linseed oil, we saw a decrease of 27.8 and 23.23 percent in the anisidine value compared to the zero moment after the thermal process. The reason for this phenomenon may be related to the high resistance of these two levels of Baneh skin oil oil to the formation of secondary oxidation compounds. During the thermal process, some of the compounds resulting from secondary oxidation, such as aldehydes, are separated from the oil due to the kinetic energy resulting from the thermal process and their volatility. But because the rate of formation of these compounds is higher than their release in the oil, we usually see an increase in the anisidine index during the

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thermal process at high temperatures. But in these two oil samples mentioned, because they had a very high resistance to the formation of secondary compounds, the rate of release of these compounds from the oil was higher than their formation during the thermal process and a decrease in these compounds was observed at the end of the thermal process. Therefore, according to the results of the anisidine value test, the best treatment was sunflower oil containing 0.05% of Baneh skin oil, followed by oil samples containing 0.1, 0.2, 0.35 and 0.5% of Baneh skin oil, oil samples containing 100 ppm of TBHQ and pure sunflower oil, respectively. The results indicate the complete superiority of Baneh skin oil in amounts less than 0.5% over the chemical antioxidant TBHQ in controlling secondary oxidation. This was while Baneh skin oil, unlike TBHQ, was not pure. It was also found that with increasing the concentration of Baneh skin oil in sunflower oil, their antioxidant activity against secondary oxidation decreased. In a study that used 1 to 6% Baneh skin oil to improve the oxidative stability of soybean oil, it was reported that the amount of carbonyl compounds in soybean oil containing 1, 2, 4, and 6% caraway seed oil increased by 317, 214, 218, and 155%, respectively, after 8 hours of thermal processing [4]. Comparison of the results of this study and the present study showed that Baneh skin oil at concentrations of less than 0.5% has a much higher antioxidant power in inhibiting secondary oxidation than at concentrations higher than 1%. Sharif et al. also reported that 2% Baneh skin oil had a higher ability to inhibit secondary oxidation of refined sunflower oil compared to 4% and 8% levels [8]. In a study conducted by Sharayei et al., it was found that after 8 hours of thermal processing, the increase in carbonyl compounds of canola oil containing 0.05, 0.1, 0.2 and 0.4% caraway kernel oil increased by 85, 11.1, 61 and 59%, respectively [5]. Comparison of this study and the present study showed that Baneh skin oil had higher antioxidant activity in inhibiting secondary oxidation compared to Baneh kernel oil. Tavakoli et al. also reported that the use of Kolkhoung fruit oil (the closest pistachio species to Baneh) at 0.5 and 1% levels resulted in higher resistance to secondary oxidation compared to TBHQ [21].

The TOTOX value indicates the total oxidation rate, including primary and secondary oxidation, in oils and fats [2]. The trend of changes in the TOTOX value of refined sunflower oil during 8 hours of thermal processing, under the influence of different amounts of Baneh skin oil and 100 ppm TBHQ, is shown in Figure 4. As is clear, there was no significant difference between the TOTOX value of different samples at time zero. Also, the trend of changes in this index in all treatments during the thermal processing was completely increasing. The increase in the TOTOX value of pure sunflower oil and sunflower oil samples containing 0.05, 0.1, 0.2, 0.35 and 0.5% of Baneh skin oil and 100 ppm of TBHQ at the end of the thermal process compared to the zero moment was determined to be 1.383, 9.65, 4.92, 6.123, 6.122, 8.129 and 4.192%, respectively. An examination of these results showed that Baneh skin oil in all the amounts used was completely superior to the synthetic and powerful antioxidant TBHQ, which was a quite significant result considering that Baneh skin oil was not pure in terms of antioxidant compounds. It was also found that by increasing the concentration of Baneh skin oil from 0.05% to 0.5%, the oxidation resistance decreased. This result means that Baneh skin oil in lower amounts improved the oxidative stability of sunflower oil during the thermal process. Certainly, increasing the amount of caraway seed oil in sunflower oil caused a peroxidative state instead of an antioxidant effect and the oxidative stability of sunflower oil decreased. Similar results were reported in previous studies. Sharif et al. reported that among different amounts of 2 to 8% Baneh skin oil added to sunflower oil, the oil sample containing 2% Baneh skin oil had the highest oxidative stability, and amounts higher than that caused prooxidant activity [8]. Also, Tavakoli and Sorbi, similar to the present study, reported that increasing more than 4% Baneh skin oil in soybean oil caused a peroxidative state during the frying process [4]. In another study, it was reported that the use of Kolkhoung fruit oil (the closest species to Baneh skin oil), in amounts greater than 1% as an antioxidant in olive oil, caused a decrease in its oxidative stability during 8 hours of thermal processing at 170 °C [21].

Since hydroperoxide compounds are fragile at high temperatures, this property can cause errors in the peroxide value test results. Therefore, in addition to the peroxide value test,

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the conjugated diene value test should be used as a supplementary test to examine the quality of edible oils during high-temperature thermal processing [22]. The conjugated diene value is a suitable indicator for indicating the degree of lipid oxidation in oils containing a significant amount of fatty acids with multiple double bonds, and its level increases with oxygen absorption and the formation of lipid peroxides. Conjugated diene is rapidly formed and accumulated in polyunsaturated oils. These compounds can react with oxygen to form conjugated hydroperoxides [2]. The measurement of the conjugated diene value is a good indicator of the initial oxidation of oil samples. The increase in absorbance at a wavelength of 234 nm is proportional to the increase in oxygen consumption and the formation of hydroperoxides during the initial stages of oxidation [23]. The trend of changes in the conjugated diene value of different oil samples studied in the present study is shown in Figure 4, which indicates the absence of significant differences at time zero and a completely increasing trend during the thermal process in different samples. The increase in the conjugated diene value of pure sunflower oil and sunflower oil samples containing 0.05, 0.1, 0.2, 0.35 and 0.5 percent of Baneh skin oil and 100 ppm of TBHQ at the end of the thermal process compared to time zero was determined to be 167.4, 128.7, 110.9, 122.8, 137.6, 152.5 and 152.5%, respectively. As is clear, the best treatment was sunflower oil containing 0.1% of the Baneh skin oil, followed by sunflower oil containing 0.2, 0.05, 0.35 and 0.5% of the Baneh skin oil and 100 ppm of TBHQ. There was no significant difference between the two oil samples containing 0.5% of the Baneh skin oil and 100 ppm of TBHQ in terms of this test. The results of this test, like the other three oxidative stability tests including peroxide value, anisidine index and TOTOX value, indicated the absolute superiority of Baneh skin oil over the synthetic antioxidant TBHQ. In a similar study; Sharif et al. used amounts of 2 to 8% of the bark oil to increase the oxidative stability of sunflower oil, and reported that after 32 hours of frying, bark oil at a level of 2% caused the greatest inhibition of the formation of conjugated diene value [8]. Another study also reported that among the amounts of 1 to 6% of caraway seed oil to increase the oxidative stability of soybean oil during 32 hours of frying, the level of 4% had the best conditions in terms of conjugated diene value test [4]. Considering that in the present study, amounts less than 0.5% were used, it indicated the superiority of Baneh skin oil in lower amounts in edible oils. In another study, Sharayei et al. (2011) used amounts of 0.05 to 0.4% Baneh kernel oil as an alternative antioxidant to TBHQ for the frying process of canola oil for 48 hours, which was found that caraway seed oil at the level of 0.1% caused the least increase in the conjugated diene value of canola oil after thermal processing, which had similar conditions to the caraway seed oil used in the present study [5]. In a study that used 0.5 to 10% of Kolkhoung fruit oil to improve the oxidative stability of olive oil, Tavakoli et al. reported that during 8 hours of thermal processing at 170°C, Kolkhoung fruit oil at levels of 0.5 and 10% created the best conditions in terms of conjugated diene value test [21].

Overall, the results of the oxidative stability tests, including peroxide value, anisidine value, TOTOX value, conjugated diene value, and acid value, showed that the 0.05% level of Baneh skin oil provided the best conditions in terms of oxidative stability during 8 hours of thermal processing in sunflower oil, and after that, the level of 0.1% of this oil was the same. An interesting point was the superiority of Baneh skin oil over TBHQ, which is a very powerful antioxidant. Only in the acid value test, TBHQ was superior, which was not significant compared to other tests, given that the increase in acid value during thermal processing was insignificant in all samples. These results were obtained while the Baneh skin oil was not pure compared to TBHQ. Therefore, in order to better understand the oxidative stability tests, changes in antioxidant compounds and antioxidant activity of sunflower oil under the influence of different amounts of Baneh skin oil were also investigated.

3-2-2- Changes in antioxidant compounds during thermal processing

Tocopherols are the main antioxidant compounds of edible oils [19]. The changes in tocopherol compounds of the oil samples studied in the present study are shown in Figure 6. The results showed that there was no

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significant difference between the amount of tocopherol compounds at time zero. The reduction rate of tocopherol compounds in pure sunflower oil and sunflower oil samples containing 0.05, 0.1, 0.2, 0.35 and 0.5% of Baneh skin oil and 100 ppm of TBHQ at the end of the thermal process was determined to be 20.2, 2.18, 6.15, 4.14, 1.21, 4.17 and 24%, respectively, compared to time zero. As is clear from this test, the oil sample containing TBHQ was the best treatment, followed by oil samples containing 0.2, 0.1, 0.5 and 0.05% of Baneh skin oil, pure sunflower oil and sunflower oil sample containing 0.35% of Baneh skin oil, respectively. These results were not consistent with the oxidative stability tests at all. Because in these tests, the antioxidant TBHQ did not perform well compared to the different levels of Baneh skin oil, but in the test of tocopherol compounds it caused a very small reduction of these compounds. In contrast, in the oxidative stability tests, the level of 0.05% caraway seed oil had the highest antioxidant power, while the reduction of tocopherol compounds in this treatment was the lowest among all samples. Various reasons can be given for the interpretation of these results. First, other antioxidant compounds may play a role in the test results. Second, by examining the results, it was found that in the worst case, about 80% of tocopherols remained in sunflower oil after thermal processing. This finding could mean that there was not much difference between the amount of tocopherol compounds in different sunflower samples. During high-temperature thermal processing, such as frying, tocopherol compounds in oils containing a high amount of acids with multiple double bonds decompose at a slower rate than in more saturated oils. Fatty acids with multiple double bonds decompose before they react with tocopherols during thermal processing at very high temperatures. It has also been reported in other studies that there is competition between tocopherol compounds and unsaturated fats in the reaction with compounds resulting from the decomposition of hydroperoxides (alkoxyl and hydroxyl radicals) [22]. Considering that the sunflower oil studied in the present study contained more than 57% of fatty acids with multiple double bonds, the reason for the small changes in tocopherol compounds can be justified. Third, in oil samples containing more tocopherol, a peroxidative state may be created during the thermal process and the oxidative stability may be reduced. For example, in a sample containing TBHQ, maintaining a high amount of tocopherol compounds in addition to the synthetic antioxidant TBHQ can cause a peroxidative state in sunflower oil during 8 hours of thermal process and reduce its oxidative stability. The effect of natural tocopherol present in edible oils or tocopherol added to them during the frying process is unpredictable. The use of these compounds at high temperatures is not always beneficial and even a peroxidative effect may be observed. Lipid oxidation at high temperatures occurs irregularly with increased activity of contaminating metals or increased prooxidative activity of tocopherols in vegetable oils [22].

Tavakoli and Sorbi reported in a study that the reduction in tocopherol compounds of soybean oil after 8 hours of heat treatment, under the influence of 1, 2, 4 and 6% of Baneh skin oil was 38, 40, 46 and 51% respectively. Also after 32 hours of heat treatment, it was found that the tocopherol compounds of the soybean oil sample containing 4% of Baneh skin oil had the highest reduction among all treatments. This was while in the oxidative stability tests, the soybean oil sample containing 4% of Baneh skin oil had the best conditions and, as in the present study, did not correspond to the changes in tocopherol compounds [4].

In another study, it was also reported that after 8 hours of heat treatment at 170 ° C, the amount of tocopherol compounds of olive oil containing 0.5, 1, 2, 5 and 10% of Kolkhoung fruit oil decreased by 81, 86, 85, 84 and 80% respectively, which was much more than the reduction in tocopherol compounds in the present study. In terms of oxidative stability tests, the sample containing 0.5% of Kolkhoung fruit oil was the best treatment [21].

Polyphenolic compounds with strong biological activities are powerful antioxidants that are widely distributed in plants [19]. The changes in polyphenolic compounds of the oil samples studied in the present study are shown in Figure 7. The results showed that the amount of phenolic compounds in different sunflower oil samples (32.9 to 33.1 mg/kg) was very low compared to tocopherols (858 mg/kg) and these compounds were identified as minor antioxidants in these oil samples. The trend of changes in these compounds during the thermal process was also completely different from

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tocopherols, and both decrease and increase were observed in them. After 8 hours of thermal process, the amount of these compounds was determined to be 25.3 to 33 mg/kg, indicating a low decrease of these compounds during the thermal process. Among the different oil samples, sunflower oil containing TBHQ was the best sample with an almost zero % decrease. This finding means that TBHQ, like tocopherols, had an excellent protective effect on polyphenolic compounds.

3-2-3- Changes in antioxidant activity during thermal processing

In the present study, the antioxidant activity of the oils used was determined using DPPH free radical scavenging and oxidative stability index tests. The changes in DPPH free radical scavenging of refined sunflower oil under the influence of different amounts of Baneh skin oil and 100 ppm TBHQ during 8 hours of thermal processing are shown in Figure 8. Although the amounts of antioxidant compounds including tocopherols and polyphenols in different oil samples before thermal processing did not differ significantly, their free radical scavenging powers were different. It should be noted that in addition to tocopherols and polyphenols, other antioxidant compounds such as carotenoids and some sterols are also present in sunflower oil, which are effective in the level of antioxidant activity. Even the synergistic phenomenon of several antioxidant compounds is also effective in their antioxidant activity. The trend of changes in antioxidant activity of different oil samples except sunflower oil containing 0.05% of Baneh skin oil in the present study was decreasing during the thermal process. The reduction in free radical scavenging power of pure sunflower oil and sunflower oil samples containing 0.1, 0.2, 0.35 and 0.5% of Baneh skin oil and 100 ppm of TBHQ was determined to be 26.3, 12.2, 14.6, 9.9, 15.9 and 8.4% at the end of the thermal process compared to the zero moment, respectively. However, in the case of sunflower oil containing 0.05% of Baneh skin oil, an increase of 7.5% in free radical scavenging power was observed after the thermal process. Considering that in the oxidative stability test, the level of 0.05% of Baneh skin oil was the best treatment, one of the reasons for this superiority can be attributed to the changes in free radical scavenging power.

In some other studies, the trend of increasing and decreasing free radical scavenging power has been reported. In a study that investigated the effect of papaya and melon oils on the oxidative stability of soybean oil during 20 hours of thermal processing at 180°C, it was found that in papaya and melon oils and a combined formula of soybean oils with papaya and melon oils, an increase in free radical scavenging power and then its decrease in some samples was observed, which was consistent with the results of the present study [24].

The trend of changes in the oxidative stability index of different oil samples during thermal processing was measured by the Rancimat device (Figure 9). A number of accelerated methods have been developed to investigate the resistance of edible oils. In all of these tests, high temperature is used to increase the rate of oxidation reaction . Also, in some methods, such as the Rancimat test, air flow is also used in addition to high temperature [25]. The Rancimat test is the most common accelerated method for evaluating the oxidative stability and antioxidant activity of edible oils and fats. However, one point about this test is that due to the use of accelerated conditions, the results of this test differ from the real conditions. For this reason, it is suggested that it be performed as a complementary test along with common oxidative stability tests such as peroxide value, anisidine index, totox index and conjugated dien value during thermal processing such as frying [22, 26]. The initial Rancimat test results of different oil samples showed that adding Baneh skin oil, like TBHQ, increased the oxidative stability index of sunflower oil, but the use of TBHQ significantly increased this index, so that the level of this index in sunflower oil increased by 327% with the addition of 100 ppm TBHQ. This was while increasing 0.05, 0.1, 0.2, 0.35 and 0.5% of Baneh skin oil to sunflower oil, the oxidative stability index increased by 27, 29, 54, 41 and 53%, respectively. However, the trend of changes in the oxidative stability index during the thermal process was completely different from the zero-time conditions. The reduction in the oxidative stability index of pure sunflower oil and sunflower oil samples containing 0.1, 0.2, 0.35 and 0.5% of Baneh skin oil and 100

[DOI: 10.22034/FSCT.22.159.1]

ppm of TBHQ at the end of the thermal process compared to the zero moment was determined to be 19.9, 12.2, 24.1, 21, 22.9, 18.8 and 47.1%, respectively, indicating the superiority of Baneh skin oil at the level of 0.05%, while in the oil sample containing TBHQ, the greatest reduction in the oxidative stability index was observed at the end of the thermal process. It should be noted that the slope of changes in the different quantities of edible oils is important, as is the condition of the oil before the thermal process. It seems that the antioxidant TBHQ in unheated sunflower oil has a very high resistance to the conditions of the Rancimat apparatus, including temperature and air flow, and therefore has the greatest effect on the oxidative stability index of sunflower oil in unheated oil samples, but after the oil samples were subjected to heat treatment, these properties of TBHQ are severely weakened and cause the greatest decrease in the oxidative stability index of sunflower oil after 8 hours of heat treatment. However, as mentioned, the Rancimat test alone is not reliable for identifying the oxidative stability of edible oils. This is because the oxidation mechanism under the conditions of the Rancimat apparatus is different from the real conditions in terms of temperature and air flow rate [22]. Also, the conditions in the Rancimat test are not suitable for measuring the real antioxidant activity of edible oils, because volatile antioxidants may be removed with the air flow. Also, the oils are severely spoiled by the end point of this test [26].

Tavakoli et al. studied the improvement of the oxidative stability of olive oil using different amounts of Kolkhoung fruit oil, which also used 100 ppm of TBHQ antioxidant for comparison. The results showed that adding all the above items increased the oxidative stability index of olive oil, but as in the present study, TBHQ had the greatest improving effect in the Rancimat test. Also, during 8 hours of thermal processing at 170 °C, the greatest reduction in the oxidative stability index was in olive oil containing 100 ppm TBHQ (83.5%). Also, the reduction in this index in olive oil containing 0.5, 1, 2, 5 and 10% was determined to be 75.6, 73, 87.6, 63.8, 59.9 and 79.2%, respectively [21]. Another study reported that the use of different amounts of unsaponifiable matter in Kolkhoung fruit oil (50 to 1000 ppm) caused different effects on the results of the Rancimat test. Among the different treatments, 50 and 100 ppm levels of unsaponifiable matter in kalkhong fruit oil had the greatest protective effect on the oxidative stability index of olive oil after 8 hours of thermal processing at 170°C. As in the present study, olive oil containing 100 ppm TBHQ was among the weakest samples after thermal processing in the Rancimat test [23]. In a study conducted by Farhoush et al., it was reported that the use of 0.5% of Baneh skin oil in sunflower oil increased the stability index of this oil by 29%, while in the present study, increasing the same amount of Baneh skin oil increased the oxidative stability index of sunflower oil by 53% [10].

4- Conclusions:

The results of various oxidative stability tests (peroxide value, anisidine value, TOTOX value, conjugated diene value and acid value) showed that the use of Baneh skin oil at a level of 0.05% created the best conditions in terms of oxidative stability tests during 8 hours of thermal processing at 170 °C compared to 100 ppm TBHQ. In order to better interpret the results of oxidative stability tests, changes in tocopherol and polyphenolic compounds as two key antioxidant compounds were examined during the thermal process. The results showed that there was no relationship between the changes in these compounds and oxidative stability tests. For example, the sample containing TBHQ had the highest protective effect on antioxidant compounds, which decreased the oxidative stability of sunflower oil due to the creation of a peroxidative state caused by the increase in antioxidants. Also, the study of changes in antioxidant activity during the thermal process using two free radical scavenging and Rancimat tests showed that the sunflower oil sample containing 0.05% of Baneh skin oil had the best conditions, which was consistent with the results of the oxidative stability tests. The value of these results is determined when the Baneh skin oil was not at all pure compared to TBHQ. Antioxidant compounds are usually among the unsaponifiables of edible oils. That is, if we consider all the unsaponifiables of Baneh skin oil to have antioxidant properties, by adding Baneh skin oil to sunflower oil at levels of 0.05%, 10.5 ppm of unsaponifiable matters has been added to sunflower oil, which indicates the very high antioxidant power of Baneh skin oil

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during a long-term thermal process at very high temperatures. Of course, this indicates that research on identifying effective compounds in these unique properties of Baneh skin oil should continue.

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Table 1 Some chemical properties of Baneh skin oil and sunflower oil

parameter	Baneh skin oil	sunflower oil
Fatty acid composition		
14:0	-	0.08±0.05
16:0	21.25±0.2 a	8.6±0.09 b
16:1	12.2±0.13 a	0.32±0.04 b
18:0	3.79±0.06 b	4.59±0.03 a
18:1	51.8±0.3 a	27.8±0.21 b
18:2	8.71±0.2 b	54.4±0.25 a
18:3	1.3±0.03 b	2.75±0.06 a
20:1	0.46±0.02 a	0.42±0.03 a
22:0	0.39±0.02 b	0.84±0.03 a
Unsaponifiable matter content (%)	2.1±0.04 a	0.65±0.03 b
Total tocopherols content (mg/kg)	734±1 b	858±19 a
Total phenolics content (mg/kg)	86±1.4 a	33±2.1 b

Mean ± SD within a row with the same lowercase letters are not significantly different at $P<0.05$.

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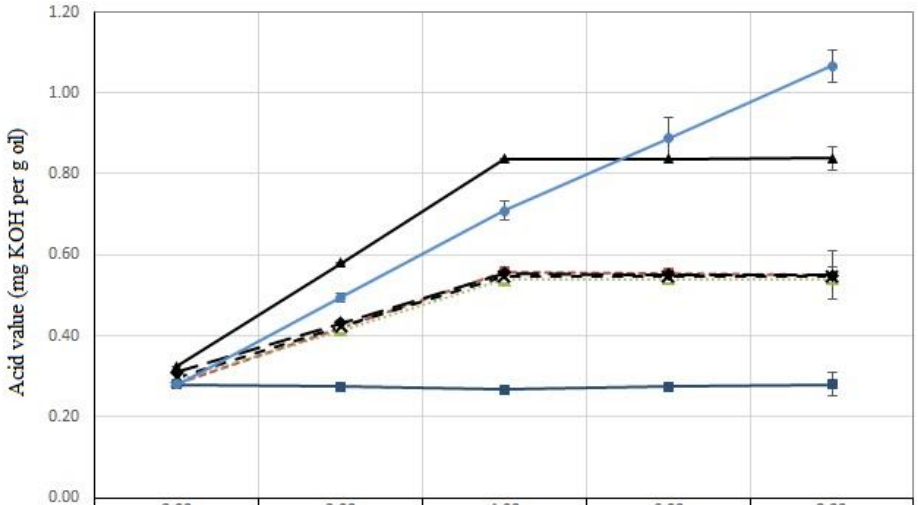
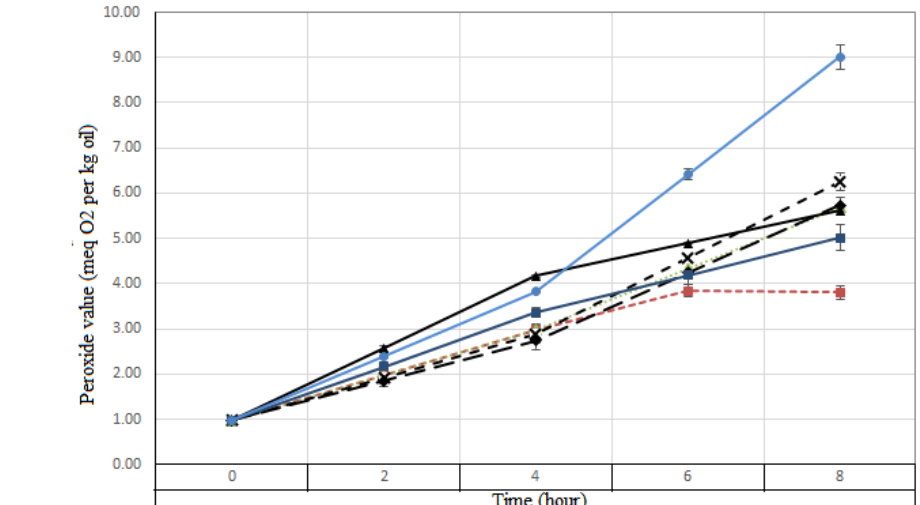


	0.00	2.00	4.00	6.00	8.00
H: 0.05	0.28	0.42	0.56	0.55	0.55
I: 0.1	0.29	0.41	0.54	0.54	0.54
K:0.2	0.30	0.42	0.55	0.55	0.55
M:0.35	0.31	0.43	0.55	0.55	0.55
R:0.5	0.32	0.58	0.84	0.84	0.84
TBHQ	0.28	0.27	0.27	0.27	0.28
pure sunflower	0.28	0.49	0.71	0.89	1.07

Figure 1- Changes in the acid value of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.



	0	2	4	6	8
H: 0.05	0.97	1.98	2.99	3.84	3.80
I: 0.1	0.97	1.98	2.99	4.34	5.69
K:0.2	0.97	1.92	2.88	4.56	6.25
M:0.35	0.97	1.86	2.74	4.24	5.74
R:0.5	0.98	2.57	4.17	4.89	5.61
TBHQ	0.97	2.17	3.36	4.19	5.01
pure sunflower	0.97	2.39	3.81	6.41	9.01

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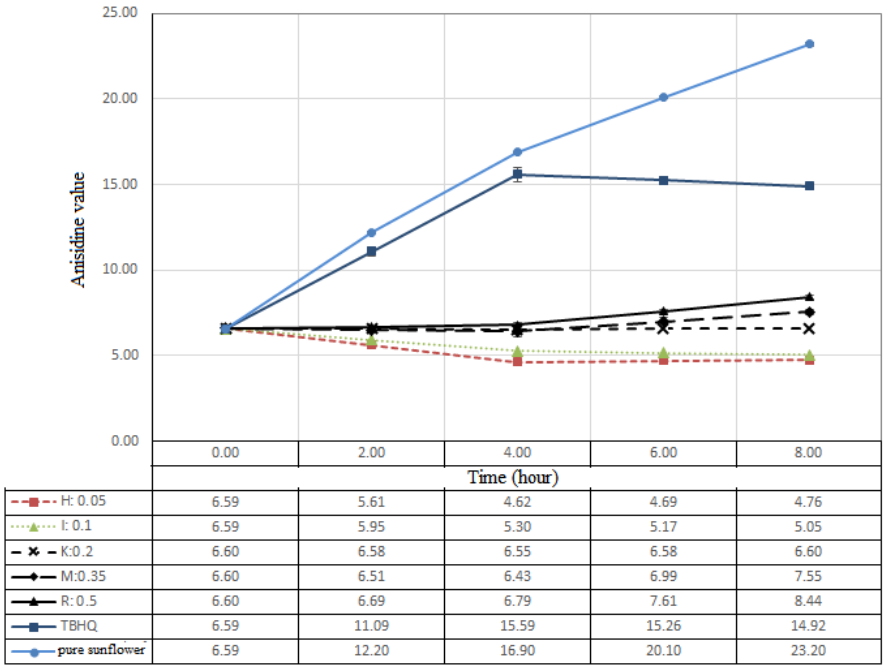
Figure 2- Changes in the peroxide value of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.



	0.00	2.00	4.00	6.00	8.00
H: 0.05	6.59	5.61	4.62	4.69	4.76
I: 0.1	6.59	5.95	5.30	5.17	5.05
K:0.2	6.60	6.58	6.55	6.58	6.60
M:0.35	6.60	6.51	6.43	6.99	7.55
R: 0.5	6.60	6.69	6.79	7.61	8.44
TBHQ	6.59	11.09	15.59	15.26	14.92
pure sunflower	6.59	12.20	16.90	20.10	23.20

Figure 3- Changes in the anisidine value of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.

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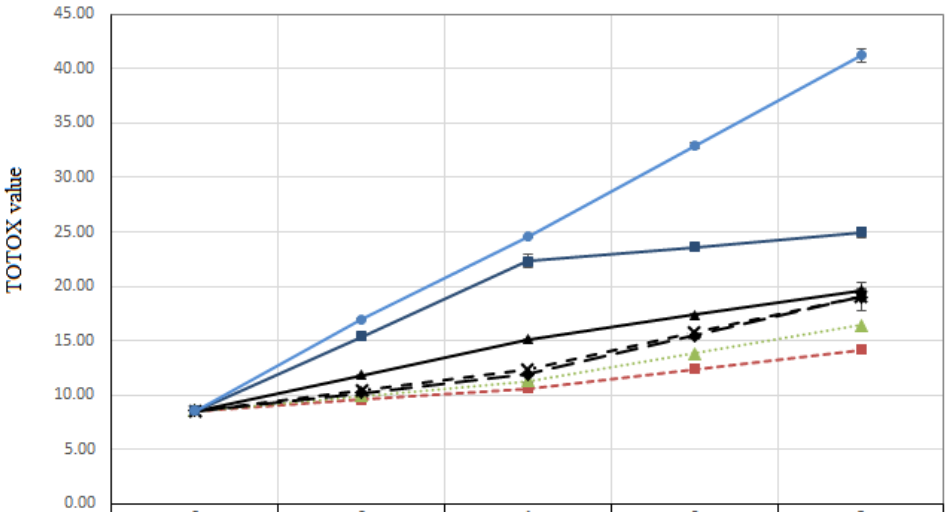
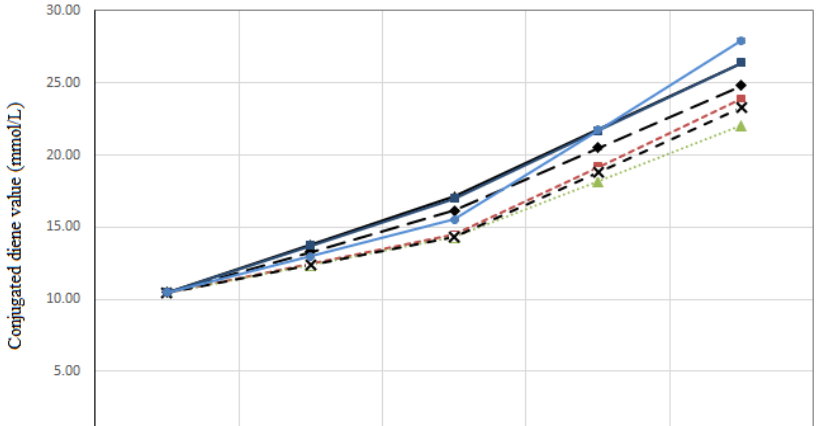


	0	2	4	6	8
	Time (hour)				
H: 0.05	8.54	9.57	10.60	12.38	14.16
I: 0.1	8.54	9.90	11.27	13.85	16.42
K:0.2	8.54	10.42	12.31	15.70	19.10
M:0.35	8.55	10.23	11.90	15.47	19.03
R: 0.5	8.55	11.84	15.12	17.39	19.65
TBHQ	8.53	15.42	22.32	23.63	24.95
pure sunflower	8.53	16.98	24.53	32.92	41.22

Figure 4- Changes in the TOTOX value of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.



	0.00	2.00	4.00	6.00	8.00
	Time (hour)				
H: 0.05	10.45	12.46	14.48	19.19	23.90
I=0.1	10.45	12.36	14.28	18.16	22.03
K=0.2	10.45	12.36	14.28	18.78	23.28
M=0.35	10.45	13.29	16.14	20.48	24.83
R: 0.5	10.45	13.81	17.17	21.78	26.38
TBHQ	10.45	13.71	16.97	21.67	26.38
pure sunflower	10.45	12.98	15.52	21.72	27.93

Figure 5- Changes in the conjugated diene value of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of

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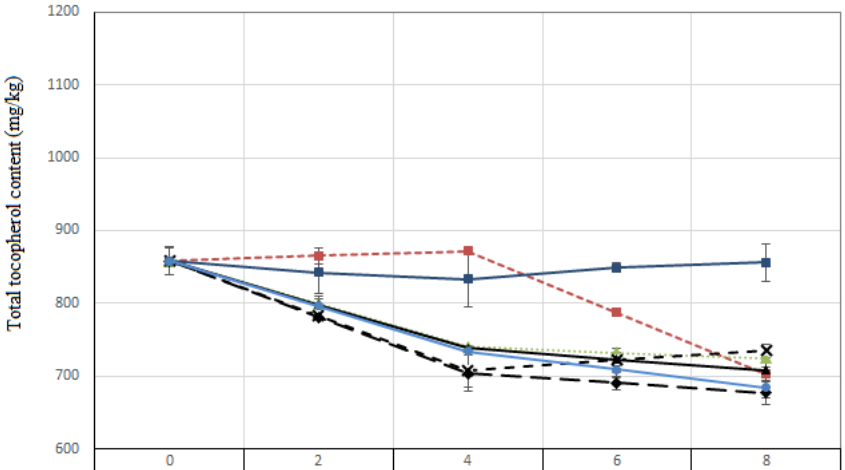
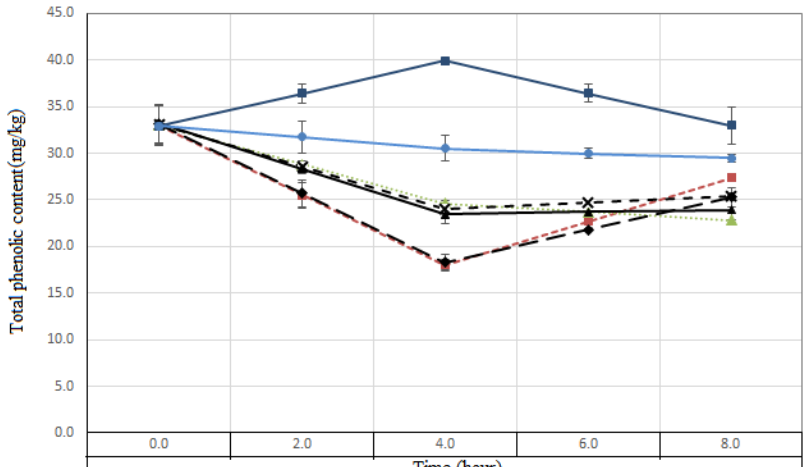
sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.



	0	2	4	6	8
H: 0.05	858	865	872	787	702
I: 0.1	858	799	740	732	724
K:0.2	858	783	708	721	734
M:0.35	858	781	704	691	677
R: 0.5	858	798	738	723	708
TBHQ	858	842	833	849	856
pure sunflower	858	796	734	709	685

Figure 6- Changes in the Total tocopherols content of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.



	0.0	2.0	4.0	6.0	8.0
H: 0.05	33.0	25.5	18.0	22.7	27.4
I: 0.1	33.0	28.8	24.6	23.7	22.8
K: 0.2	33.1	28.5	24.0	24.7	25.4
M: 0.35	33.1	25.7	18.3	21.8	25.3
R: 0.5	33.2	28.3	23.5	23.7	23.9
TBHQ	32.9	36.4	39.9	36.4	33.0
pure sunflower	32.9	31.7	30.5	30.0	29.5

Figure 7- Changes in the Total phenolic content of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of

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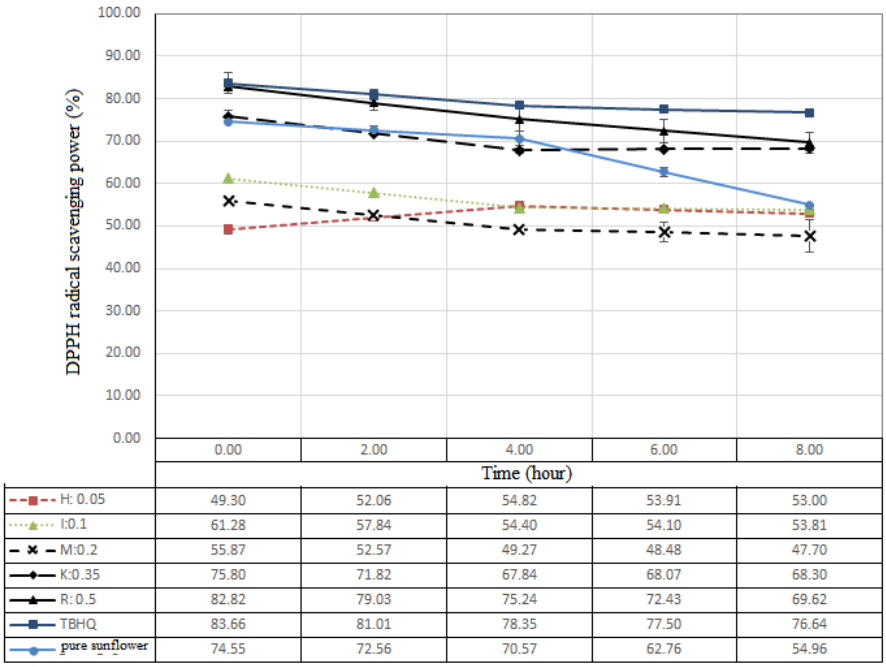
sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.



	0.00	2.00	4.00	6.00	8.00
H: 0.05	49.30	52.06	54.82	53.91	53.00
I:0.1	61.28	57.84	54.40	54.10	53.81
M:0.2	55.87	52.57	49.27	48.48	47.70
K:0.35	75.80	71.82	67.84	68.07	68.30
R: 0.5	82.82	79.03	75.24	72.43	69.62
TBHQ	83.66	81.01	78.35	77.50	76.64
pure sunflower	74.55	72.56	70.57	62.76	54.96

Figure 8- Changes in the DPPH radical scavenging power of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.

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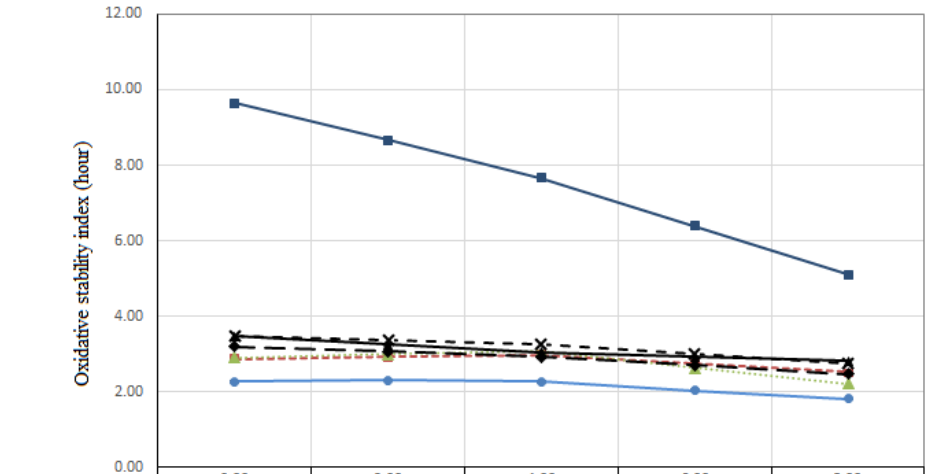

	0.00	2.00	4.00	6.00	8.00
	Time (hour)				
H: 0.05	2.87	2.93	2.98	2.75	2.52
I=0.1	2.91	2.99	3.07	2.64	2.21
K=0.2	3.47	3.37	3.27	3.01	2.74
M=0.35	3.19	3.06	2.93	2.70	2.46
R: 0.5	3.46	3.26	3.05	2.93	2.81
TBHQ	9.65	8.66	7.66	6.39	5.11
pure sunflower	2.26	2.31	2.26	2.04	1.81

Figure 9- Changes in the oxidative stability index (measured with Rancimat device) of refined sunflower oil under the influence of different amounts of crude Baneh skin oil and 100 ppm of TBHQ during 8 hours of thermal processing at 170 °C. H: sample of sunflower oil containing 0.05% of crude Baneh skin oil; I: sunflower oil sample containing 0.1% of crude Baneh skin oil; K: sample of sunflower oil containing 0.2% of crude Baneh skin oil; M: sample of sunflower oil containing 0.35% of crude Baneh skin oil, R: sample of sunflower oil containing 0.5% of crude Baneh skin oil.

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بهبود پایداری اکسایشی روغن آفتابگردان با استفاده از روغن پوست بنه در مقادیر بسیار کم

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چکیده

در تحقیق حاضر، اثر مقادیر مختلف روغن پوست بنه (۰/۰۵ تا ۰/۵ درصد) بر پایداری اکسایشی روغن آفتابگردان تصفیه‌شده طی ۸ ساعت فرایند حرارتی در ۱۷۰ درجه‌سانتیگراد بررسی شد که از آنتی‌اکسیدان سنتزی TBHQ به میزان ۱۰۰ پی‌پی‌ام جهت مقایسه استفاده گردید. بررسی نتایج آزمونهای مختلف پایداری اکسایشی (عدد پراکسید، اندیس آنیزیدین، اندیس توتوکس، عدد دی-انمزدوج و عدد اسیدی) نشان‌داد که استفاده از روغن پوست بنه باعث بهبود پایداری اکسایشی روغن آفتابگردان شد. بهترین شرایط پایداری اکسایشی در روغن آفتابگردان حاوی ۰/۰۵درصد روغن پوست بنه مشاهده شد و بعد از ان نمونه روغن حاوی ۰/۱ درصد روغن پوست بنه قرار داشت که هر دو دارای اثر آنتی‌اکسیدانی برتر از TBHQ بودند. به منظور تفسیر بهتر نتایج آزمونهای پایداری اکسایشی، تغییرات ترکیبات توکوفرولی و پلی‌فنلی به عنوان دو ترکیب آنتی‌اکسیدانی شاخص طی فرایند حرارتی بررسی شد. نتایج نشان داد بین تغییرات این ترکیبات و آزمونهای پایداری اکسایشی ارتباط وجود ندارد. نمونه حاوی TBHQ دارای بیشترین اثر حفاظتی بر ترکیبات آنتی‌اکسیدانی بود که به علت ایجاد حالت پراکسیدانی ناشی از افزایش آنتی‌اکسیدانها، باعث کاهش پایداری اکسایشی روغن آفتابگردان شد. همچنین بررسی تغییرات فعالیت آنتی‌اکسیدانی طی فرایند حرارتی به کمک دو آزمون قدرت مهارکنندگی رادیکال آزاد و رنسیمت نیز نشان داد که نمونه روغن آفتابگردان حاوی ۰/۰۵ درصد روغن پوست بنه بهترین شرایط را داشت که با نتایج آزمونهای پایداری اکسایشی همخوانی داشت.. ارزش این نتایج زمانی مشخص می‌شود که روغن پوست بنه در مقایسه با TBHQ اصلا خالص نبود.

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