Italian Journal of Food Science, 2021; 33 (SP1): 69–77

Incorporation of essential oils in Iranian traditional animal oil: an assessment of physicochemical and sensory assessment

Maliheh Azizi[1], Fatemeh Fazeli[1*], Mehrdad Mohammadi[2*], Amin Mousavi Khaneghah[3*]

[1]Department of Food Science and Technology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran; [2]Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [3]Department of Food Science and Nutrition, Faculty of Food Engineering, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil

***Corresponding Authors:** Fatemeh Fazeli, Department of Food Science and Technology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran. Email: fatemeh_fazeli2002@yahoo.com; Mehrdad Mohammadi, Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: mohammadi@sbmu.ac.ir; Amin Mousavi Khaneghah, Department of Food Science and Nutrition, Faculty of Food Engineering, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil. Email: mousavi@unicamp.br

Submitted: 10 March 2021; Accepted: 5 May 2021; Published: 29 June 2021

PAPER

Abstract

This study aimed to incorporate the herbal essential oils (*Ziziphora tenuior* L. (*Z. tenuior*), *Ferulago angulata* (*F. angulata*), and *Bunium persicum* (*B. persicum*)) all in three levels (5, 7, and 10% w/w) and tertiary butyl hydroquinone (100, 200, and 300 ppm) to evaluate the oxidation stability of Iranian animal oil (IAO). *Z. tenuior*, *F. angulata*, and *B. persicum* (5, 7, and 10% w/w) and tertiary butyl hydroquinone (100, 200, and 300 ppm) were added to IAO. The physicochemical properties and color analysis, and sensory characteristics (odor, taste, rancidity, and overall acceptability) of the treatments were investigated on days 0, 7, 14, 21, and 28. The results showed that increasing the amounts of *Z. tenuior*, *F. angulata*, and *B. persicum* was associated with reducing acid, peroxide, and thiobarbituric acid values. It also lowered brightness and yellowness while the oxidative stability of the IAO significantly was increasing. It was concluded that the incorporation of *B. persicum* had the highest effectiveness regarding the proposed criteria with the least effect on sensory properties.

Keywords: bioactive compounds; herbal antioxidant; oil stability; natural preservative; color analysis

Introduction

Oxidation in food, especially in fats and oils, is a destructive process with adverse effects on their nutritional values and chemical composition (Henry and Heppell, 2002). It can also have undesirable effects on the color, taste, and texture of foods, while degrading the essential vitamins and fatty acids or creating toxic compounds (Stoilova *et al*., 2007; Wang *et al*., 2008). Antioxidants are natural or synthetic substances added to food products to prevent or delay the damage caused by oxygen (Sharififar *et al*., 2010). Although compounds such as tocopherols are naturally available in some oils, synthetic antioxidants with a phenolic structure are commonly used to prevent the oxidation of oils (Agregán *et al*., 2019; Mousavi Khaneghah *et al*., 2016).

Synthetic antioxidants, such as tertiary butyl hydroquinone (TBHQ), are inexpensive, easily accessible, and have high efficacy in inhibiting oxidation. TBHQ at 75 ppm is used in the oil industry in many countries, including Iran. It affects the free radicals of lipids in oxidative chain reactions. Its phenolic group stabilizes free fatty radicals by using hydrogen and thus preventing the oxidation of fats (Wang *et al*., 2008).

However, some concerns have been raised regarding the mutagenicity and the role of synthetic antioxidants in the disruption of liver enzymes' activity as well as the development of diseases such as cancer and cardiovascular disease (Iqbal and Bhanger, 2007; Tavakoli *et al*., 2018). As a result, replacement of these compounds with natural antioxidants of plant origin has been considered. Antioxidant properties of plant extracts mainly depend on their phenolic compound content (Ahmed *et al*., 2019; Burt, 2004). In this regard, herbs and their derivatives (essential oils and extracts) with potent antioxidant properties are widely used to prevent the oxidative degradation of food (Hashemi *et al*., 2017; Tiwari *et al*., 2009).

"Kermanshahi Roghan," known as the "Roghan-*e*-Heyvani," is an Iranian animal oil (IAO) and a yogurt by-product, like ghee and yayik butter commonly used in India and Turkey, respectively (Mostafaie *et al*., 2018). It is a traditional anhydrous milk fat product with a golden yellow color because of carotenoids. As an expensive dairy product, IAO is one of the most favorable oils consumed in many regions of Iran (Salarabadi *et al*., 2015). Produced from cow, sheep, or goat's milk, IAO is exported to many countries worldwide (Chalabi *et al*., 2018). The fatty acid composition of IAO (99% fat and < 0.2% moisture) is different from that of butter, as the lipids are extracted directly from milk. Therefore, it has lower long-chain fatty acids and cholesterol (Najafi *et al*., 2011).

Z. tenuior, *Ferulago angulata* (Chavill), and *Bunium persicum* (Black Caraway) belong to *Lamiaceae*, *Apiaceae*, and *Chattrian*, respectively. *Z. tenuior* (Bakhtiar *et al*., 2021) and *F. angulata* (Ghasemi Pirbalouti *et al*., 2016) are widely distributed in Iran. Also, *B. persicum* grows in areas with a Mediterranean climate such as Central and Western Asia (e.g., Iran) (Hassanzadazar *et al*., 2018). Due to their phenolic and flavonoid compounds, these plants have antioxidant and antibacterial activities, and gastrointestinal transit accelerating properties (Amiri and Joharchi, 2016; Dakah *et al*., 2019; Ehsani *et al*., 2016; Hazrati *et al*., 2019; Mahboubi, 2019).

Considering the increasing awareness among consumers regarding the harmfulness of synthetic additives in food, as well as studies on the use of herbal extracts to increase the shelf life of vegetative oils, the present study is designed to compare the oxidation of IAO treated with *Z. tenuior*, *F. angulata*, *B. persicum*, with that of synthetic antioxidant TBHQ.

Materials and Methods

Extraction of essential oil

Fresh IAO, free of any antioxidants, was purchased from a local market. The dried leaves of *Z. tenuior, F. angulata*, and *B. persicum* were obtained from the Iranian Institute of Medicinal Plants, Karaj, Iran. The essential oils were extracted from these leaves using a Clevenger-type apparatus (Biogenic, Brasilia, Brazil), and based on the steam distillation method of Gharibzahedi *et al*. (2015). The brief protocol is as follows:

One-hundred grams of the leaves were placed in the distillation flask, and the oil was extracted from the vegetal substrate at 5-, 15-, 30-, 60-, and 100-min intervals. In order to completely isolate the water from the essential oil, 0.5 g of sodium sulfate was added to the separation column each time. The extracted essential oils were stored at 4 ± 1°C until experiment time. TBHQ and other chemicals were purchased from Merck Co. (Darmstadt, Germany).

Preparation of the treatments

Different concentrations of the essential oils and TBHQ were added to 100 g of IAO based on the values indicated in Table 1 and mixed using RH Basic 2 magnetic stirrer (Staufen, Staufen Germany). StaufenThe IAO samples were evaluated on days 0, 7, 14, 21, and 28 of storage. All tests were performed in three replications.

Table 1. Iranian animal oil treatments with incorporation of different essential oils and TBHQ[a].

Treatments	*Z. tenuior* (%)	*F. angulata* (%)	*B. persicum* (%)	TBHQ (ppm)
T1	5	–	–	–
T2	7	–	–	–
T3	10	–	–	–
T4	–	5	–	–
T5	–	7	–	–
T6	–	10	–	–
T7	–	–	5	–
T8	–	–	7	–
T9	–	–	10	–
T10[b]	–	–	–	100
T11[b]	–	–	–	200
T12[b]	–	–	–	300

[a]TBHQ: Tertiary butyl hydroquinone, artificial antioxidant.
[b]T10–T12 treatments were used as control.

IAO analysis

IAO with a peroxide value of less than 5 meq O^2/kg was purchased from the Pegah Golpayegan Dairy Company (Golpayegan, Iran). It was kept at 4°C during the whole study, as recommended by the producer.

Acid value

The AV was defined as the amount (mg) of potassium hydroxide (KOH) required to neutralize the free fatty acids in 1 g of IAO samples dissolved in a mixture of ethanol. The titration method was used to determine the AV according to ISO 660 (ISO, 2009) using the following equations:

$$\text{Acid value (mL KOH/g IAO)} = \frac{56.1 \times N \times V}{M} \times 100 \quad (1)$$

N: KOH normality; V: Consumed KOH volume (mL); M: IAO weight (g); 56.1 = KOH molecular weight (g/mol).

Peroxide value

IAO was dissolved in a solution of isooctane and glacial acetic acid and then mixed with potassium iodide. Free iodine via peroxides was measured (ISO 3960, ISO, 2017) using iodometry in the presence of starch and sodium thiosulfate solution. PV was determined through sodium thiosulfate titration and was calculated using the following equation:

$$\text{Peroxide Value (meq } O^2\text{/kg IAO)} = \frac{V \times N}{M} \times 1000 \quad (2)$$

V: Consumed thiocyanate volume; N: Thiosulfate normality; M: IAO weight.

Thiobarbituric acid value

Thiobarbituric acid (TBA) value shows the amount of malondialdehyde (MDA) present in each 100 g of oil. MDA, commonly used as an oxidation marker, is one of the most abundant aldehydes generated during secondary lipid peroxidation in foods (Reitznerová *et al*., 2017). This method allows direct measurement of the TBA levels in fats and oils without the MDA's need for prior isolation. MDA reacts with TBA and forms a color complex with absorption maxima at a wavelength of 530 nm (AOCS, 2017).

$$\text{TBA (mg MDA/kg IAO)} = \frac{50 \times (A - B)}{M} \quad (3)$$

A: Absorption of IAO sample; B: Absorption of TBA as the blank sample; M: IAO weight

Oxidative stability

The ability of the oil against oxidation is known as Os and is expressed as h. The test was performed based on the Rancimat technique using a Metrohm 743 Rancimat (FanAzma Gostar, Alborz, Iran) at 110°C and 20 l/h airflows (ISO 6886, ISO, 2016). Oxidation resistance ends when a rapid increase is seen in the specific conductance due to the decomposition of the carboxylic acids resulting from lipid oxidation and their absorption in deionized water.

Color analysis

The color evaluation was performed using Hunter lab colorimeter (Hunter lab D25-DP9000, Germany) with black and white calibration plates. L* (lightness) from black (0) to 100 (white), a* (green-red), and b* (blue-yellow) was calculated from −120 to 120 (Guo *et al.*, 2016).

Sensory evaluation

The sensory characteristics of the treatments such as odor, taste, rancidity, and overall acceptability of coded IAO samples during 28 days of storage were evaluated based on a 5-point hedonic scale from 1 (dislike very much) to 5 (like very much) (Mohammadi *et al*., 2011).

Statistical analysis

A randomized complete block design was applied. The data were analyzed by analysis of variance (ANOVA) using SAS 9 (SAS Institute Inc., Cary, NC, USA) followed by Duncan's multiple range test (Koushki *et al*., 2011). Values were reported as mean ± standard deviation (SD) of six repetitions for each treatment. P values < 0.05 were considered statistically significant for all comparisons (Amiri-Rigi *et al*., 2011).

Results and Discussion

AV, PV, TBA values and Os analysis of different IAO treatments

AV value depends on the type and the amount of the applied essential oils. In other words, the difference in the AV is due to the difference in the antioxidant content of the active compounds as well as their ability to trap water. According to Figure 1A, there was a significant difference between the AV values of the controls (T10–T12) and that of the treatments containing essential oils (P < 0.05). A decreasing trend was observed in AV values with increasing concentrations of essential oils. Treatments containing *B. persicum* and *Z. tenuior* were reported to have significantly lower AV values than those containing *F. angulata*. This is mainly because the high polyphenolic, flavonoid, and antioxidant content of treatments containing *B. persicum* deactivates free radicals derived from the environmental oxidation process (Hassanzadazar *et al*., 2018). AV indicates the progress of the oxidation process, and therefore its value decreases with the increasing flavonoid content of the oil. This is mainly because of the increased concentration of applied essential oils.

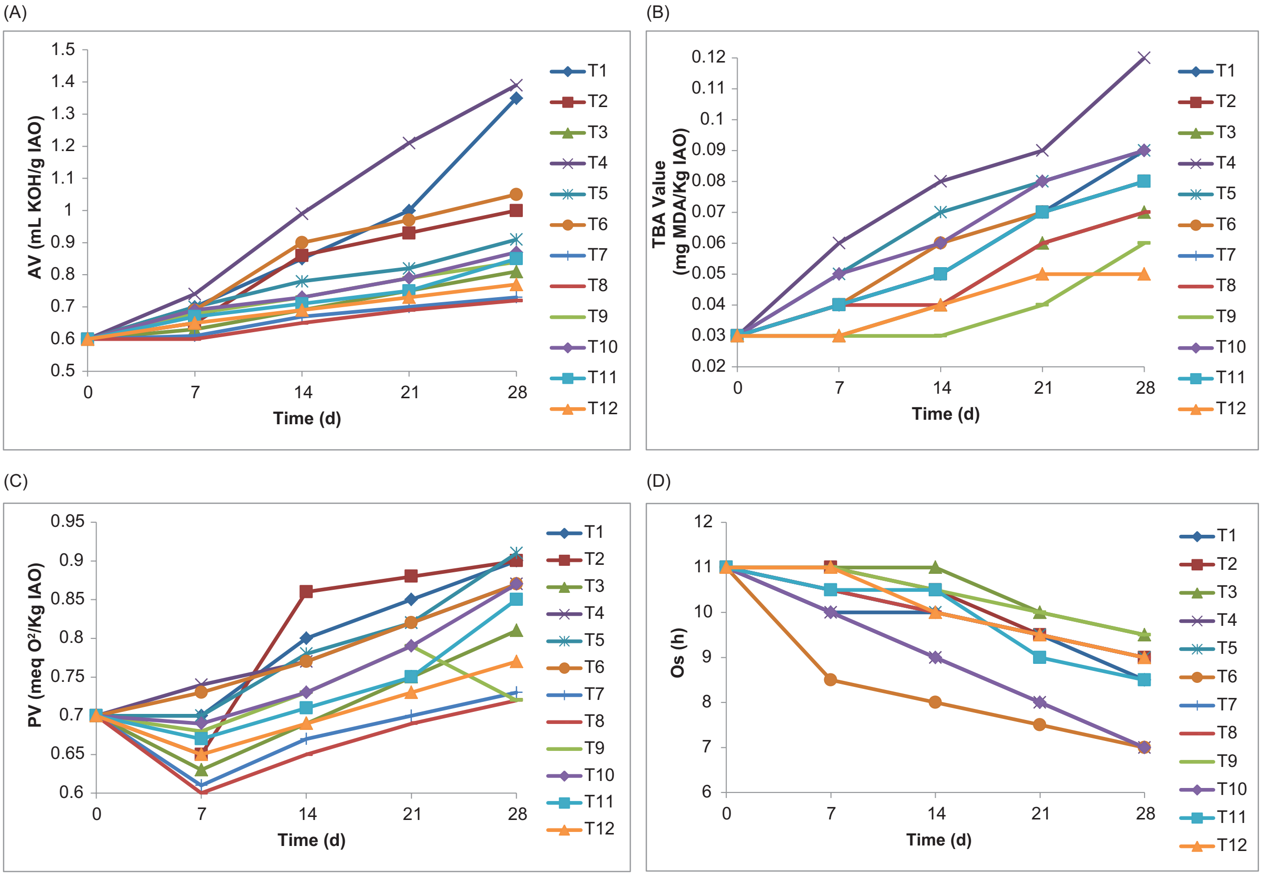

Figure 1. Acid value (AV) (A), peroxide value (PV) (B), Thiobarbituric acid (TBA) value (C), and oxidative stability (Os) (D) of Iranian animal oil treated with herbal essential oils during storage time.
Treatments (T1–T12) are described in Table 1.

There was an overall increase in the AV of IAO treatments with increased storage time that negatively affects the oil quality. This can be secondary to the increase of IAO's free fatty acid content during this time, either because of the hydrolysis of triglycerides or their formation as an end product of the oxidation process (Guo *et al.*, 2016). Moreover, the increased exposure of oil to oxygen, light, and acidic compounds as the oxidation process progresses could also be responsible for the significant increase noted in the AV values. For instance, the increase noted in AV of IAO treatments containing *B. persicum* and *Z. tenuior* was lower than that of controls containing TBHQ. Treatments containing 10% *B. persicum* and *Z. tenuior* had the lowest AV enhancement (28 days) ($P < 0.05$).

On the other hand, the AV of treatments containing *F. angulata* increased significantly during storage but was negatively correlated with the amount of *F. angulata* ($P < 0.05$). Compared with *B. persicum* and *Z. tenuior*, *F. angulata* is high in aromatic compounds but low in polyphenolic ones. In line with our findings, Najafi *et al.* (2011) showed similar results while studying the antioxidant effect of olive leaf and its extract on soybean oil stability (Najafi *et al.*, 2011).

Unsaturated fatty acids such as oleic and linoleic acids with double bonds in their chemical structure are highly susceptible to oxidation-accelerating factors such as heat, light, oxygen, and lipoxygenase activity (Pradhananga and Manandhar, 2018). Hydroperoxides are the primary products of the oxidation reactions and are thus used to evaluate the oxidation reactions' progression (Alizadeh *et al.*, 2016). IAO is very susceptible to oxidative corruption. According to Figure 1B, the use of essential oils changed the PV of the treated IAO significantly compared with the TBHQ-containing controls ($P < 0.05$). The treatments containing *B. persicum* had the lowest PV. This can be due to the presence of active compounds such as limonene and Trans-Carveol and limonene derivatives (Mohammadi *et al.*, 2021). PV of all treatments increased with longer storage time, depending on the type and the amount of applied essential oil. Among the applied essential oils, *F. angulata* had the least effect on PV values. In other words, there was no significant difference between the PV of the *F. angulata*–treated IAO and the control containing 100 ppm TBHQ. The least amount of change in PV values was reported in IAO containing 10% *B. persicum* after 28 days of storage ($P < 0.05$). Darughe *et al.* (2012) also found that the antioxidant compounds in *B. persicum* significantly inhibited any increase in PV values of the cake during storage (Darughe *et al.*, 2012).

PV breaks down after reaching a certain level and the by-product compounds are formed (Pradhananga and Manandhar, 2018). For this reason, TBA value, alongside PV, was also investigated. According to Figure 1C, there was a significant difference between the TBA values of different IAO treatments based on the type and the amount of applied essential oil ($P < 0.05$). TBA followed an increasing trend during the 28 days of storage. The treatment containing 5% *F. angulata* had the highest TBA value, whereas treatments with *B. persicum* had the lowest increase in TBA value during storage ($P < 0.05$). This can be due to the increase in the latter's phenolic and limonene compounds that inhibited oxidation more effectively (Mohammadi *et al.*, 2021). The high aromatic compound content of *F. angulata*, on the other hand, was not as effective in inhibiting the oxidation process. The increase noted in the TBA values of the IAO treatments indicates the formation of products such as aldehydes and ketones that have an adverse effect on the organoleptic properties of the oil (Serfert *et al.*, 2010). In corroboration with our results, Hassanzadazar *et al.* (2018) also concluded that *B. persicum* could be used as a natural alternative to synthetic antioxidants based on the results of PV and TBA values of their investigation (Hassanzadazar *et al.*, 2018).

Figure 1D illustrates a significant difference between the treatment's oxidation resistance time, and the controls ($P < 0.05$). Treatments containing 10% of each essential oil had the highest Os compared with those with 5% and 7% of the corresponding essential oils. The Os rate in treatments containing *B. persicum*, regardless of their concentration, was higher than that of IAO samples treated with *F. angulata* and *Z. tenuior*. The lowest Os was reported in the IAO samples treated with *F. angulata*. Also, it was observed that the treatments with *B. persicum* had an Os equivalent to TBHQ. While increasing the storage time was associated with a significant decrease in the Os values, increasing the concentration of essential oils had the opposite effect ($P < 0.05$). The former could be secondary to the partial inactivation of phenolic compounds as well as an increase in the levels of secondary hydroperoxide compounds due to storage-related oxidation (Hashemi *et al.*, 2016). Our results were also supported by the research on rosemary extract's antioxidant effects on soybean oil (Casarotti and Jorge, 2014).

Color analysis of IAO treatments

Color is one of the apparent qualitative properties of the food products with a substantial impact on their marketability. This points out the importance of evaluating IAO's color indices treated with herbal essential oils as part of the quality control study. In this study, a* value did not change significantly due to the absence of red pigment and lycopene compounds in *Z. tenuior*, *F. angulata*, and *B. persicum*. L* and b* values of treated IAO samples during the 28 days of storage are shown in Figure 2 (A,B). Figure 2A illustrates that IAO samples treated with *Z. tenuior* and *F. angulata* had lower L* values than the control and *B. persicum*–treated ones. In addition, treatment with higher concentrations of *F. angulata* and *Z. tenuior* resulted in a lower L* value than those containing 5% essential oils. Therefore, it could be concluded that the L* value is significantly but inversely correlated with the concentration of the essential oils ($P < 0.05$). This could be due to the significant but negative effects of the chlorophyll content and pigment content of herbal essential oils on the light reflection, and thus the L* value. Compared with *B. persicum*–treated IAO samples, the control IAO samples were not reported to have significantly different L* values.

The L* values of treatments decreased significantly with increasing storage time ($P < 0.05$). This reduction, which is believed to be secondary to oxidative and lipolytic corrosion, was not significant in IAO samples treated with

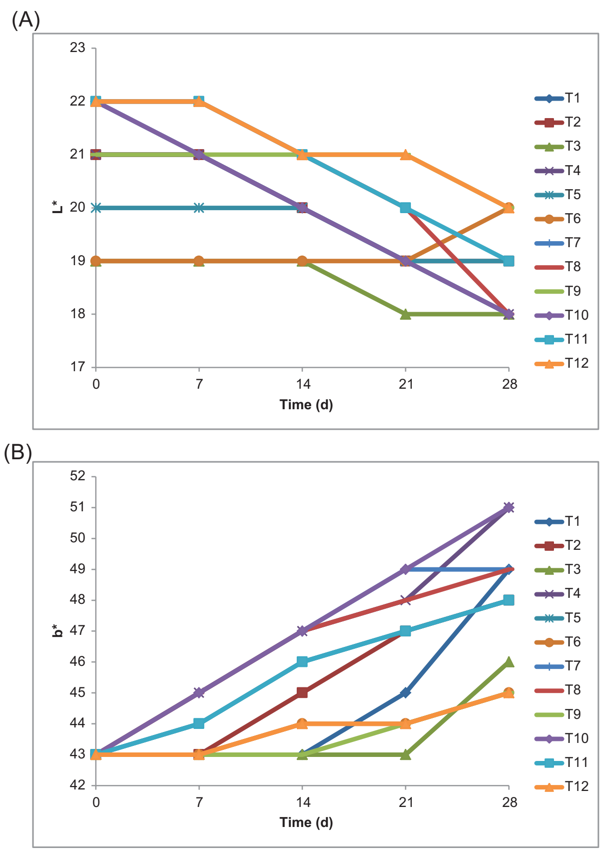

Figure 2. L* value (A) and b* value (B) of Iranian animal oil treated with herbal essential oils during storage time. Treatments (T1–T12) are described in Table 1.

essential oils at concentrations higher than 5%. These findings are in line with that of Guo *et al.* (2016), who reported that the rosemary ethanol extract had a significant effect on the L* value of treated palm oil samples (Guo *et al.*, 2016). They also reported that an increase in rosemary ethanol extract could decrease the L* value of palm oil treatments. Similarly, Hozhabri *et al.* (2014) investigated the effect of fish oil and *Z. tenuior* on the quality and oxidation rate of the Mahabadi goat meat. They showed that the addition of *Z. tenuior* could significantly reduce L* value (Hozhabri *et al.*, 2014).

Figure 2B shows significant differences observed between the b* values of various treatments ($P < 0.05$). The highest b* value was observed in the treatment containing 5% *F. angulata* essential oil, whereas the lowest values were found in those with 10% of *B. persicum*, *F. angulata*, and *Z. tenuior* ($P < 0.05$). An increasing trend in b* values was reported in the treated IAO samples with increased storage time; the increase, however, was lower in the IAO samples containing 10% of essential oils and 300 ppm TBHQ. Similar results were reported in previous research into the antioxidant effects of rosemary extract on soybean oil (Casarotti and Jorge, 2014).

Sensory evaluation of IAO treatments

According to Figure 3A, a significant decrease in taste scores of treatments increased the essential oil concentration. One of the reasons for this may be the dominance of the plant's taste over IAO's special taste. Guo *et al.* (2016) also found that rosemary ethanolic extract in high amounts in palm oil caused a vegetable taste in palm oil. With increasing storage time, there was a decreasing trend in the taste scores of the treatments. Treatments containing higher than 5% essential oils had lower taste scores on day 0, but they kept their scores steadily during storage time. Treatments with *F. angulata* and then *Z. tenuior* showed the lowest scores, but the treatments containing *B. persicum* had the same score as the control treatment with 300 ppm TBHQ. Even treatments with 200 and 100 ppm TBHQ had lower taste scores than those with *B. persicum* during storage until the end of day 28 ($P < 0.05$).

According to Figure 3B, increasing essential oil concentration led to a significant decrease in the odor scores in IAO treatments. Treatments with *B. persicum* had the least effect on the odor characteristic because the aromatic compounds in *Z. tenuior* and *F. angulata* are

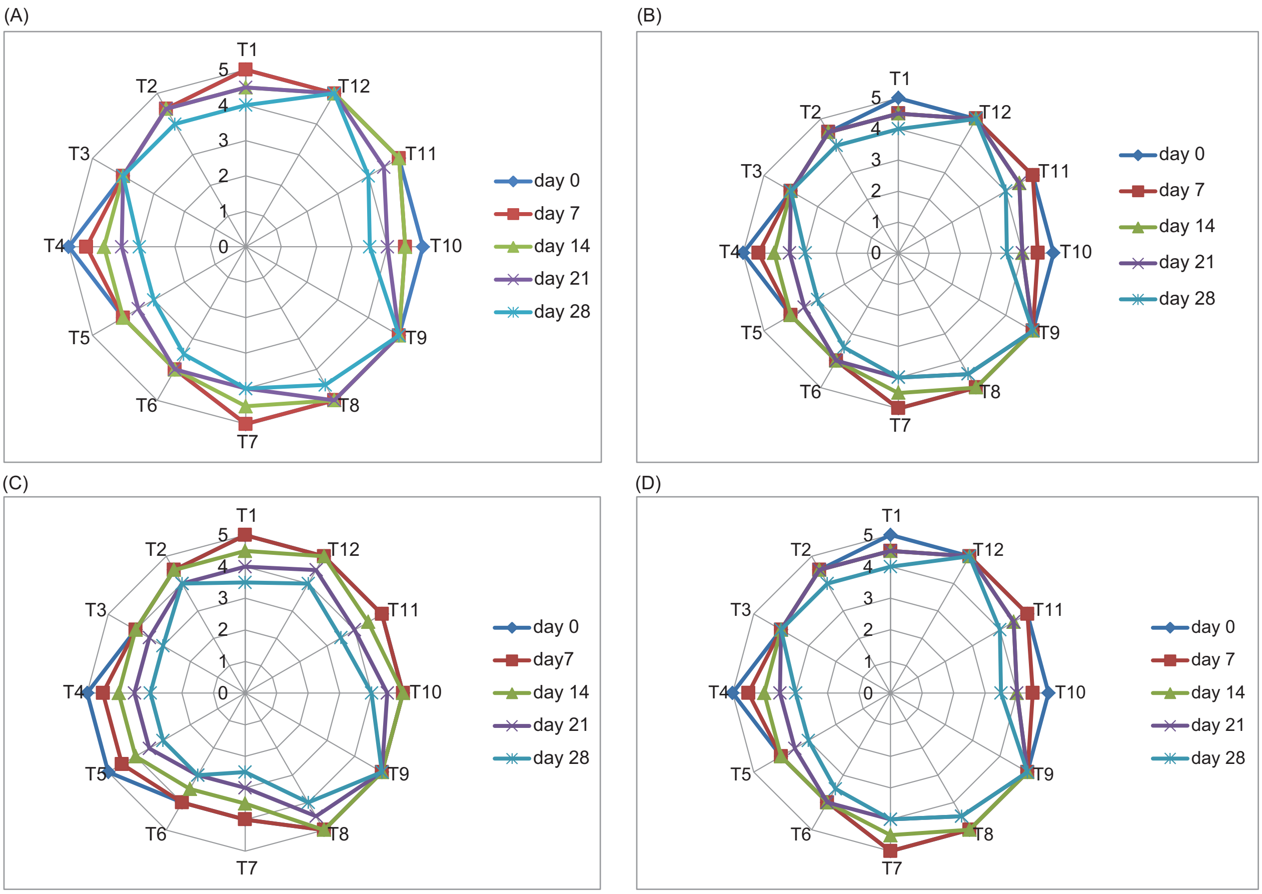

Figure 3. Taste scores (A), Odor scores (B), Rancidity scores (C), and Overall acceptability (D) of Iranian animal oil treated with herbal essential oils during storage time.
Treatments (T1–T12) are described in Table 1.

higher than the *B. persicum*, which reduces IAO's odor. Also, during the storage period, the flavor and odor scores of all treatments significantly decreased ($P < 0.05$). This decrease in the treatments containing *F. angulata* was more than in the other treatments.

According to Figure 3C, minimum scores of rancidity belonged to treatments containing *F. angulata* and then to *Z. tenuior*; the highest scores belonged to treatments with 300 ppm TBHQ and 10% *B. persicum*. The results showed that the effect of *F. angulata* on the rancidity character of IAO was lower than the effect of *B. persicum* and *Z. tenuior*, which is due to the difference in the chemical content of these essential oils. All the essential oils extracted from three medicinal herbs have high amounts of bioactive compounds. Mohammadi reported that the main chemical constituents in *Z. tenuior*, *F. angulata*, and *B. persicum* essential oils were pulegone (12.77%), ferulagon (14.97%), and (+)-*trans*-carveol (57.70%), respectively. However, higher total phenolic content, total flavonoid content, total carotenoid, and total chlorophyll in *B. persicum* essential oil were found compared to *Z. tenuior* and *F. angulata* essential oils (Mohammadi *et al*., 2021).

Also, increasing the essential oil concentration led to an increase in rancidity scores of treatments. During the storage period, the reduction of rancidity scores was lower in treatments with 10% essential oils. This can be due to the prevention of oxidation by essential oils during storage. Treatments containing 10% *B. persicum* had higher rancidity scores on the 28th day in comparison to other treatments.

According to Figure 3D, treatments containing *B. persicum* and TBHQ had the highest overall acceptability among the other treatments. The overall acceptability of treatment containing 5% *F. angulata* was significantly reduced because of the sensitivity of the *F. angulata* aroma and aromatic compounds of the *F. angulata*. Overall acceptability of all treatments decreased by increasing storage time, and increased by increasing essential oil content. Only the treatments containing 10% *B. persicum* and 300 ppm TBHQ maintained their overall acceptability until the end of the 28th day of storage time. Mehraban Sangatash *et al*. (2006) investigated the effect of *Ziziphora Clinopodioides* essential oil and extract on the activity of yogurt starter bacteria and found that the use of high concentration of essential oil reduced the final overall acceptability of yogurt treatments, which is consistent with the results of the present study.

Conclusions

It is concluded that increasing concentrations of *Z. tenuior*, *B. persicum*, and *F. angulata* are associated with a significant decrease in AV, PV, TBA value, L*, and b* of the treated IAO. The addition of herbal antioxidants, regardless of their concentrations, also improved the Os of the treated IAO; their effects though started to decrease after 28 days of storage. Moreover, by increasing the essential oils' content, taste and odor of IAO decreased, while rancidity and overall acceptability were increased. The bioactive effects of the applied essential oils was found to be in the order *B. persicum* > *Z. tenuior* > *F. angulata*. The addition of 10% *B. persicum* to the IAO was found to be an applicable and safe replacement for TBHQ. Our results are beneficial for developing strategies for producing edible oils and lipid-rich foods containing natural antioxidants with appropriate oxidative stability and pleasing sensory characterizations. In general, more study should be done to evaluate the antimicrobial effects of edible oils and emulsions enriched with the studied essential oils.

Acknowledgments

The authors would like to thank the National Nutrition and Food Technology Research Institute (Iran, Tehran) for providing the equipment required for this study.

Conflict of Interest

The authors of this manuscript wish to declare no conflict of interest associated with the submission and its content.

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