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Research Article

The Extended Oxidative and Sensory Stability of Traditional Dairy-Based Oil with Steam-Distilled Essential Oils Extracted from the Bioactive-Rich Leaves of *Ziziphora tenuior*, *Ferulago angulata*, and *Bunium persicum*

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The oxidation rate and overall sensory acceptability of Iranian animal oil (IAO) during storage were evaluated after adding the bioactive essential oils extracted from medicinal herbs of *Ziziphora tenuior*, *Ferulago angulata*, and *Bunium persicum*. Results showed that the most dominant 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. IAO contained more saturated fatty acids (67.43%, mainly palmitic and myristic acids) than unsaturated (32.27%, mostly oleic acid) ones. *B. persicum* essential oil compared to the other two oils significantly had more total flavonoid (1.08 mg quercetin equivalent/g), phenolic (123.2 mg GAE/g), carotenoid (591.31 mg/kg), and chlorophyll (24.32 mg/kg) contents. A significant dose-dependent increase in the overall sensory acceptability of IAO was found by increasing the concentration of *B. persicum* essential oil. Similar to tertiary butyl hydroquinone, the oil blend of IAO+10% *B. persicum* essential oil obtained the maximum overall sensory acceptability scores during 28 d cold storage due to the remarkable *in vitro* DPPH inhibition (83.45%) and ferric-reducing power (0.754 at $Å_{700nm}$). A much slower formation rate in primary and secondary oxidation compounds in IAO rich in *B. persicum* essential oil during the storage was associated with the overall sensory acceptability data ($p < 0.01$, $r = 0.951$). Thus, this bioactive additive as a bio-preservative may well stabilize crude oils and emulsions.

1. Introduction

Oxidation of fats and oils is an adverse process that leads to undesirable effects on sensorial and nutritional properties by forming toxic compounds in foods [1]. Antioxidants are natural or synthetic substances that can be added to oil-containing foodstuff products to prevent or delay the oxidation process [2]. Tertiary butyl hydroquinone (TBHQ) is considered a widely used antioxidant in edible oil industries. The usual concentration of this synthetic preservative in Iranian oil refining factories is approximately 75 ppm [3]. However, due to consumer concerns about the harmful effects of synthetic antioxidants on human health (i.e., the prevalence of cancer types and cardiovascular diseases) and safety standards, substituting these substances with plant antioxidants is an interesting strategy to develop safe and natural edible oil products [4].

"Roghan-*e*-Heyvani" known as "Kermanshahi Roghan" is a yogurt by-product with a yellow golden color similar to butter oil, clarified butter, or ghee, which is typically produced from cow, sheep, or goat milk containing 99% fat and less than 0.2% moisture [5]. Iranian animal oil (IAO) is

produced under a similar process to Yayik butter in Turkey and ghee in India with a difference in the last unit operation. For instance, IAO is obtained with cold clarification of yogurt, while ghee is produced by heating. IAO is taken into account as the most famous dairy product of Iran to export to other countries in the world [6]. However, the economic value of this commercial product can be profoundly reduced due to the formation of undesirable aroma/flavor and toxic components as a result of the high content of saturated fatty acids (SFAs) and cholesterol [7, 8]. Therefore, it is necessary to apply some practical solutions for decreasing/preventing the development of off-flavor, off-odor, and other negative organoleptic characteristics.

The addition of natural antioxidants such as essential oils (EOs) extracted from plant sources is one of the safest practices to maintain the natural taste/flavor of oils rich in SFAs (like IAO) during storage. In this study, EOs obtained from three native medicinal herbs including *Ziziphora tenuior* L. (ZT), *Ferulago angulata* (Schlecht.) Boiss (FA), and *Bunium persicum* Boiss (BP) were added to IAO. ZT, FA, and BP belong to the family of Lamiaceae, Apiaceae, and Apiaceae, respectively. *Z. tenuior* L., as an annual herb and edible ethnomedicinal plant, is widely distributed in Iran, Central Asia, Turkey, Eurasian countries, and West Siberia [9]. There are 35–40 species of the genus *Ferulago* in the globe that about eight species exist in the flora of Iran. *F. angulata* Boiss is more widespread in Iran (mainly in western and southwestern parts), Turkey, Greece, Yugoslavia, Macedonia, and Australia [10]. Also, *B. persicum* Boiss grows in areas with a Mediterranean climate such as central and Western Asia (e.g., Iran, Turkey, Syria, Pakistan, Tajikistan, and Afghanistan), North India (Kashmir and Pamir), China, and some parts of Europe, Northern Africa, and South America [11]. The phenolic and flavonoid compounds present in these plants implied positive biological features such as antioxidant, antimicrobial, antiallergic, and antilipidemic activities [12–16]. It was also demonstrated that phenols are mainly responsible for the antioxidant properties of essential oils and extracts obtained from these medicinal plants [12, 15]. Thus, it was assumed that IAO enrichment would be an effective approach to prevent its oxidative degradation by promoting sensory attributes.

To the best of our knowledge, there are a few studies on IAO production and the role of EOs in improving the overall quality of consumer's preference for this butterfat concentrate developed from yogurt. Hence, this study was aimed to evaluate the blending effect of IAO with ZT-, FA-, and BP-EOs compared to TBHQ on the organoleptic characteristics and the formation rate of oxidation products during the storage.

2. Materials and Methods

2.1. Plant Material and Raw Milk. The dried leaves of the herbs of ZT, FA, and BP were obtained from the Iranian Institute of Medicinal Plants (Karaj, Iran). Raw cow's milk with $3.5 \pm 0.2\%$ fat was provided from an animal farm in Golpayegan city (Isfahan Province, Iran).

2.2. Chemicals and Reagents. 2, 2- Diphenyl-1-picrylhydrazyl (DPPH), gallic acid (GA), Folin–Ciocalteu reagent (FCR), aluminium chloride ($AlCl_3$), and TBHQ were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada). Other chemicals including sodium phosphate, anhydrous sodium sulfate (Na_2SO_4), sodium carbonate (Na_2CO_3), potassium acetate (CH_3CO_2K), trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), potassium ferricyanide ($K_3Fe(CN)_6$), methanol, chloroform, *iso*-octane, toluene, and quercetin were purchased from Merck Chemical Co. (Darmstadt, Germany). The fatty acid standards including SFAs (C4–C20) and mono- (MUFAs, C18:1*t* n-7 and C18:1*c* n-9) and poly- (PUFAs, C18 : 2 n-6 and C18 : 3 n-3) were provided by Sigma-Aldrich Chemical Co. (Steinheim, Germany).

2.3. Extraction Process of Essential Oils. The EO was extracted from dried leaves of each medicinal herb by the steam-distillation method [17]. In brief, the sun-dried leaves of each herb were initially oven-dried at $60 \pm 1^{\circ}C$ for 24 h until attaining a constant weight. The EOs were extracted after the appearance of the first drop of distillate at the time intervals of 5, 15, 30, 60, and 100 min. The oil was separated from water by adding 0.5 g Na_2SO_4 and then stored at $4 \pm 1^{\circ}C$ until analysis time by gas chromatography-mass spectrometry (GC-MS). The EO yield was estimated on a dry-weight basis (v/w).

2.4. GC-MS Analysis of Essential Oils. The procedure described by Sereshti et al. [18] with small modifications was used to identify and quantify chemical components present in the different EOs. An online GC (model 3700, Varian, Palo Alto, CA) coupled with a 4,000 series mass detector (Varian, Palo Alto, CA) was used to separate bioactive compounds present in EOs. The used column was an HP5-MS capillary fused-silica column (length, 30 m; internal diameter, 0.25 mm; film thicknesses, 0.25 μm) with the stationary phase of 5% phenyl polysiloxane and 95% methyl polysiloxane. The temperature program was as follows: $40^{\circ}C$ for 1 min, $10^{\circ}C\,min^{-1}$ up to $250^{\circ}C$, and hold for 10 min. The flow rate, split ratio, and injector temperature were 1 mL/min, 1 : 30, and $250^{\circ}C$, respectively. In the MS analysis, 50–550 m/z was determined for the scanned mass range, while 70 eV ionization energy and full scan mode were considered to take the mass spectra [19].

2.5. Phenolic Content Measurement. The total phenolic content (TPC) of EOs was assessed with the FCR according to the explained method by Moghaddam et al. [20]. After mixing 0.1 mL of the solutions containing 1.0 mg of each EO with 46 mL of deionized water, 1.0 mL FCR was added to the mixture and shaken completely. After holding the sample for 3 min at $23 \pm 1^{\circ}C$, 50 mL of 2% (w/v) solution of Na_2CO_3 was added and then incubated for 120 min at $23 \pm 1^{\circ}C$. The mixture absorbance was determined at 760 nm using a UV-visible spectrophotometer (UNICO UV-2100, Shanghai,

China). The TPC results were expressed as mg GA equivalent (GAE) per g of EO based on the drawn standard curve.

2.6. Flavonoid Content Assessment. The procedure of Dhaiwal et al. [21] with the standard of quercetin was used to measure the total flavonoid content (TFC) of EOs. In brief, 0.5 mL of each EO with 0.1 mL of 10% (w/v) $AlCl_3$ and 0.1 mL of 1 M CH_3CO_2K was mixed, and subsequently, 4.3 mL of 80% methanol was added to reach the total volume to 5.0 mL. The mixture absorbance at 415 nm was measured using a UV-visible spectrophotometer. The TFC was calculated according to the standard curve and expressed as mg quercetin equivalent (QE)/g EO.

2.7. Evaluation of Chlorophyll and Carotenoid Content. The method of Mínguez-Mosquera et al. [22] was used to estimate the content of chlorophyll and carotenoid pigments. In a visible absorption spectrum, the chlorophyll and carotenoid fractions were, respectively, measured at 670 and 470 nm using the spectrophotometer cells with a thickness (L) of 10 mm. Then, the total chlorophyll (TChC, mg/kg EO) and carotenoid (TCaC, mg/kg EO) content was, respectively, estimated by the following equations:

$$\text{TChC (mg/kg)} = \left(\frac{A_{670} \times 10^6}{613 \times 100 \times L}\right), \quad (1)$$

$$\text{TCaC (mg/kg)} = \left(\frac{A_{470} \times 10^6}{2000 \times 100 \times L}\right). \quad (2)$$

2.8. Iranian Animal Oil (IAO) Preparation. The yogurt was initially prepared after pasteurizing (at 95°C for 5 min), cooling, and fermenting with the starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. The prepared yogurt with an equal volume of water was diluted to obtain doogh. The doogh was churned for 8 h at 23 ± 1°C and stored for 18 h at 4°C. Then, the butter in a water bath at 45 ± 1°C was separated and centrifuged at 10,000 rpm for 10 min. The upper layer, as IAO, was collected and stored at −18°C until before the analysis.

2.9. Lipid Extraction and Fatty Acid Profile Analysis. The lipid extraction from IAO was performed in a mixture of methanol and chloroform (2 : 1 v/v) according to the modified method of Bligh and Dyer [23]. The modified method of Chalabi et al. [6] was used to analyze the fatty acid methyl esters (FAMEs) from IAO prepared by transesterification using a GC system (Varian 3400, Palo Alto, CA, USA) equipped with a fused-silica capillary column DB-23 (60 m length, 0.25 mm internal diameter, 0.2 μm film thickness), a flame ionization detector (FID), and a split/splitless injector. The differences in the applied procedure were as follows: the carrier gas of helium at a flow rate of 1.2 mL/min, the split ratio of 1 : 60, and the injector temperature of 265°C. The fatty acid composition was determined based on the comparison of retention times of detected peaks between samples and standards.

2.10. IAO Enrichment with Essential Oils. IAO was treated with different concentrations (5, 7, and 10%) of EOs obtained from ZT (T1–T3), FA (T4–T6), and BP (T7–T9) and stored for four weeks at 4 ± 1°C. TBHQ (T10–T12) as control was also used to treat IAO at concentrations of 100, 200, and 300 ppm. Our preliminary studies showed that the addition of the studied concentration levels to IAO resulted in desirable changes of oxidative and sensory attributes.

2.11. Antioxidant Activity of EO-Treated IAO

2.11.1. Scavenging Capacity Assessment of DPPH Free Radical. In this antioxidant test, 100 mg of each IAO in 1.0 mL toluene was shaken with the DPPH solution in toluene (10^{-4} M, 3.9 mL) for 30 min. The mixture absorbance was assessed using a UV-visible spectrophotometer at 515 nm. As control, the DPPH solution was used by mixing 3.9 mL of DPPH solution and 1.1 mL pure toluene [23]. The scavenging percentage of DPPH radical was calculated as follows:

$$\text{DPPH inhibition (\%)} = 100 - \left(\frac{A_C - A_S}{A_C} \times 100\right), \quad (3)$$

where A_C and A_S are the absorbance amounts of the control and sample solutions, respectively.

2.11.2. Reducing Power Measurement. The method described by Mau et al. [24] with small modifications was used to analyze the reducing power of ferric ions. In brief, the equal volume (2.5 mL) of each IAO formulation in methanol, sodium phosphate buffer (200 mM, pH 6.6), and 1% $K_3Fe(CN)_6$ was mixed and incubated at 52 ± 2°C for 25 min. After adding 2.5 mL of 10% TCA, the mixture was centrifuged at 5,000 rpm for 10 min. Five milliliters of the collected supernatant were mixed with distilled water (5.0 mL) and 0.1% $FeCl_3$ (1.0 mL), and then the spectrophotometric absorbance was measured at 700 nm.

2.12. Determination of Oxidation Values. The peroxide value (PV) of each cold-stored IAO was assessed according to the procedure of the International Dairy Foundation [25]. Based on the standard method of AOCS (no., CD 18–90), a mixture of oil blend (1.0 g) and *iso*-octane (100 mL) was prepared and its spectrophotometric absorbance was read at 350 nm to determine the anisidine value (AnV) [26]. Finally, the total oxidation (TOTOX) value was calculated as follows: TOTOX = 2 × PV + AnV.

2.13. Sensory Acceptability Evaluation. The overall sensory acceptability (OSA) of coded oil samples at 0, 7, 14, 21, and 28 days of storage was evaluated by 25 trained panelists based on a 5-point hedonic scale from 1 (dislike very much) to 5 (like very much). Each sample was presented twice in

Table 1: Concentration of the main chemical constituents of triple EOs and fatty acids of IAO[a].

ZT-EO		BP-EO		FA-EO		IAO	
Component	Conc. (%)	Component	Conc. (%)	Component	Conc. (%)	Fatty acid	Conc. (%)
Bornyl acetate	5.73 ± 0.03	β-Caryophyllene	0.41 ± 0.01	Carvacrol methyl ether	12.05 ± 0.06	Butyric acid (C4:0)	9.52 ± 0.11
Camphene	4.25 ± 0.02	Carvacrol	0.33 ± 0.01	Trans-Chrysanthenyl acetate	5.06 ± 0.02	Caproic acid (C6:0)	1.45 ± 0.02
1, 8-Cineole	5.99 ± 0.08	(−)-*trans*-Carveol	57.70 ± 0.84	*p*-Cymene	14.45 ± 0.07	Caprylic acid (C8:0)	3.09 ± 0.05
Limonene	5.73 ± 0.11	Carvone	1.48 ± 0.04	2, 5-Dimethoxy-*p*-cimene	8.31 ± 0.03	Capric acid (C10 : 0)	3.42 ± 0.05
(β)-Myrcene	5.47 ± 0.01	*trans*-(+)-Dihydrocarvone	0.31 ± 0.00	Ent-3 β-hydroxy-13-epi-manoyl oxide	10.62 ± 0.07	Lauric acid (C12 : 0)	1.35 ± 0.03
Isomenthone	7.48 ± 0.12	Limonene	35.50 ± 0.32	Ferulagon	14.97 ± 0.02	Myristic acid (C14 : 0)	13.10 ± 0.21
Neomenthone	4.30 ± 0.06	(+)-*trans*-Limonene oxide	2.01 ± 0.07	(*Z*)-β-Ocimene	11.58 ± 0.12	Palmitic acid (C16 : 0)	30.06 ± 0.35
Piperitenone	5.47 ± 0.08	(β)-Myrcene	0.48 ± 0.04	α-Pinene	11.07 ± 0.31	Stearic acid (C18 : 0)	3.46 ± 0.06
(E)-β-Ocimene	6.14 ± 0.04	Perillyl alcohol	0.34 ± 0.02	γ-Terpinene	11.86 ± 0.05	Arachidic acid (C20 : 0)	1.98 ± 0.04
α-Pinene	3.40 ± 0.07	α-Terpinolene	0.10 ± 0.00			*trans*-Vaccenic acid (C18:1*t* n-7)	5.02 ± 0.14
Pulegone	12.77 ± 0.14					Oleic acid (C18:1*c* n-9)	22.91 ± 0.19
Terpinen-4-ol	7.75 ± 0.23					Linoleic acid (C18 : 2 n-6)	4.11 ± 0.07
γ-Terpinene	6.24 ± 0.08					Linolenic acid (C18 : 3 n-3)	0.23 ± 0.01
α-Terpinolene	5.50 ± 0.04						
α-Thujene	3.28 ± 0.03						
Sabinene	4.48 ± 0.05						
Spathulenol	5.94 ± 0.07						

[a]EOs: ZT = *Z. tenuior*, FA = *F. angulata*, and BP = *B. persicum*; IAO = Iranian animal oil.

random order. The panelists used water to rinse their mouths after tasting each sample, while unsalted crackers were available [27, 28].

2.14. Statistical Analysis. All experiments were carried out in triplicate, and the results were expressed as mean ± SD. All of the data were analyzed with the SAS software, version 9.0 (SAS Institute, Inc., Cary, NC). Assumptions of normal distribution and variance homogeneity were tested graphically using residual plots. The OSA data were statically analyzed by the Kruskal–Wallis test. The other collected data were subjected to analysis of variance (ANOVA), whereas Duncan's multiple range test was used to determine the significant difference between treatments at the 95% confidence interval ($p < 0.05$). The correlation analysis was also conducted employing Pearson's test with the SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Fatty Acid Profile of Extracted Lipids. Table 1 exhibits the type and content of fatty acids of IAO. 99.7% of fatty acids present in IAO were identified and quantified by the GC-FID analysis. The total content of SFAs, MUFAs, and PUFAs in IAO was 67.43, 27.93, and 4.34%, respectively. The percentage of short-chain, medium-chain, and long-chain SFAs in IAO was determined to be 9.52, 9.31, and 48.60%, respectively. Hence, long-chain SFAs were dominant in the fatty acids profile of IAO ($p < 0.05$). The most frequent SFAs in IAO were palmitic (30.06%), myristic (13.10%), and butyric (9.52%) acids, respectively. The major unsaturated fatty acid (USFA) found in IAO was oleic acid (22.91%). Also, two PUFAs of linoleic and linolenic acids were quantified to be 4.11 and 0.23%, respectively (Table 1). Earlier, Chalabi et al. [6] found similar results with the analysis of fatty acids of Kermanshahi Roghan. Palmitic (C16 : 0) and oleic (C18:1*c* n-9) acids were, respectively, reported to be the most frequent fatty acids in this animal-based oil. They also realized that the content of short-chain SFAs (C4–C10), long-chain SFAs (C12–C20), and USFAs was 18.13, 47.74, and 34.10%, respectively [6]. However, in the present work, a lower percentage of caproic, lauric, and linolenic acids and more content of butyric, myristic, and palmitic acids were found. This discrepancy may be ascribed to the difference in livestock diet and dietary lipid as well as the storage and preparation conditions of IAO before the GC injection. Bahrami et al. [29] demonstrated that the intake of Kermanshahi Roghan compared to industrial edible milk-based oils could substantially reduce the low-density lipoprotein (LDL) cholesterol levels.

Table 2: The content of bioactive compounds in EOs extracted from medicinal herbs.

EO type	TFC (mg QE/g)	TPC (mg GAE/g)	TChC (mg/kg)	TCaC (mg/kg)
ZT	0.09 ± 0.03^{b}	100.70 ± 3.96^{b}	11.09 ± 0.78^{b}	342.21 ± 6.38^{b}
FA	0.05 ± 0.02^{b}	67.80 ± 3.31^{c}	9.97 ± 1.42^{b}	202.54 ± 1.90^{c}
BP	1.08 ± 0.07^{a}	123.23 ± 2.65^{a}	24.32 ± 2.44^{a}	591.31 ± 8.42^{a}

All data represent the mean of three replications (mean ± SD). The different superscript letters (a, b, and c) in each column indicate the significant statistical difference ($p < 0.05$). ZT = *Z. tenuior*, FA = *F. angulata*, and BP = *B. persicum*.

3.2. Identification and Quantification of Chemical Components of EOs. Table 1 also shows the main chemical components analyzed by the GC-MS in the three EOs extracted from ZT, FA, and BP herbs. The highest number of chemical constituents was detected in ZT-EO. ZT-EO consists of bornyl acetate, camphene, eucalyptol (1, 8-cineole) limonene, (β)-myrcene, isomenthone, neomenthone, piperitenone, (E)-β-ocimene, α-pinene, pulegone, terpinen-4-ol, γ-terpinene, α-terpinolene, α-thujene, sabinene, and spathulenol. The lowest count of chemical components was identified in FA-EO, which included carvacrol methyl ether, *trans*-chrysanthenyl acetate, *p*-cymene, 2, 5-dimethoxy-*p*-cimene, ent-3 β-hydroxy-13-epi-manoyl oxide, (Z)-β-ocimene, α-pinene, γ-terpinene, and monoterpene ester of ferulagon. Also, BP-EO contained β-caryophyllene, carvacrol, (−)-*trans*-carveol, carvone, *trans*-(+)-dihydrocarvone, limonene, (+)-trans-limonene oxide, (β)-myrcene, perillyl alcohol, and α-terpinolene. There were three common components (i.e., limonene, (β)-myrcene, and α-terpinolene) between ZT- and BP-EOs. Moreover, α-pinene and γ-terpinene were present in both EOs obtained from ZT and FA. The most dominant constituents in ZT-, FA-, and BP-EOs were determined to be pulegone (12.77%), *p*-cymene (14.45%), and ferulagon (14.97%), as well as (-)-*trans*-carveol (57.7%), respectively (Table 1). Other researchers previously reported the presence of similar polyphenolic components in BP-EO [30, 31]. Although (-)-*trans*-carveol in this study was quantified as the major component in BP-EO, limonene by Mahboubi [16] and α-terpinolene by Hassanzad Azar et al. [32] were mainly determined in this EO. Ghasempour et al. [33] reported that α-pinene, cis-ocimene, and bornyl acetate were the major components of FA-EO from two different habitats of Western Iran. The difference in chemical components of EOs can be due to the variances in environmental and genetic factors, geographical conditions, chemical polymorphic structure, plant maturation stage, and nutritional status of the plant [34].

3.3. Antioxidant Bioactive Compounds of Different EOs. Table 2 compares the content analysis results of bioactive compounds such as TFC, TPC, TChC, and TCaC among the three EOs. BP-EO significantly showed the highest TFC (1.08 mg QE/g), TPC (123.23 mg GAE/g), TCaC (591.31 mg/kg), and TChC (24.32 mg/kg) ($p < 0.05$). There was no significant difference in TFC and TChC between ZT- and FA-EOs. However, ZT-EO compared to FA-EO significantly presented more TPC (100.7 vs. 67.8 mg GAE/g) and TCaC (342.21 vs. 202.54 mg/kg) ($p < 0.05$). In general, the order of EOs in the content of bioactive compounds was as follows: BP > ZT > FA (Table 2).

3.4. Storage-Dependent Changes of Oxidative and Antioxidant Indices of Oil Blends. Figures 1(a)–1(c) show the storage-dependent changes of PV, AnV, and TOTOX values of IAO samples containing EOs and TBHQ. An increase in the storage time led to a significant increase in all the oxidation indices of oil blends. The addition of natural (EOs) and synthetic (TBHQ) antioxidants to IAO could retard the oxidation process. The oxidative stability of IOA was better maintained at high concentrations of antioxidants. However, TBHQ and BP-EO were superior preservatives compared to FA- and ZT-EOs. The highest oxidative rate was observed in IAO enriched with FA-EO, particularly at a 5% addition level (Figures 1(a)–1(c)). Figures 1(d) and 1(e) illustrate the DPPH• scavenging activity and ferric ion-reducing ability of different IAO-based blends during the storage. The DPPH inhibition activity of more than 90% was found for fresh samples formulated with BP-EO (90.24–91.25%) and TBHQ (94.25–95.65), while the lowest antiradical potential against DPPH free radical belonged to IAOs containing 5% FA-EO on the 28th day of storage (35.65%). There was no significant difference in the DPPH scavenging capacity of 10% BP-EO (83.45%) and 300 ppm TBHQ (84.24%) at the end of the storage period. Similar results concerning the reducing ability of EOs and TBHQ were obtained. IAOs formulated with 5 and 7% FA-EO showed the minimum reducing power of ferric ions on the 28th day of storage, while two IAO samples enriched with 10% BP-EO and 300 ppm TBHQ maintained 91.50 and 95.56% of this antioxidant potential after 4 weeks of storage. The correlation analysis showed that the DPPH scavenging activity was positively associated with TFC ($r^2 = 0.665$), TPC ($r^2 = 0.837$), TCaC ($r^2 = 0.545$), and TChC ($r^2 = 0.519$) levels ($p < 0.01$). Also, the reducing power value was directly correlated with the TFC ($r^2 = 0.549$), TPC ($r^2 = 0.765$), TCaC ($r^2 = 0.481$), and TChC ($r^2 = 0.457$) levels ($p < 0.01$).

As a result, phenolics compared to flavonoids induced more antioxidant effects to stabilize IAO during the storage. Accordingly, the presence of different forms of carvacrol, carvone, and limonene in BP-EO strongly acted to quench radical chain reactions on the autoxidation of organic substrates. On the other hand, lipid oxidation products such as malondialdehyde alter the odor and color of IAO and reduce its nutritional value [35, 36]. Phenolic compounds actively prevent lipid oxidation via elimination of free oxygen and free radicals, as well as chelation and reduction of

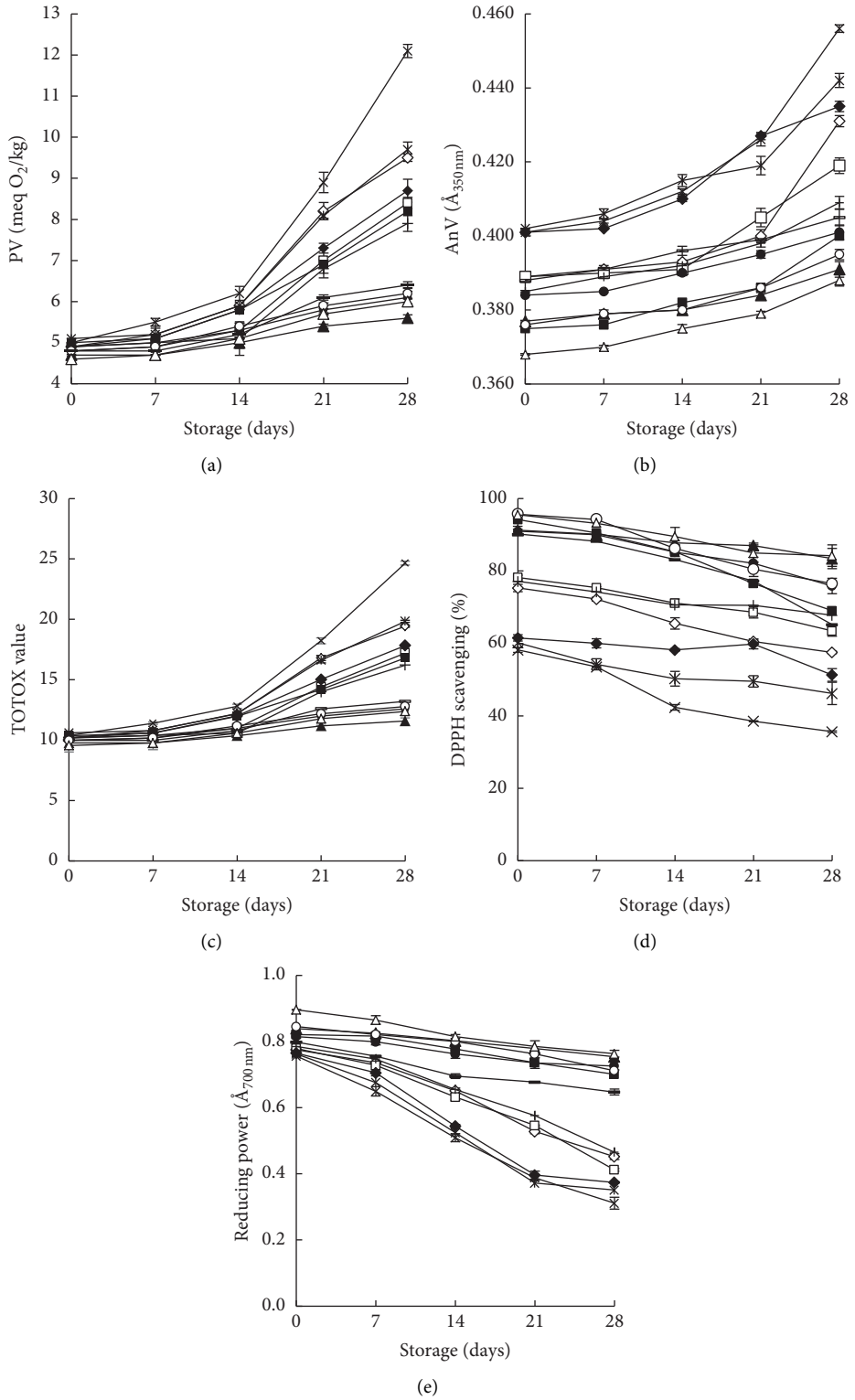


Figure 1: Storage-dependent changes of PV (a), AnV (b), TOTOX value (c), DPPH inhibition percentage (d), and reducing power of ferric ions (e) of the prepared oil blends for 4 weeks (T1 (◊), T2 (□), T3 (+), T4 (×), T5 (*), T6 (◆), T7 (▬), T8 (●), T9 (▲), T10 (■), T11 (○), and T12 (Δ)). Different treatments (T1–T12) are described in Table 3.

Table 3: The OSA scores of different oil formulations prepared by blending IAO and EOs/TBHQ during 28 d cold storage.

Treatment	IAO-based formulation	Storage (days)				
		0	7th	14th	21st	28th
T1	+5% ZT-EO	5.0 ± 0.0^{a}	4.6 ± 0.1^{b}	4.6 ± 0.1^{b}	4.4 ± 0.2^{b}	4.0 ± 0.0^{c}
T2	+7% ZT-EO	4.6 ± 0.1^{a}	4.5 ± 0.1^{a}	4.4 ± 0.2^{ab}	4.2 ± 0.2^{b}	4.0 ± 0.0^{b}
T3	+10% ZT-EO	4.4 ± 0.0^{a}	4.3 ± 0.0^{a}	4.2 ± 0.0^{ab}	4.0 ± 0.0^{b}	4.0 ± 0.0^{b}
T4	+5% FA-EO	5.0 ± 0.0^{a}	4.6 ± 0.1^{b}	4.0 ± 0.0^{c}	3.5 ± 0.4^{d}	3.0 ± 0.0^{e}
T5	+7% FA-EO	4.5 ± 0.0^{a}	4.3 ± 0.0^{a}	3.8 ± 0.0^{b}	3.5 ± 0.4^{b}	3.0 ± 0.0^{c}
T6	+10% FA-EO	4.0 ± 0.0^{a}	4.0 ± 0.0^{a}	4.0 ± 0.0^{a}	4.0 ± 0.0^{a}	3.5 ± 0.4^{b}
T7	+5% BP-EO	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	4.6 ± 0.1^{b}	4.0 ± 0.0^{c}	4.0 ± 0.0^{c}
T8	+7% BP-EO	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	4.7 ± 0.2^{b}	4.6 ± 0.1^{b}
T9	+10% BP-EO	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}
T10	+100 ppm TBHQ	5.0 ± 0.0^{a}	4.6 ± 0.1^{b}	4.0 ± 0.0^{c}	4.0 ± 0.0^{c}	3.5 ± 0.4^{d}
T11	+200 ppm TBHQ	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	4.6 ± 0.2^{b}	4.4 ± 0.1^{b}	4.0 ± 0.0^{c}
T12	+300 ppm TBHQ	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}

ZT = *Z.* tenuior, FA = *F. angulata*, and BP = *B. persicum*. Means followed by different letters (a–d) within each row are significantly ($p < 0.05$) different.

metal ions. Therefore, the presence of reductones in EOs, mainly at high concentrations, well stabilized free radicals and terminated the chain reaction due to an aromatic ring having electron repelling substituents [37]. In general, most natural compounds such as phenolics, flavonoids, and carotenoids synergistically present a wide spectrum of antioxidant and antiradical activities [38].

3.5. Storage-Dependent Organoleptic Changes of IAO-Based Formulations. Table 3 reveals the OSA scores of oil blends made of IAO and EOs/TBHQ at 0, 7, 14, 21, and 28 days of storage. According to the trained panelists' preference, an increase in the concentration of ZT- and FA-EOs resulted in a significant decrease in OSA scores, while the addition of BP-EO at the maximum dose could maintain the sensory quality until the 28th day storage. The reduced IAO-OSA at high incorporation levels of ZT- and FA-EOs might be due to their aromatic composition as well as the predominance of herbal aroma and flavor. Guo et al. [39] also found that the use of rosemary ethanolic extract in palm oil at high concentrations caused a reduction in OSA scores owing to the induction of vegetable taste to palm oil. Overall, the storage negatively affected the OSA score of IAO-EO/TBHQ blends. As a result, the lowest OSA scores were for IAO-based formulations enriched with ZT- and FA-EOs on the 28th day of storage. However, samples formulated with FA-EO compared to those enriched with ZT-EO significantly showed worse OSA scores at the end of storage time (Table 3).

Interestingly, samples containing 10% BP-EO similar to IAOs preserved with 300 ppm TBHQ received the highest OSA rate over the storage period. Furthermore, a significantly direct association was identified between OSA scores and DPPH scavenging activities ($r^2 = 0.788$, $p < 0.01$) and TPC ($r^2 = 0.715$, $p < 0.01$). It could be concluded that the antioxidant properties of BP-EO were associated with higher doses of phenolic compounds. Thus, phenolic compounds prevent harmful changes in sensory properties by preventing lipolysis and oxidation processes, the activity of oxidation-induced radicals, and/or removal of free oxygen [38, 40].

4. Conclusion

The present work evaluated bioactive effects of EOs extracted from three medicinal herbs of ZT, FA, and BP using the steam-distillation technique to extend the shelf life of IOA. The content of SFAs in IAO was more than quantities of short-chain and unsaturated fatty acids. Although all the EOs showed high amounts of bioactive compounds, much more TPC, TFC, TCaC, and TChC in BP-EO were found compared to ZT- and FA-EOs. The GC-MS analysis proved that different forms of carvacrol, carvone, and limonene were dominant in BP-EO. The weak preservative potential of FA-EO may be attributed to the low number of identified bioactive constituents. An increase in the concentration of EOs could significantly reduce the PV, AnV, and TOTOX values due to the more functional compounds and biological activities. The formation prevention of primary and secondary oxidation compounds at the incorporation level of 10% BP to IAO highly maintained the appropriate OSA for 28 days of cold storage. Therefore, BP-EO as a rich source of antioxidant components could be used as a natural alternative to chemical antioxidants (such as TBHQ) in food, cosmetic, and pharmaceutical industries. Our results are beneficial for producing other edible oils and emulsions containing natural antioxidants because consumers' health can be maintained by reducing the consumption and harmful effects of chemical and synthetic additives. The preparation of stable edible oils using bioactive EOs obtained from herbs/spices provides the main strategy to manufacture lipid-rich foods with good oxidative stability and pleasing sensory attributes. However, further work should be performed to assess the antimicrobial effects of flavored edible oils and emulsions enriched with the studied EOs, particularly BP-EO.

Abbreviations

AnV: Anisidine value
BP: *Bunium persicum*
EO: Essential oil
FA: *Ferulago angulata*
FAMEs: Fatty acid methyl esters

FCR: Folin–Ciocalteu reagent
FID: Flame ionization detector
GAE: Gallic acid equivalent
GC-MS: Gas chromatography-mass spectrometry
IAO: Iranian animal oil
LDL: Low-density lipoprotein
UFA: Monounsaturated fatty acid
OSA: Overall sensory acceptability
PUFA: Polyunsaturated fatty acid
PV: Peroxide value
QE: Quercetin equivalent
SFAs: Saturated fatty acids
TBHQ: Tertiary butyl hydroquinone
TCA: Trichloroacetic acid
TCaC: Total carotenoid content
TFC: Total flavonoid content
TOTOX: Total oxidation value
TPC: Total phenolic content
TChC: Total chlorophyll content
USFA: Unsaturated fatty acid
ZT: *Ziziphora tenuior*.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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