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Stabilizing fish oil during storage with *Satureja bachtiarica* Bunge

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Abstract:
Fish oil is highly susceptible to lipid oxidation, which leads to safety loss during storage. Natural antioxidants can prevent lipid oxidation. *Satureja bachtiarica* Bunge, also known as savory, is an endemic species plant that contains the necessary bioactive compounds and possesses antioxidant activity suitable for this purpose. This study featured the effects of savory extract and its essential oil as stabilizing agents on kilka fish oil.

We assessed the oxidative stability of fish oil fortified with of savory extract and essential oil in amounts of 0.5 and 1%. Then we compared their oxidative activity with that of samples treated with a synthetic antioxidant during 35 days at 40°C. The fish oil samples were tested for antioxidant activity, acid degree value, thiobarbituric acid-reactive substances, para-anisidine value, conjugated dienoic acids, peroxide value, total oxidation value, and free fatty acids.

Savory essential oil at the concentration of 1% was more effective than other samples in reducing the rate of lipid oxidation in fish oil. On storage day 35, the control sample yielded the following data: peroxide value = 14.79 meq O_2/kg, acid degree value = 32.49 mL/g, thiobarbituric acid-reactive substances = 5.82 mg MDA/g, para-anisidine value = 116.03, total oxidation index = 136.27. These results were significantly ($p < 0.05$) higher than those in the sample with 1% savory essential oil: peroxide value = 9.52 meq O_2/kg, acid degree value = 22.41 mL/g, thiobarbituric acid-reactive substances = 3.46 mg MDA/g, para-anisidine value = 78.3, total oxidation index = 108.09. The fish oil samples contained more unsaturated fatty acids (66.76–68.83%) than saturated fatty acids (31.13–32.6%).

Savory essential oil demonstrated good potential as an effective natural antioxidant that extends the shelf life of fish oil.

Keywords: *Satureja bachtiarica* Bunge, *Bachtiarica* spice, *Bakhtiari* savory, essential oil, extract, fish oil, lipid oxidation, natural antioxidant

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INTRODUCTION

Marine fish oils are a source of popular saturated dietary fatty acids, e.g., lauric, palmitic, myristic, and stearic acids [1, 2]. In addition, fish oil yields long-chain ω-3 (n-3) polyunsaturated fatty acids, e.g., eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) [3]. The high content of ω-3 fatty acids promotes its beneficial effects on human health. Fish oil is good for heart, brain, and nervous system, which makes it a valuable functional product. Fish oil fatty acids are effective against obesity, type 2 diabetes, depression, non-alcoholic fatty liver disease, and inflammation. Also, ω-3 fatty acids improve heart rate and reduce the risk of cardiovascular diseases [4]. Unfortunately, ω-3 fatty acids obtained from fish oil are sensitive to oxidation, which limits its use in the food industry. The rate of oil oxidation depends on the oil structure, temperature, and micro components, e.g., pigments, hydroperoxides, and free fatty acids. Therefore, fish oil needs to be protected from oxidation during consumption and storage. Oil oxidation can be prevented or inhibited by antioxidants and special conditions, e.g., thermal processing, exposure to light, oxygen, and storage at ≥ 20°C, etc. [4].

Antioxidants prevent oil oxidation by inhibiting the formation of free radicals or by stopping their release. According to Diniz do Nascimento *et al.*, synthetic antioxidants must be limited in animal studies [5]. Therefore, natural antioxidants of plant origin may replace

synthetic substitutes in the food industry to improve the oxidative stability of fish oil [6, 7].

Several compounds used in sufficient concentrations may act as antioxidants, e.g., plant extracts obtained from dried plant materials by steam distillation, cold pressure, or solvent extraction [8]. This list also includes essential oils and aromatic oily liquids that are composed of volatile compounds with low molecular weight [9].

Hrebień-Filisińska & Bartkowiak added 25% (w/w) sage extract to fish oil and managed to reduce the oxidation rate, both during refrigerated storage and at room temperature storage [10].

The genus *Satureja* consists of 30 species. It belongs to aromatic plants that are distributed in the Mediterranean, Africa, Asia, and North America. Iran has 12 wild species of this genus. *Bakhtiari* savory (*Satureja bachtiarica* Bunge.) belongs to the *Lamiaceae* family. Its leaves are used as spices, nutrients, and herbal pharmaceuticals. This plant grows in the central part of the Zagros Mountains, Iran. *Bakhtiari* savory is a one-year herbaceous semi-shrub 20–45 cm tall, with numerous stems and short branches covered with gray lashes. Medicinal and aromatic plants are members of the mint family and contain a wide range of bioactive molecules that inhibit free radicals and possess antioxidant activity [11].

Polyphenols are valuable natural products obtained from plant extracts. They can protect cells from damage caused by free radicals. They demonstrate numerous pharmacological properties, e.g., they prevent atherosclerosis, cancer progression, or pathogen growth [12].

Most savory species contain essential oils. Savory varieties are rich in natural preservatives, including monoterpenes, e.g., thymol, carvacrol, and cimen [13]. The major phenolic acid compounds in *Bakhtiari* savory are rosmarinic acid, *p*-coumaric acid, parmentin B, 12-hydroxyjasmonic acid, tuberonic acid, *β*-D-glucopyranoside, methylrosmarinic acid, and caffeic acid ethyl ester [14].

Currently, there is a strong global interest in exploring new sources of natural antioxidants that are both safe and cheap. Natural antioxidants cause no adverse effects typical of their synthetic analogues [15]. As far as we know, no publications have featured *Bakhtiari* savory to enhance the oxidative stability of edible oils. Our research investigated the antioxidant compounds in savory extract and savory essential oil. We also evaluated their antioxidant potential against a synthetic antioxidant, namely tertiary buthylhydroquinone. The research parameters assessed during 35 days of storage included peroxide value, acid value, thiobarbituric acid, p-anisidine, total oxidation value, and conjugated dienoic acids. In addition, we also studied the antioxidant activity and fatty acid profile of the fish oil samples.

STUDY OBJECTS AND METHODS

Plant and fish oil. *Bakhtiari* savory (*Satureja bachtiarica* Bunge.) was gathered from the natural surroundings of the city of Sadra (Shiraz, Fars Province, Iran). This plant was identified using the botanical herbarium compiled by the Fars Research and Academic Center of Agricultural and Natural Resources. Kilka (*Clupeonella cultriventris caspia*) fish oil was obtained from Apsa Trading Company in Qaimshahr (Mazandaran, Iran) and contained no antioxidants.

Chemicals. 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium thiosulfate, potassium acetate, gallic acid, Folin-Ciocalteu's reagent, ethanol, chloroform, acetic acid, and aluminum chloride were obtained from Merck (Darmstadt, Germany). Quercetin and methanol came from Applichem (Darmstadt, Germany). Other chemical materials and reagents of analytical grade were purchased from Sigma Aldrich (St. Louis, United States) and Merck (Darmstadt, Germany).

Extracting savory essential oil and savory extract. The savory essential oil and the savory extract were prepared based on the procedures described by Hashemi & Khodaei and Khademvatan *et al.* [16, 17].

Total phenol content. The total phenolic content in the savory essential oil and the savory extract was measured using the Folin-Ciocalteu method. It involved a calibration curve of gallic acid prepared in methanol. The results obtained using a calibration curve regression equation ($Y = 15.575x - 0.0176$, $R^2 = 0.9985$) were expressed as gallic acid in mg/g sample [18].

Total flavonoid content. The total flavonoid content in the savory essential oil and the savory extract was measured based on the aluminum chloride colorimetric method [18]. A calibration curve of quercetin was made in methanol. The results were calculated using a regression calibration curve equation ($Y = 0.0237x + 0.0867$, $R^2 = 0.9886$). It was expressed as quercetin mg/g sample [19].

DPPH analysis. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was employed to measure the antioxidant activity concerning free radical inhibition percentage. TBHQ made it possible to construct a standard curve at various concentrations. The equation below served to represent a percentage of scavenging activity:

$$\% \text{ Scavenging} = [(A_0 - A_1)/(A_0)] \times 100 \quad (1)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Finally, we calculated IC_{50}, i.e., the absorbance value of 50% in the reducing power assay [20].

Determining total phenolic content. High-performance liquid chromatography (HPLC) (Agilent Technologies, 1200 series, Germany) was used to determine the number of polyphenolic compounds in the savory extract.

Gas chromatography – mass spectrometry test. We used gas chromatography – mass spectrometry (GC–MS) to identify the chemical components in the savory essential oil (Varian, 450-GC/MS: 1200, USA). The length of column HP-5MS (phenylmethyl silox) was 30 m, its diameter was 250 nm, and its thickness was 0.25 mm. The test involved an electron ionization system with an ionizing energy of 70 eV. The temperature in the oven stayed 50°C for 2 min to be adjusted to 70°C

at 5°C/min and heated to100°C at 20 and 10°C/min. Finally, it remained 290°C for 2 min. The detector and injector temperatures were 300 and 200°C, respectively. Helium served as carrier gas at a flow degree of 0.8 mL/min, and the 0.5% samples were injected physically in the splitless style. Summits area percents were applied for achieving numerical information. The mass array was determined from 50 m/z to 550 amu. Holding directories were restrained for compounds by homologous types of n-alkanes (C5–C24) injected in circumstances equivalent to those applied to the samples.

In the savory essential oil, chemical components were identified using GC–MS with a similar device. Helium was applied as carrier gas with a persistent flow rate of 1 mL/min. The temperature mode was the same as described above. Four microliters of oil were injected as split; the split relation was 1:100. The MS functional restrictions were as follows: 200°C interface temperature, 70 eV ionization potential, 50–800 mass array acquisition. The oil compounds were determined in line with the protocol described by Fathimoghaddam *et al.* [18]. Table 1 illustrates the gradient program used to measure the polyphenolic compounds in the essential oil.

Preparing fish oil and storage conditions. The fish oil samples and their storage conditions were in line with the method introduced by Lizárraga-Velázquez *et al.* with some modifications [19]. The savory essential oil and the savory extract were added separately to fish oil samples in concentrations of 0.5 and 1% (w/v). Tween 20 (10% w/v) served as emulsifier for the extract [21]. TBHQwas added (100 ppm) to the fish oil as a synthetic antioxidant. One group contained no antioxidants and served as control. The essential oil, the extract, and the synthetic oxidant were dispersed slowly in the oil and mixed until homogeneous emulsion. The oil samples were poured into dark glass bottles and kept in an incubator at 40°C and 75% relative humidity for 35 days. The samples were evaluated on storage days 0, 7, 14, 21, 28, and 35. Each treatment was performed in triplicate [19].

Peroxide value. To determine the peroxide value in fish oils, we applied the method described by Sarojini *et al.* [22]. The resulting peroxide value, meg$\frac{O_2}{kg}$fat , was expressed as milliequivalents of oxygen per 1 kg of fat as in the Eq. (2):

$$\text{Peroxide value} = \frac{(S - B) \times N \times 1000}{W} \quad (2)$$

where S is the sample volume, mL; B is the blank sample volume, mL; N is the normality of sodium thiosulfate solution, mol/L; and W is the sample weight, g [22].

Table 1 Total phenolic, total flavonoid, and IC_{50} in savory extract and savory essential oil

Sample	Total phenol, mg/g	Total flavonoid, mg/g	IC_{50}, mg/mL
Savory extract	104.269	13.825	0.206
Savory essential oil	70.882	0.801	2.865
Gallic acid	–	–	0.025

Acid degree value. We weighed 10 g of the oil sample in a 250 mL Erlenmeyer flask. Then, we added 50 mL of ethanol and diethyl ether (1:1) into the flask, followed by three drops of a phenolphthalein indicator solution. For titration, we applied 0.1 M potassium hydroxide until the mix turned pink. The same conditions were replicated for the blank sample. The acid degree value, mg KOH/g, was determined as follows (ISO 660):

$$\text{Acid degree value} = \frac{(V - b) \times N \times 56.1}{W} \quad (3)$$

where 56.1 is the molecular weight of potassium hydroxide, g/mol; V is the potassium hydroxide volume in for the oil sample, mL; b is the potassium hydroxide volume in the blank sample, mL; N is normality of potassium hydroxide solution, mol/L; and W is the sample weight, g.

The ***thiobarbituric acid reactive substances*** were measured as proposed by Sarojini *et al.* and represented as mg malonaldehyde (MDA)/kg [22].

The ***para-anisidine value*** in the fish oil was measured according to the method described by Yeşilsu & Özyurt [23]:

$$Para\text{-}anisidine\ value = \frac{25 \times (1.2 \times A_s \times A_b)}{m} \quad (4)$$

where A_s is the fat solution absorption; A_b is the absorption of fat solution after its reaction with para-anisidine reagent; and m is the sample weight, g.

Total oxidation value (TOTOX index). We used the TOTOX number, or total oxidation value, to determine the total fat and oil oxidation. It was calculated according to the method described by Jairoun *et al.* [24]:

$$\text{TOTOX index} = (2 \times PV) + pAV \quad (5)$$

where pAV is the para-anisidine value and PV is the peroxide value.

Conjugated dienoic fatty acid. We studied the conjugated dienoic fatty acid, %, in the fish oil samples in line with the equation proposed by Na *et al.* [25]:

$$\text{Conjugated dienoic fatty acid} = \\ = 0.84\ \{A_s / (b \times c) - 0.03\} \quad (6)$$

where A_S is the absorbance observed; b is the cuvette length, cm; and c is the concentration of test sample, g/L.

The ***antioxidant activity (DPPH(*** was assessed based on the method proposed by Hrebień-Filisińska & Bartkowiak [10].

The ***fatty acid profile*** of the fish oil was assessed using the method described by Soltaninejad & Sekhavatizadeh [26]. The results were presented as a percentage of the relative peak area.

Statistical analysis. For data analysis, we used a one-way ANOVA and SPSS Statistics 19.0 (Chicago, USA) ($p < 0.05$). To determine the significant difference, we applied Duncan's test. The graphs were constructed in Microsoft Excel 2016.

RESULTS AND DISCUSSION

Identifying chemical compounds by GC–MS. Table 2 summarizes the gas chromatography – mass spectrometry results for the savory essential oil. The test revealed 12 compounds that made up 100% of the total composition. Carvacrol (58.019%) and *γ*-terpinene (25.148%) appeared to be the main components. The other compounds were o-cymene (4.836%), *α*-terpinene (4.361%), *β* Myrcene (1.772%), and carvacrol acetate (1.242%). The savory essential oil also contained *α*-pinene (1.219%), *α*-Thujene (1.133%), *trans*-anethole (1.009%), *β*-pinene (0.448%), phellandrene (0.44%), and limonene (0.374%).

Memarzadeh *et al.* identified 28 chemical compounds in savory essential oil, which occupied 98.59% of the total essential oil [11]. They found carvacrol (31.25%) and *γ*-terpinene (10.65%(to be the most abundant chemical compounds, which was different from our results. In their study, the amount of carvacrol and *γ*-terpinene was between 28.18–35.71 and 6.05–8.25%, respectively. In addition, o-cimen and thymol were on their list of the main components. The major difference between the results may be related to the number of identified chemical compounds, type, and concentration; however, carvacrol and *γ*-terpinene proved to be the main components in both studies. Other researchers also reported carvacrol (31.25–14.20%) as the most common chemical component in savory essential oil [16, 18]. The different geographical location, soil structure, consistency, and climate were the most important factors that caused differences in the chemical composition of the savory essential oil [18].

Antioxidant activity, total flavonoid content, and total phenolic content. The results showed that the total flavonoid and phenolic contents in the savory extract were 104.269 and 13.825 mg/g of extract, respectively. These amounts exceeded those detected in the savory essential oil (70.882 and 0.801 mg/g). The IC_{50} level in the savory essential oil was higher than in the gallic acid sample and the savory extract, which indicates that the antioxidant activity of the savory essential oil was lower than that of the savory extract and the gallic acid sample.

The total phenolic contents were 104.269 and 70.882 mg GAE/g for the extract and the essential oil, respectively. The composition and content of herbal extracts and essential oil are known to depend on various factors, such as temperature, time, extraction method, solvent, etc. The type of solvent is the most important factor due to its polarity and the tendency to combine with different substances [27]. Fathimoghaddam *et al.* measured the total phenolic and flavonoid contents in the savory essential oil as 88.33 ± 1.69 mg GAE/100 g DW and 20.63 ± 1.24 mg QU /100 g DW, respectively [18]. These values were higher than those obtained by us in this research.

These differences may be related to many factors, including extraction method, cultivation conditions, the type of extraction solvent, maturity of plants, geographical location, environment, genetics, variety, part of the plant used, and harvesting season [28].

Table 2 Chemical composition of savory essential oil, GC–MS

Retention time, min	Chemical components	Amount, %
5.592	*α*-thujene	1.133
5.835	*α*-pinene	1.219
7.164	*β*-pinene	0.448
7.396	*β*-myrcene	1.772
8.067	Phellandrene	0.44
8.446	*α*-terpinene	4.361
8.785	o-cymene	4.836
8.874	Limonene	0.374
10.138	*γ*-terpinene	25.148
19.907	*trans*-anethole	1.009
21.166	Carvacrol	58.019
23.124	Carvacrol acetate	1.242
Total		100.00

In this study, the savory extract had a lower IC_{50} compared to the savory essential oil: as a result, the antioxidant activity was higher. The IC_{50} results ranged between 0.025 and 2.865 mg/mL. The lower IC_{50} content in the savory extract was due to the higher total phenolic and flavonoid contents. It had the highest antioxidant activity compared to the savory essential oil. In this regard, Fathimoghaddam *et al.* linked the high antioxidant effect in savory essential oil to the greater total phenolic and flavonoid contents [18].

In our study, the DPPH radical inhibition was 76.72%. In a similar study, Memarzadeh *et al.* explained the high value of the savory radical scavenging capacity of essential oil by the hydroxyl groups present in the chemical structure of phenolic compounds, which provided a radical scavenger [11].

Table 3 shows the polyphenol content in savory. The essential oil antioxidant activity was probably due to *γ*-terpinene terpenoids and carvacrol, which were the main contents in savory. Memarzadeh *et al.* also found many polyphenolic compounds in the savory family, especially flavonoids and phenolic acids, including caryophyllene and borneol [11].

Table 3 shows polyphenolic compounds in the savory extract identified by HPLC. Carvacrol (21 250.81 mg/L) proved to be one of the main components in this plant extract. It was followed by rosmarinic acid (3753.279 mg/L), *trans*-ferulic acid (82.60816 mg/L), and catechin (68.24201 mg/L).

In the present study, carvacrol and rosmarinic acid proved to be two major polyphenolic compounds. Phenolic compounds and flavonoids are often reported in scientific publications. For instance, rosmarinic acid and rutin were the most abundant of their kind in *Satureja montana*: 7.85 and 17.29%, respectively. The total polyphenolic content varied from 100.65 to 420.68 mg/100 g among three species [29]. In another research on savory, the total phenolic content was 177.92 mg/100 g [30]. This polyphenolic profile was different from that obtained in our study, probably, due to different HPLC standards and extraction methods [31].

Table 3 Polyphenolic content of savory extract: HPLC

Retention time, min	Polyphenol content	Savory extract, mg/L
3.3	Gallic acid	n.d.
8.3	Catechin	68.24201
11.6	Caffeic acid	n.d.
13.5	Vanilin	n.d.
15.6	*p*-coumaric acid	n.d.
16.3	*trans*-ferulic acid	82.60816
16.5	Sinapic acid	n.d.
17.4	Coumarin	n.d.
18.5	Hesperedin	n.d.
19.02	Ellagic acid	n.d.
19.2	Rosmarinic acid	3753.279
21.6	Quercetin	n.d.
22.4	Hesperetin	n.d.
23.7	Eugenol	n.d.
28.4	Carvacrol	21 250.81
28.9	Thymol	n.d.

n.d. – not detected

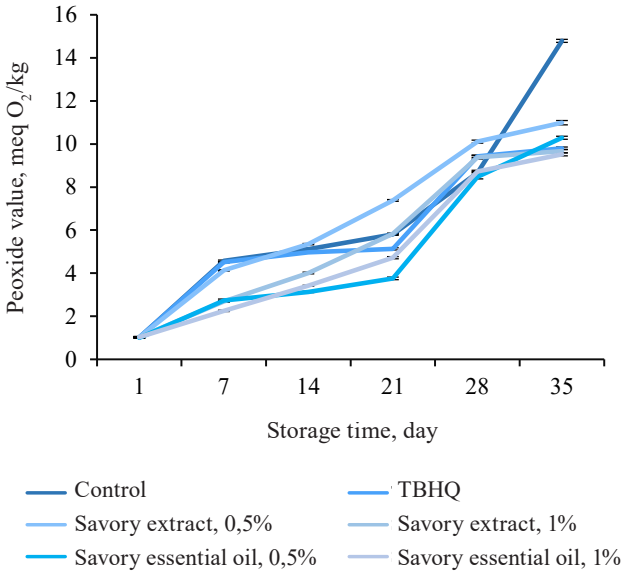

Figure 1 Peroxide values in fish oil during storage

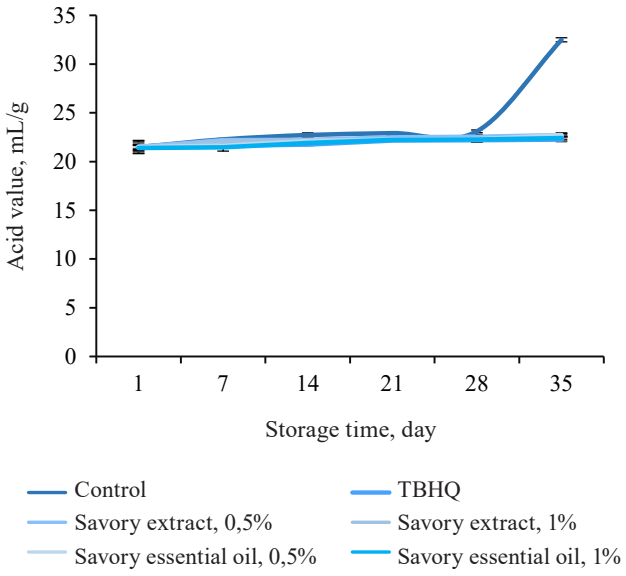

Figure 2 Acid degree values in fish oil during storage

Peroxide value. Figure 1 illustrates the peroxide values in fish oil samples fortified with different concentrations of savory extract and savory essential oil during 35 days of storage. The peroxide values in all fish oil samples increased significantly during the storage period ($p < 0.05$). The initial peroxide value was 1–1.3 meq O_2/kg, but it increased from to 9.38 to14.79 meq O_2/kg on storage day 35. We detected no significant difference between the fish oil samples with 1% savory extract and the oil sample with TBHQ ($p > 0.05$), but the control sample had the highest peroxide value on storage day 35 ($p < 0.05$).

Therefore, 0.5 and 1% savory essential oil added to fish oil during storage affected its oxidative parameters. Antioxidants usually delay the rate of oxidation due to their ability to chelate metals, quench singlet oxygen, and destroy free radicals [32]. The mint family is known to contain phenolic compounds. For instance, Sayyad & Farahmandfar reported that *Teucrium polium* L. essential oil contained mono and sesquiterpene compounds [33]. They were applied as antioxidants in canola oil and showed higher protective effects than BHA against oxidation during storage.

Acid degree value. Figure 2 demonstrates the changes in acid degree value that occurred in the fish oil samples fortified with different concentrations of savory extract and savory essential oil during 35 days of storage. All samples of fish oil revealed a significant increase ($p < 0.05$) in acid degree value. On storage day 35, the lowest acid degree value belonged to the samples with TBHQ (22.3 ± 0.26 mL/g) and 1% savory essential oil (22.41 ± 0.18 mL/g) ($p < 0.05$).

Acid degree value in the control samples of fish oil increased during storage, except those with TBHQ, savory extract, and savory essential oil. Özkan & Özkan reported that the acid values of oils containing 600 and 1200 ppm of savory (*Satureja thymbra*) and marjoram (*Origanum onites*) extracts were similar to each other [34]. Also, they had a lower acidity compared to the control sample. The presence of monoterpenoid phenolic compounds, e.g., carvacrol, are probably related to the higher number of hydroxyl groups in essential oil molecules.

Thiobarbituric acid index. Figure 3 shows the analysis of the thiobarbituric acid index in the fish oil samples fortified with different concentrations of savory extract and savory essential oil during 35 days of storage. The values of the thiobarbituric acid index in all fish oil samples demonstrated a significant increase ($p < 0.05$) and rose from 1.19–1.28 to 3.46–5.82 mg MDA/kg during storage time. On storage day 35, the lowest thiobarbituric acid index belonged to the sample with 0.5 and 1% savory essential oil (4.19 ± 0.09 and 3.46 ± 0.08 mg MDA/kg, respectively).

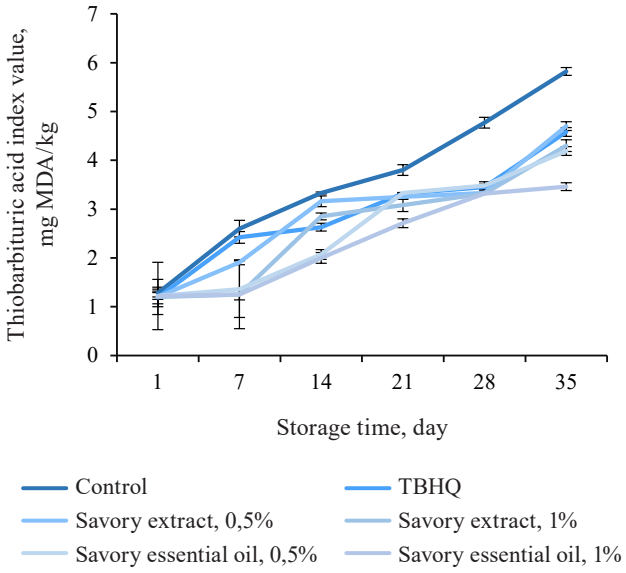

Figure 3 Thiobarbituric acid index in fish oil during storage

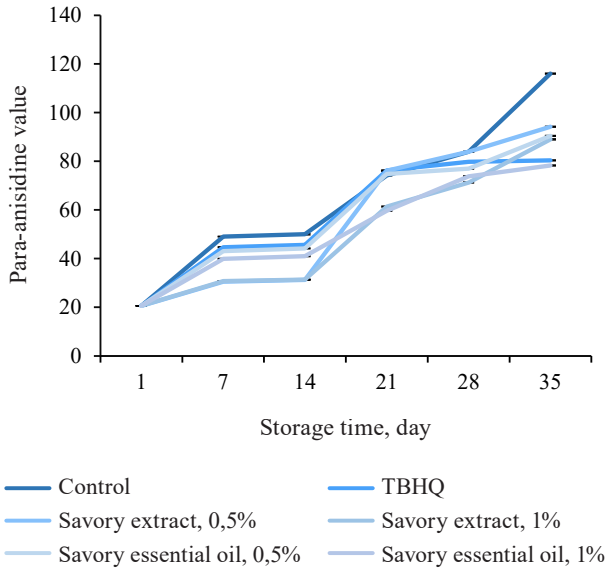

Figure 4 Para-anisidine values in fish oil during storage

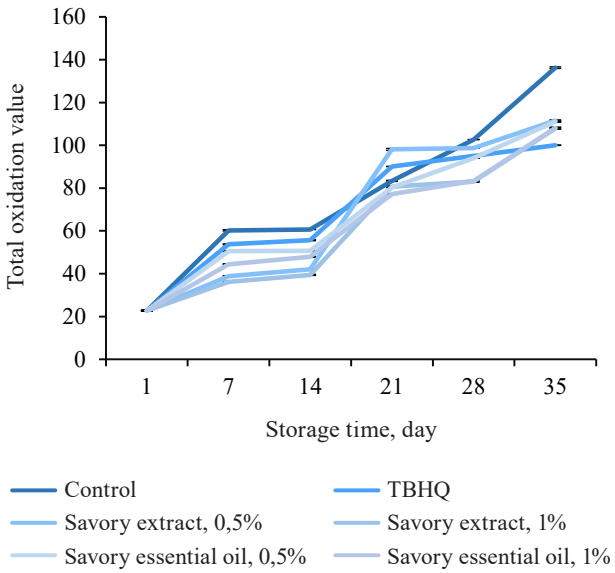

Figure 5 Total oxidation index in fish oil during storage

In our research, the thiobarbituric acid index increased during the storage time. However, the thiobarbituric acid index in all samples treated with savory extract and savory essential oil was lower compared to the control and the samples with tertiary buthylhydroquinone, which can be related to their phenolic compound [18]. Kamkar *et al.* added methanolic and ethanolic extracts of summer savory (*Satureja hortensis* L.) to soybean oil to increase its oxidation stability [35]. García-Pérez *et al.* added *Bryophyllum* plant extracts to fish oil to increase the antioxidant efficiency due to the incorporation of polyphenols [36].

Para-anisidine value. Figure 4 reports para-anisidine values in the fish oil samples. The para-anisidine values increased significantly in all fish oil samples during the storage period. On storage day 35, the highest value was observed in the control oil sample (116.05 ± 0.23). In our study, para-anisidine values increased significantly ($p < 0.05$) with storage. Santos *et al.* linked para-anisidine increase to the degradation of primary lipid oxidation products (hydroperoxides) that turned to secondary oxidation products (carbonyls) [37]. On storage day 35, the lowest para-anisidine values belonged to the sample with TBHQ (76.22 ± 0.22) and 1% savory extract (78.00 ± 0.23). Hwang *et al.* reported an increase in para-anisidine when they raised the concentration of coffee acetone extract (0.1, 0.25, and 0.5%) added to fish oil as antioxidant during 14 days of storage [38].

Total oxidation value (TOTOX index). Figure 5 shows the changes in the total oxidation value. The TOTOX index ranged from 22.64 to 22.85 on the first day and increased to 100.01–136.27 on storage day 35. At the end of the storage time, the highest total oxidation value belonged to the control sample (136.27 ± 0.38). The lowest was observed in the samples with TBHQ (100.01 ± 0.09) and 1% savory essential oil (108.09 ± 0.40).

In a similar study, low concentrations of sage extract (5, 10, 25, and 50%) in cod fish liver oil caused a significant decrease ($p < 0.05$) in the TOTOX index due to the appropriate amount of antioxidant polyphenols [39]. However, in our research, this parameter increased significantly ($p < 0.05$) during the storage period.

Conjugated dienoic acid. Figure 6a illustrates the contents of conjugated dienoic acids in the fish oil samples fortified with different concentrations of savory extract and savory essential oil. The amount of conjugated dienoic acid increased significantly in all fish oil samples during storage ($p < 0.05$), rising from 0.64–1.01 to 1.7–1.75%. We detected no significant difference in conjugated dienoic acid between the samples at the end of storage ($p > 0.05$).

In the present study, synthetic and natural antioxidants produced no significant effect on conjugated dienoic acid. However, other scientists reported that

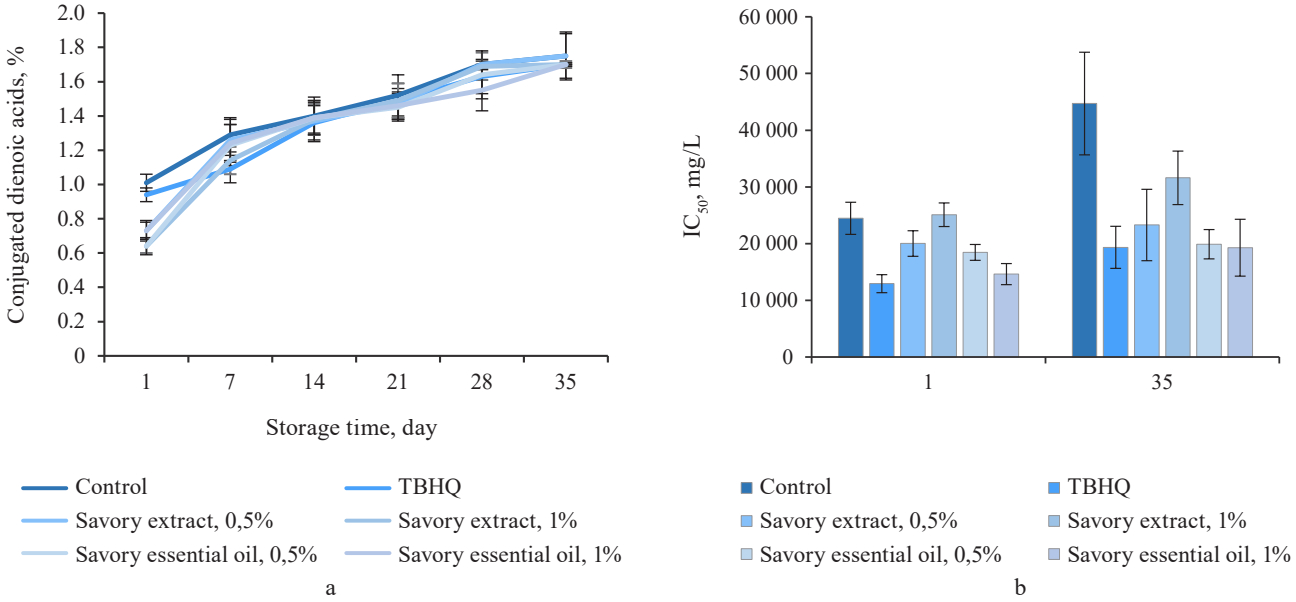

Figure 6 Conjugated dienoic acid (a) and antioxidant activity (b) in fish oil during storage

Table 4 Fatty acids in fish oil during storage

Fatty acid, type	Fatty acid, name	Day 1	Day 35		
		Control	Control	Savory extract (1%)	Savory essential oil (1%)
		Saturated fatty acids (SFA)			
C12:0	Lauric acid	0.13	0.12	0.06	0.07
C14:0	Myristic acid	3.24	3.23	3.39	3.66
C15:0	Pentadecylic acid	0.63	0.63	0.64	0.66
C16:0	Palmitic acid	19.58	19.18	19.82	20.31
C17:0	Margaric acid	1.52	1.46	1.48	1.48
C18:0	Stearic acid	4.86	4.86	4.71	4.95
C20:0	Arachidic acid	0.31	0.27	0.40	0.33
C22:0	Behenic acid	0.74	0.72	0.76	0.72
C24:0	Lignoceric acid	0.63	0.66	0.14	0.42
		Monounsaturated fatty acids (MUFA)			
C14:1	Myristoleic acid	0.51	0.53	0.54	0.49
C15:1	Pentadecenoic acid	0.16	0.16	0.16	0.14
C16:1	Palmitoleic acid	5.82	6.33	6.27	5.97
C17:1	heptadecenoic acid	0.77	0.77	0.78	0.75
C18:1*trans*	Vaccenic acid	0.22	0.22	0.26	0.63
C18:1*cis*	Oleic acid (*ω*-9)	36.80	37.07	35.50	36.61
C20:1	Gondoic acid	1.93	1.94	1.90	1.88
C22:1	Erucic acid	0.20	0.19	0.18	–
C24:1	Nervonic acid	0.47	0.45	0.58	0.43
		Polyunsaturated fatty acids (PUFA)			
C18:2*cis*	linoleic acid (*ω*-6)	1.91	2.00	1.91	2.29
C18:3*cis*	*α*-Linolenic acid (*ω*-3)	1.66	1.70	1.59	1.66
C20:2	Eicosadienoic acid	–	0.10	0.19	0.18
C22:5	docosapentaenoic acid (DPA)	0.44	0.50	0.44	–
C20:5	Eicosapentaenoic acid (EPA)	6.46	6.30	6.55	6.01
C22:6	Docosahexaenoic acid (DHA)	11.00	10.57	11.19	9.72
ω-6/*ω*-3		1.15	1.17	1.20	1.37
ΣSFA		31.64	31.13	31.4	32.60
ΣMUFA		46.88	47.66	46.17	46.90
ΣPUFA		21.47	21.17	21.87	19.86
ΣUFA		68.35	68.83	68.04	66.76
ΣPUFA/ΣSFA		0.67	0.68	0.69	0.60
Polyene index (EPA + DHA)/palmitic acid)		0.89	0.87	0.89	0.77

synthetic and natural antioxidants curbed the amount of conjugated dienoic acid produced. The list of substances that reduced the amount of conjugated dienoic acid in fish oil included different concentrations of such plant extracts as rosemary, garlic, and bryophyllum, as well as 5% roasted or unroasted pumpkin seed oil [36, 40, 41].

Antioxidant activity (DPPH). Figure 6b demonstrates antioxidant activity in the fish oil samples during storage. The sample with lower IC_{50} revealed the highest antioxidant activity. In our study, the samples with TBHQ and 1% savory essential oil had the highest antioxidant activity on storage days 1 and 35. In general, the antioxidant activity in all samples decreased during the storage period.

The IC_{50} in all oil samples increased significantly after 35 days of storage ($p < 0.05$), which indicated a gradual decrease in antioxidant activity during storage. Thus, the storage time had a significant effect on the antioxidant activity of kilka fish oil. The sample with 1% savory essential oil was not significantly different from the sample with TBHQ ($p > 0.05$). Other samples with natural antioxidants, except for the 1% savory extract sample, had a significant difference with the TBHQ sample on storage day 35. Other reports on the high antioxidant activity of different savory species confirm the results obtained in this study [42]. Moreover, Jafari *et al.* linked the total antioxidant capacity not only to phenolic compounds but also to enzyme systems, vitamins, organic acids, and other compounds in the plant [43].

Fatty acid profile. Table 4 gives data on the fatty acid profile. During storage, the fatty acid content was in the range of 31.13–32.6% (saturated fatty acids), 46.17–47.66% (monounsaturated fatty acids), and 19.86–21.87% (polyunsaturated fatty acids). Monounsaturated fatty acids proved to be the most abundant of their kind both in the control sample and in the samples fortified with savory extract and savory essential oil.

Golmakani *et al.* reported the fatty acid composition of kilka oil as mainly composed of monounsaturated fatty acids, followed by saturated fatty acids and polyunsaturated fatty acids, which was in line with our research [44]. In the present research, the n-6 to n-3 ratio in the fish oil samples was 1.15–1.37%.

In this regard, Golmakani *et al.* also reported that the ratio of polyunsaturated to saturated fatty acids in kilka fish oil was 0.54%, which also confirmed our results [45].

CONCLUSION

In the present study, we obtained *Bakhtiari* savory extract and *Bakhtiari* savory essential oil using traditional methods and added them separately to kilka fish oil in concentrations of 0.5 and 1%.

Satureja bachtiarica Bunge proved to be rich in bioactive compounds, including active phenolic ones, e.g., carvacrol with its high antioxidant activity and oxygenated monoterpenes.

Fish oil, which is highly susceptible to oxidative degradation, was mixed with the savory extract and savory essential oil with high antioxidant content. As a result, the oxidative stability of fish oil increased. Savory essential oil in a higher concentration (1%) inhibited the oxidation process more effectively than the samples fortified with savory extract and 0.5% savory essential oil. In addition, 1% savory essential oil had the same potential as TBHQ in delaying the formation of secondary oxidation products. As a result, savory essential oil could be used as an effective natural antioxidant to increase the shelf-life of kilka fish oil.

CONTRIBUTIONS

Mohammad Mehdi Ghanbari supervised the project. Atefeh Matbooe performed the research, as well as collected and interpreted the data. Seyed Saeed Sekhavatizadeh drafted the manuscript and proofread the final version. Mehdi Nikkhah proofread the manuscript.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests related to the publication of this article.

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