

Article

Antioxidant Performance of *Borago officinalis* Leaf Essential Oil and Protective Effect on Thermal Oxidation of Fish Oil

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Abstract: This study aimed to determine the antioxidant activity of *Borago officinalis* essential oil in the thermal oxidation of fish oil. The volatile compound profile of *B. officinalis* essential oil (BEO) was determined using gas chromatography and mass spectrometry. As a result of the analysis, 97.27 percent of the volatile components of the product were characterized. The product's major components were benzene acetaldehyde (28.59 percent) and linalool (13.60 percent). As a result of the free radical scavenging activity determined using 2-diphenyl-1-picrylhydrazyl (DPPH) analysis, its antioxidant activity was determined, and a 50 percent inhibitory concentration value was calculated as 736.06 ppm. In order to determine the protective effect of the BEO on fish oil oxidation, 0% (BEO0), 0.1% (BEO0.1), 0.5% (BEO0.5), 1% (BEO1), and 3% (BEO3) ratios of BEO were added to the fish oil, and the experimental groups were kept at 70 °C for 24 h with continuous ventilation for the thermal oxidation process. As a result of the oxidation study, it was determined that the addition of BEO suppressed fish oil oxidation, and the oxidation radicals in the product decreased significantly ($p < 0.05$) depending on the BEO concentration. In conclusion, it was determined that 1 percent BEO supplementation minimized the oxidation of fish oil under various temperature and ventilation conditions.



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1. Introduction

Fish oil is an important ingredient in the animal feed industry, particularly in aquaculture, due to its high content of omega-3 fatty acids. These fatty acids are essential for fish's growth, health, and reproduction and also benefit other animals such as poultry, swine, and dairy cows [1–4]. Omega-3 fatty acids, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), are important for fish growth and development and for the health and nutrition of humans who consume fish. These fatty acids are also beneficial for other animals, including improving reproductive performance in dairy cows and reducing inflammation in poultry [5]. In addition, fish oil is also an important source of energy and essential fatty acids, which are important for overall animal health [6]. However, there are concerns about the sustainability of fish oil, as overfishing and environmental degradation have led to declining fish populations. Therefore, alternative sources of omega-3 fatty acids, such as algae-based supplements, are being developed and tested in animal feed formulations [7]. In recent years, it has become increasingly essential to utilize fish oil efficiently, as a valuable component due to its limited production and to prevent its degradation. Fish oil is highly susceptible to oxidation due to its high content of polyunsaturated fatty acids. Oxidation is a chemical reaction that occurs when the oil comes into contact with oxygen in the air, and this can cause the oil to become rancid and lose its nutritional value. Therefore, it is important to protect fish oil against oxidation. When fish oil undergoes oxidation, the fatty acids in the oil can become damaged, resulting in a decrease in quality, taste, and

nutritional value [8]. In addition, oxidation products can harm animals that consume the oil [9,10]. Therefore, protecting fish oil against oxidation is crucial to maintaining its quality and nutritional value. In order to prevent oxidation, it is important to store fish oil under specific conditions. It should be kept in a cool, dark, and dry place to minimize exposure to light, oxygen, and moisture. Additionally, antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) are often added to fish oil to prevent oxidation [11]. Antioxidants work by scavenging free radicals that can cause oxidation and stabilizing the oil to preserve its quality. Protecting fish oil against oxidation is essential to maintaining its nutritional value and preventing harmful oxidation products from forming [12]. The use of chemical antioxidants, including in animal feed and human food, has been debated. While chemical antioxidants can prevent oxidation and extend the shelf life of nutritional products, there is some evidence that some of these antioxidants may have negative health effects on animals and humans [13,14]. The most important example of this is ethoxyquin (EQ), once used to prevent the oxidation of lipids and stabilize fat-soluble vitamins. For many years in the European Union, the use of EQ was authorized under Directive 70/524/EEC and Regulation EC 1831/2003. However, this authorization was suspended under Regulation EC 2017/962 and is now banned, as its use has been found to have adverse effects on human and animal health [15]. Similar legal regulations may be applied in the future to some synthetic antioxidants that are currently available for use. Some antioxidants, such as BHA and BHT, have been linked to potential carcinogenic effects in animal studies [16,17]. These antioxidants may also have negative effects on the immune system and liver function in animals. However, the safety of these antioxidants for human consumption is still a matter of debate and has yet to be fully established [18]. Therefore, it is important to carefully consider chemical antioxidants in animal feed and human food and ensure that they are used safely. Additionally, alternative natural antioxidants, such as vitamin E and rosemary extract, can be used in animal feed and human food to prevent oxidation and extend shelf life without some chemical antioxidants' potential negative health effects [19]. However, it is necessary to increase product diversity to meet industrial demand for natural antioxidants.

Due to their high phenolic component content, shown to display substantial antioxidant activity, essential oils have attracted considerable interest as possible sources of natural antioxidants [20]. Free radicals are highly reactive chemicals that may produce oxidative stress in cells and tissues, which can result in cell damage, aging, and several illnesses. Antioxidants are molecules that can stop or slow down this damage [21–23]. The volatile and intricate combinations of aromatic chemicals that make up essential oils come from numerous plant sources. Many *in vitro* and *in vivo* investigations have shown the powerful antioxidant activity of several essential oils, including clove, oregano, and thyme [24]. These oils include significant concentrations of phenolic compounds, renowned for their antioxidant qualities. Examples of these chemicals include carvacrol, thymol, eugenol, and rosmarinic acid [24–27]. In several experimental models, essential oils have been shown to scavenge free radicals, lessen lipid peroxidation, and protect against oxidative stress. According to certain research, the activity of endogenous antioxidants like glutathione, superoxide dismutase, and catalase may be increased by essential oils, which might provide further protection against oxidative stress in *in vivo* investigations [28–31]. Additionally, using essential oils as antioxidants is considered safer than synthetic antioxidants, which have been linked to possible negative effects [32]. However, it is crucial to remember that when misused or in excessive dosages, essential oils may also have hazardous consequences, so care should be taken while utilizing them. Because an essential oil overdose might be as hazardous as synthetic antioxidants, dosage is the most crucial aspect of using essential oils [33,34]. Overall, essential oils have potential uses in the food, pharmaceutical, and cosmetic sectors and may be a great source of natural antioxidants [35]. Further study is necessary to completely comprehend their antioxidant capabilities and ideal utilization in various applications.

Borago officinalis, popularly known as borage or starflower, is an annual herb used in local cuisines, and for studies on its medicinal effects. It is collected from nature and/or cultivated for these purposes [36]. Research on this plant focuses on the antioxidant properties of products obtained from *Borago officinalis* and reports that these products show strong antioxidant properties [37]. Therefore, the aim of this study was to determine the usability of the essential oil obtained from the leaves of the *Borago officinalis* plant to stabilize the oxidative quality of fish oil, which is of great importance for the animal feed industry.

2. Materials and Methods

The leaves of *B. officinalis* utilized in this research were procured from the nearby marketplace in Kastamonu, Turkey, June of the year 2020.

2.1. *Borago officinalis* Essential Oil (BEO) Extraction

Borago officinalis leaves were cleaned with drinkable water and cut into small pieces using laboratory scissors. The leaves were placed in a round-bottomed flask with distilled water (100 g 300 mL⁻¹) [38], and hydrodistillation was started using a Clevenger apparatus. After a three-hour distillation process under 90 °C, the upper phase of the extract collected in the collector (essential oil) was transferred to amber glass vials and stored at −20 °C under a modified atmosphere with nitrogen gas for analysis and use in the study.

2.2. Volatile Compounds of *B. officinalis* Essential Oil

The volatile profile of *Borago officinalis* leaf essential oil was determined using gas chromatography/mass spectrometry (GC/MS) (Shimadzu GCMS QP 2010 ULTRA). During the analysis, an RTX-5MS brand capillary column (30 m; 0.25 mm; 0.25 μm) and helium as the carrier gas were used in the device. The column oven temperature was set to 40 °C, the interface temperature to 250 °C, the ion source temperature to 200 °C, and the injection temperature to 250 °C. The injection volume was 1 μL, and the split (1/5) injection method was used. During the analysis, a 78 min oven program was applied, consisting of a 3 min hold at 40 °C, a 4 °C min⁻¹ increase from 40 °C to 240 °C, a 10 min hold at 240 °C, a 4 °C min⁻¹ increase from 240 °C to 260 °C, and a 10 min hold at 260 °C [38].

2.3. Antioxidant Performance of *B. officinalis* Essential Oil

To determine the scavenging effect of BEO volatile compounds on DPPH radicals, a mixture of 0.2 mL of volatile extract, 0.5 mL of DPPH solution, and 4 mL of 80% ethanol was prepared by mixing for 15 s in a vortex mixer and left at room temperature for 15 min. At the end of the maceration period, the mixture was read against a blank at 517 nm using a UV-VIS spectrophotometer, and the absorbances were recorded. The % scavenging effect values were calculated using the following formula [39].

$$\% \text{ scavenging effect} = [1 - (A_{\text{Sample}}/A_{\text{Blank}})] \times 100$$

The same procedure was applied to butylated hydroxytoluene (BHT), ascorbic acid (ASC), and BEO at different concentrations, and the 50% inhibitory concentration (IC₅₀) values of the products were calculated and compared using the created curves. Thus, it was applied to compare dose performance with BEO and commercially commonly used antioxidants.

2.4. Experimental Thermal Oxidation Process

In order to determine the protective effect of BEO on the thermal oxidation of fish oil, different experimental groups were prepared by adding 0% (BEO0), 0.1% (BEO 0.1), 0.5% (BEO 0.5), 1% (BEO1), and 3% (BEO3) concentrations of BEO to fish oil. The experimental groups were placed in temperature-resistant bottles under thermal oxidation conditions at 70 ± 0.5 °C for 24 h while continuously ventilated.

2.5. Determination of the Oxidation Level of the Experimental Groups

The peroxide value determination method (Cd 8b-90) published by the American Oil Chemists' Society (AOCS) was used to determine the effect of thermal oxidation and the protective effect of BEO [40]. For this purpose, samples were taken from the control (new fish oil, not thermal oxidation process) and experimental groups dissolved in 5 mL chloroform. After adding 15 mL of acetic acid and 1 mL of saturated potassium iodide, the samples were kept in a dark environment at room temperature for 10 min. Following the waiting period, titration was carried out using 0.01 N sodium thiosulfate with a few drops of 1% starch as an indicator in 75 mL of deionized water. The peroxide value was calculated using the following formula based on the titrant used at the point of clear color formation, indicating the end of the titration.

$$\text{Peroxide value (PV) (\%)} = [(V1 - V0) \times N] / V$$

V1 and V0 are the volumes of titrant used for the sample and blank, respectively, N is the normality of the titration solution, and M is the weight of the sample. The peroxide value analysis results, performed in triplicate, were evaluated as meqO₂ kg⁻¹ of oil.

2.6. Statistical Analysis

The data obtained in the experiment were analyzed using the Minitab 18 statistical program. One-way analysis of variance (ANOVA) was applied to the data and subjected to the Tukey multiple comparison test. Differences between groups were considered significant at $p < 0.05$. The IC50 value for each sample of *B. officinalis* essential oil, ASC, and BHT was determined using a linear regression of the concentration–response curve of the percentage of inhibition versus the antioxidant concentration.

3. Results

3.1. *Borago officinalis* Essential Oil and Volatile Compounds

At the end of steam distillation, 1.13 ± 0.04 mL (11.32 ± 0.57 mL kg⁻¹) of essential oil was extracted from 100 g fresh leaves. The volatile components of the essential oil were identified as 97.27 percent and the profile was presented in Table 1.

Table 1. Volatile compounds of *Borago officinalis* leaf essential oil.

Sn.	Compound	Retention Time (min.)	Concentration (%)
1	Benzaldehyde	10.093	3.51
2	D-Limonene	12.692	0.83
3	1.8-Cineole (eucalyptol)	12.806	7.22
4	Benzeneacetaldehyde	13.387	28.59
5	Linalool	15.667	13.60
6	Nonanal	15.772	4.09
7	3-Acetylheptane-2,6-dione	15.906	5.19
8	α -Terpineol	19.169	7.34
9	Undecane	19.39	1.92
10	α -Terpinenyl acetate	24.833	7.01
11	β -ionone	29.404	3.88
12	Caryophyllene oxide	32.478	2.67
13	Heptadecane	35.707	2.01
14	Limonene dioxide	36.692	6.21
15	2-Pentadecanone, 6, 10, 14-trimethyl	39.754	3.18

3.2. Antioxidant Activity of *Borago officinalis* Leaf Essential Oil Volatile Compounds

There was a direct linear relationship between the concentrations of BEO, BHT, and ASC samples in the DPPH solution and their scavenging percentages, as calculated by correlation factors $R^2 \leq 0.92, 0.90,$ and 0.90 , respectively (Figure 1). Utilizing the set of linear equations provided, IC50 were computed. BHT and ASC were utilized for comparison. As determined

by the calculation, the BEO IC₅₀ value is 736.06 ppm. The calculated BHA and ASC values are 481.63 and 198.51, respectively. The IC₅₀ value of BEO is 736.06, thereby rendering it 1.5 times less active than BHT and 3.5 times less active than ASC. Compared to synthetic antioxidants, the DPPH radical scavenging activity of BEO was calculated to be feeble.

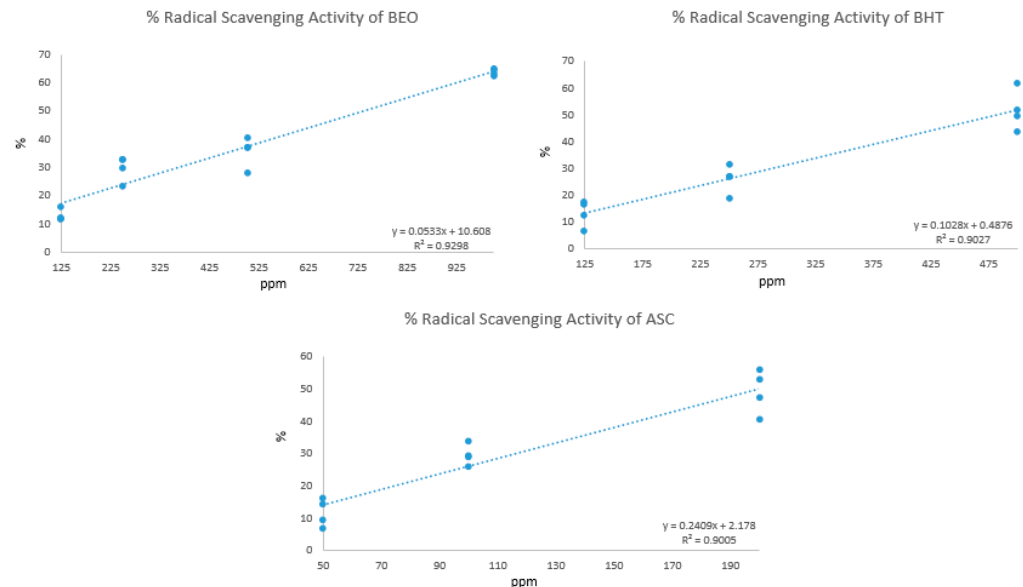


Figure 1. Percentage of radical scavenging activity in *Borago officinalis* leaf essential oil volatile extract, butylated hydroxytoluene (BHT), and ascorbic acid (ASC) (n = 4).

3.3. Changes in Lipid Oxidation of Experimental Groups

Following thermal treatment, the PV value of fish oils with different ratios of BEO addition and fish oils without addition and thermal oxidation (control) was measured, as shown in Figure 2. As expected, the significantly lowest PV value was measured in the control group that was not thermally oxidized ($p < 0.05$). At the end of the thermal oxidation process with continuous aeration for 24 h at a constant temperature of 70 °C, it was observed that PV formation was inversely proportional to the amount of BEO added. It was determined that PV values showed significant differences between the experimental groups ($p < 0.05$) and the significantly lowest PV values were found in fish oils with 1 percent and 3 percent BEO addition ($p < 0.05$).

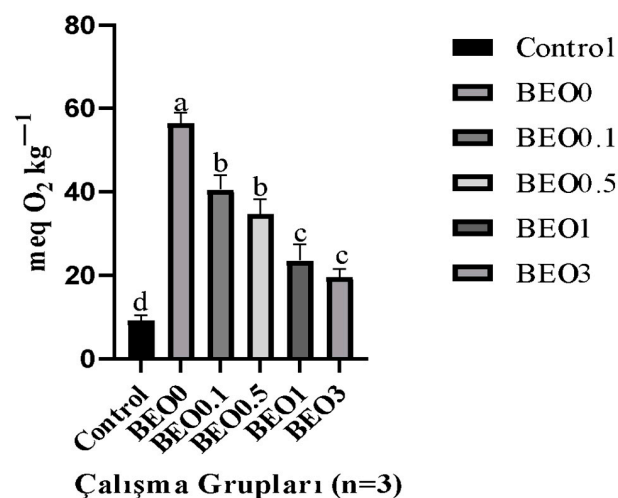


Figure 2. Effect of BEO addition at different ratios to fish oils continuously aerated and heated at 70 °C on peroxide formation at the end of 24 h (n = 3). Values with different letters indicate significant differences between their group ($p < 0.05$).

4. Discussion

The consumption of oxidized fish oil by farm animals can have negative effects on their health [41] and may also impact human health through consumption of meat and other animal products [42]. Oxidation of fish oil can result in the formation of harmful compounds, such as free radicals and reactive aldehydes, which can damage cells and tissues in the body [43]. In animals, consumption of oxidized fish oil can lead to oxidative stress, inflammation, and impaired immune function [10,44]. These effects can impact growth, feed efficiency, and disease susceptibility, ultimately affecting animal production's productivity and profitability [45]. In addition, consuming meat and other animal products from animals fed oxidized fish oil may result in adverse health effects on humans. In other words, individuals who consume oxidized oil may be affected directly or indirectly. However, there are few studies on this topic. Five human subjects were fed a supper containing 100 g of unprocessed soybean oil, and an identical meal containing 100 g of oxidized soybean oil the following day. They demonstrated that the consumption of oxidized soybean oil altered the composition of triacylglycerol-rich lipoproteins but the consumption of fresh soybean oil did not [46].

Therefore, it is important to ensure that fish oil used in animal production is protected from oxidation to prevent negative health effects on animals. Inhibition or agitation of the oxidation process can be achieved through the use of natural antioxidants, such as plant extracts and essential oils, or the use of synthetic antioxidants, such as BHT. However, synthetic antioxidants are considered controversial due to concerns about their safety and potential negative effects on human health [18]. Alternatively, different antioxidants for fish oil, such as plant-based sources, are being researched, as they have antioxidant potential and do not have an environmental and/or negative health impact [11]. The results of studies on the antioxidant activity of plants and plant-based products such as essential oils and extracts in food and feed grades have shown that these products are a promising alternative to synthetic antioxidants [47,48]. Many research investigations have been conducted on the potential of extracts and other natural products, particularly those derived from plants, to provide protection against oxidation-sensitive substrates such as fish oil. In the conducted research on the antioxidant properties of rice and wheat bran against fish oil oxidation, varying ratios of wheat and rice bran were added to fish oils, which were subsequently stored at a temperature of 38 °C for 30 days. In the antecedent investigation, which lacked a thermal process as implemented in the present study, it was noted that wheat bran exhibited superior antioxidant activity in comparison to rice bran. According to the paper, the efficacy of wheat bran can be attributed to its bioactive constituents [49]. A study investigated the protective effect of aqueous extracts of sugar grassroots and leaves on fish oil oxidation using oxidation treatment. The experiment involved subjecting fish oils to varying ratios of root and leaf extracts, followed by heating at a temperature of 50 °C. The results indicated that the root extract exhibited a lower PV value than the leaf extract, offering superior protection against oxidation [50].

Borage (*Borago officinalis*) is an annual plant cultivated for medicinal and culinary purposes and for borage seed oil production. Borage seed oil is an abundant source of gamma-linolenic acid [36,51]. In addition, it has been reported that the different types of extracts obtained from different parts of the borage plant, such as leaves, flowers [52], and seeds [53], that are not used for oil, have many benefits, especially antioxidant activity. A previous study on *Borago officinalis* leaf extract found it to have antioxidant activity in a dose-dependent manner. The extract was able to scavenge free radicals and inhibit lipid peroxidation in an in vitro rapeseed oil study. The study also reported that the extract had high levels of phenolic and flavonoid compounds, which are also known for their antioxidant properties [54]. The current study confirms this information. The essential oil obtained for this study is rich in volatile constituents, most of which are benzeneacetaldehyde and linalool. Linalool is a molecule with known antioxidant activity. Many studies have reported that linalool [55] or products containing linalool [56] show antioxidant activity. The other major component in the current study is benzeneacetaldehyde; this molecule has been

detected in essential oil in previous studies of *Borago officinalis* [52]. In addition, studies on many products known to contain benzeneacetaldehyde have reported that the products have antioxidant activity. This effect may be due to the benzeneacetaldehyde contained in the product [57–60]. These studies suggest that *Borago officinalis* leaf extract and essential oil have significant antioxidant activity. However, it is important to note that the effectiveness of these compounds may vary depending on factors such as the method of extraction and the concentration of active compounds. A disadvantage of research on the antioxidant activity of plant resources is that the products studied are natural resources; they are not cultivated in agricultural practices, or if they are, they are produced for another industry and/or consumer group. Consequently, there is concern that the basic materials required for an antioxidant product's sustainable production cannot be sourced sustainably. Borage is a productive product in this regard; as previously mentioned, borage is an agriculturally grown crop for the gamma-linoleic acid in its seeds, and its leaves have no industrial use. In other words, the production of a product from the essential oil extracted from borage leaves would be an example of adding value to refuse. In addition, according to reference, essential oils extracted from wild and cultivated Borage species were found to exhibit antioxidant properties [37].

The most widely used product for protection against lipid oxidation in natural sources, especially fish oil, is undoubtedly tocopherol groups. However, in addition to these, many plant-derived products show antioxidant activity; some are even accepted by the authorities. The most popular of these products is rosemary. Rosemary is listed as an additive with antioxidant activity in EC Directive 1129/2011, published by the European Union. In order to increase the variety and number of herbal resources like rosemary, much research has been carried out on the antioxidant efficacy of many herbal products, and this research has even increased to the extent that review papers can be written [47,48]. The variability of the antioxidant capacity of leaf essential oils is contingent upon the particular botanical species and the constitution of the essential oil. Certain essential oils derived from leaves have demonstrated significant antioxidant properties, whereas others may possess a less pronounced or insignificant antioxidant impact. Numerous research activities have been conducted to explore the antioxidative capacity of essential oils extracted from the leaves of diverse botanical species. The essential oil derived from *Rosmarinus officinalis* (commonly known as rosemary) leaves has been discovered to possess potent antioxidant properties owing to its significant concentration of phenolic compounds, including rosmarinic acid, carnosic acid, and carnosol [61]. The antioxidant activity of *Melaleuca alternifolia* (tea tree) leaf essential oil has been demonstrated to be due to its elevated levels of terpinen-4-ol and 1,8-cineole [62].

The antioxidant potential of various leaf essential oils has been investigated, including those derived from *Eucalyptus globulus* [63], *Mentha piperita* (peppermint) [64], and *Origanum vulgare* (oregano) [65]. These essential oils comprise diverse phenolic compounds, including eucalyptol, menthol, menthone, pulegone, carvacrol, and thymol. These compounds have demonstrated the ability to eliminate free radicals and safeguard against oxidative stress. In general, the capacity of leaf essential oils to act as antioxidants can be attributed to their elevated concentration of phenolic compounds, which have demonstrated potent antioxidant properties. The antioxidant capacity of essential oils may differ based on their botanical origin and chemical composition. However, the antioxidant activity of herbal products should also be tested at the source of their use. For this reason, many studies have been carried out on natural sources of antioxidants to protect fish oil, which is susceptible to oxidation [66–68]. Although many methods have been used to measure the resistance of fish oil to oxidation in these studies, the peroxide value has been the most widely used.

Peroxide value is one of the reliable methods used for many years to determine the quality of fish oil. Calculating the amount of peroxide in the product, it is used to determine the oxidation of fish oil in the usual expression of rancidity [69]. Many research efforts examining the protective impact of various natural antioxidants on fish oil oxidation have utilized peroxide value as the primary oxidation indicator. The preceding research assessed

the effectiveness of laurel, rosemary, and thyme as antioxidants in the preservation of fish oil by inhibiting oxidation. The findings of the study suggest that each of the herbal products evaluated demonstrated a significant capacity to mitigate oxidative stress. The experimental group that received a supplementation of 1500 ppm rosemary exhibited the lowest peroxide value at the end of the investigation. The findings indicate a noteworthy reduction in PV levels of fish oils that were fortified with rosemary extract after a duration of 40 days, as opposed to the cohort that received 250 ppm BHT [70]. Essential oils have the potential to serve as a direct means of preserving fish oil from oxidation as an additive to the capsulation material during the encapsulation process. The study conducted previously examined the impact of citrus essential oils, such as orange, mandarin, grapefruit, and lemon, as additives to capsulation materials on the temporary preservation of fish oil microencapsulates. The findings of the study revealed that the fish oil groups that were administered orange and lemon essential oils as supplements demonstrated the least PV upon completion of the 14-day storage duration [71]. Considering the peroxide measurement results of the current study, the significantly lowest peroxide value was naturally observed in the control group without a thermal procedure. However, the peroxide values after oxidation were inversely proportional to the amount of BEO supplemented, and the lowest peroxide values were observed in BEO1 and BEO3. The current finding showed the protective effect of BEO against the thermal oxidation of fish oil, which has been proven to be an antioxidant using radical scavenging activity analysis in previous studies [51,52] and the current study. Similar results were reported in another study in which ginger essential oil was used against fish oil oxidation. In the study investigating the protective effect of ginger essential oil and aqueous extract against fish oil oxidation, it was reported that ginger essential oil showed higher anti-oxidant activity and a lower peroxide value than aqueous extract [72].

Much research has been carried out on the protection of products extracted from plant sources with known antioxidant activity, not only directly in only oil as in the current study, but also oil sources in different oily products such as animal feed [73,74] and human food [75,76]. Several studies have utilized essential oils to preserve the quality of feed and food. However, it has been observed that essential oil additives exceeding 1 percent have resulted in unfavorable physiological reactions in animals [77] and decreased the desirability of food products due to their potent aroma [76]. Therefore, limiting the use of essential oil additives to below 5 percent is recommended. The present study restricted the doses to 3 percent due to this rationale. The results indicated a reduction in the PV value subsequent to oxidation within the range of 1–3 percent. However, this reduction did not demonstrate statistical significance. Exceeding a BEO concentration of 3 percent could have led to an increase in antioxidant activity; however, this would have had a negative impact on the overall acceptability of the fish oil.

5. Conclusions

Consequently, the consumption of oxidized fish oil by animals can cause adverse effects on animal health and may also affect human health through the consumption of animal products. Therefore, it is important to protect fish oil from oxidation in animal production to prevent adverse health effects. According to the results obtained in this study, supplementation of fish oil with 1% and 3% *Borago officinalis* essential oil protects fish oil and prevents oxidation. However, it is a fact that essential oils may reduce the consumability of the product to which they are added due to their high concentration of aroma components. For this reason, since the effect of 1% and 3% additives on PV formation was not statistically different, the findings of this study determined that the use of 1% BEO in fish oils is an efficient dose for combating oxidation.

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