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A Natural Solution Against Fish Oil Oxidation: The Antioxidant Effect of Satureja montana Essential Oil

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Abstract

Fish oil, due to its high content of polyunsaturated fatty acids (PUFA), is extremely sensitive to oxidation, which can negatively affect its nutritional quality and shelf life. In this study, the effects of Satureja montana (SMont EO) essential oil on fish oil oxidation were investigated. Within the scope of the experiment, different concentrations (100, 200, 400, 800 ppm) of Satureja montana essential oil were added to fish oils, and the commercial antioxidant BHT (200 ppm) was used as a positive control. The experimental groups were subjected to accelerated oxidation for 96 hours under 55°C temperature, 70% humidity, and 7000 lux illumination. Oxidation levels were determined by measuring peroxide value (PV) and malondialdehyde (MDA), and the obtained data were statistically analyzed using one-way ANOVA and Tukey's test. According to the GC-MS analysis results, Satureja montana essential oil contains the highest amounts of carvacrol (42.62%), γ-terpinene (12.44%), and p-cymene (14.09%). It has been reported in the literature that carvacrol is a strong free radical scavenger, γ-terpinene can prevent lipid peroxidation, and p-cymene plays an important role in combating oxidative stress. According to the peroxide value measurements, it was determined that the group containing 400 ppm Satureja montana oil had the lowest oxidation level. The peroxide value of the 400 ppm dose was determined to be 16.08 meq O_2/kg, which is similar to the commercial antioxidant BHT (25.48 meq O_2/kg). MDA levels were also measured at the lowest level in the 400 ppm essential oil group, but a slight increase was observed at the 800 ppm dose. The results indicate that Satureja montana essential oil is an effective natural antioxidant in preventing fish oil oxidation. It has been determined that a concentration of 400 ppm provides the best oxidative stability. Compared to the commercial antioxidant BHT, Satureja montana essential oil provided a similar level of oxidative protection, therefore it can be considered as an alternative to synthetic antioxidants in aquaculture feeds.

Key Words: *Fish oil oxidation, Satureja montana essential oil, Natural antioxidants, Peroxide value, Lipid peroxidation*

Introduction

Aquaculture is an essential sector for fulfilling the animal protein requirements of the swiftly expanding global population (Nasopoulou & Zabetakis, 2012). In this sector, fish oil provides a crucial nutritional source owing to its elevated quantity of polyunsaturated fatty acids (PUFAs). Omega-3 fatty acids (EPA and DHA) are known for their beneficial benefits on human health and are deemed critical components of feeding protocols in aquaculture (Harris, 2004; Rizliya & Mendis, 2013). Fish oil is particularly sensitive to oxidation because of its elevated levels of polyunsaturated fatty acids (Frankel, 1984).

Lipid oxidation results in the degradation of fat content via free radicals, leading to diminished food quality and modifications in flavor and odor (Shahidi & Zhong, 2010). This procedure reduces the shelf life of fish oil-containing nutrition and adversely impacts growth performance and feed efficiency in fish (Oliva-Teles et al., 2022).

Therefore, the use of antioxidants is crucial in order to avert the oxidation of fish oil. A common antioxidant in current commercial products is the synthetic compound butylated hydroxytoluene (BHT). Nevertheless, research indicates that certain synthetic antioxidants may exhibit toxic side effects and potentially detrimental effects on human and fish health over time (Wang et al., 2021).

This circumstance requires the investigation of natural antioxidant sources and the formulation of alternative treatments (Gulcin, 2020; Kesbiç, 2023). Essential oils, phenolic compounds, and terpenoids are prominent natural antioxidants due to their composition (Ben Hsouna et al., 2017). This study assessed the potential preventive effect of Satureja montana essential oil (SMont EO) on the oxidation of fish oil. SMont EO exhibits antioxidant and antibacterial properties thanks to its elevated carvacrol concentration.
(Trifan et al., 2015).

This research aims to clarify the potential advantages of including natural antioxidants into aquaculture feeds and to empirically validate the efficacy of Satureja montana essential oil as a substitute for synthetic antioxidants.

Materials and Methods

The SMont EO utilized in the study was procured from a commercial source, and the volatile component composition of the product was qualitatively analyzed using GC/MS (Kesbiç 2019).
The objective is to examine the efficacy of various concentrations of SMont EO (100 ppm, 200 ppm, 400 ppm, and 800 ppm) in inhibiting the oxidation of fish oil. To achieve this objective, fish oils from various experimental groups were subjected to accelerated oxidation conditions, and their impacts on oxidative stability were assessed by quantifying the peroxide value (PV) and malondialdehyde (MDA) levels. The study employed commercial antioxidant BHT at 200 ppm (BHT200) as the positive control, while fish oil without additions served as the negative control. To facilitate accurate comparisons and assess the quality of the oil utilized in the study, the peroxide (Kesbiç, 2023) and MDA (Okhawa et al., 1979) levels of the fresh fish oil were measured.
The rapid oxidation study was conducted under conditions of temperature, humidity, and light that would trigger oxidation. The prepared experimental groups were subjected to an accelerated oxidation process for 4 days at a temperature of 55.0±0.5°C, in a 70% humid atmosphere, and under 7000 lux illumination. Subsequently, peroxide and MDA measurements were conducted.

Results and Discussion

This study studied the effects of Satureja montana essential oil on the oxidation of fish oil and examined its antioxidant activity. The GC-MS analysis identified the three main components of the essential oil as carvacrol (42.62%), γ-terpinene (12.44%), and p-cymene (14.09%). Carvacrol has been identified as a potent free radical scavenger owing to its phenolic structure, and prior research has demonstrated its substantial effect in inhibiting lipid peroxidation (Trifan et al., 2015).
This molecule, extensively utilized as a natural antioxidant in food preservation, has also been examined as an effective agent against oxidative stress (Burt, 2004). γ-Terpinene is known for its capacity to neutralize highly reactive oxygen species (ROS), and it has been demonstrated that this characteristic enhances the oxidative stability of oils by inhibiting lipid peroxidation (Ruberto & Baratta, 2000). The p-Cymene molecule, owing to its lipophilic nature, demonstrates a protective impact on cellular membranes and inhibits oxidative degradation by disrupting free radical chain reactions (Gülçin et al., 2012). The existence of these components scientifically validates the antioxidant capacity of Satureja montana essential oil and demonstrates that the observed antioxidant activity in the study is grounded in its biochemical composition.
The peroxide values (PV) assessed across several experimental groups have yielded significant insights into the oxidative stability of the fish oil samples (Table 1).
The peroxide value in the control group was measured at 75.48 meq O_2/kg, considerably higher than that of fresh fish oil. This means that oxidation is advancing and lipid peroxidation has increased markedly. The group that administered the commercial antioxidant BHT (200 ppm) exhibits a peroxide value of 25.48 meq O_2/kg and demonstrates comparable antioxidant efficacy to the groups utilizing essential oils. The application of Satureja montana essential oil has resulted in a notable reduction in peroxide values. At a concentration of 100 ppm, the peroxide value was measured at 26.59 meq O_2/kg; at 200 ppm, it was 18.87 meq O_2/kg; and at 400 ppm, it was 16.08 meq O_2/kg. The peroxide value was lowest at a dosage of 400 ppm, indicating that this concentration provides the most efficient oxidative protection. At a concentration of 800 ppm, the peroxide value measured 22.41 meq O_2/kg, suggesting that volatile oil may demonstrate pro-oxidant activity at elevated levels. Research indicates that a concentration of 400 ppm of essential oil ensures adequate oxidative stability, however elevated doses may result in erratic oxidative effects (Kesbiç, 2023).
The study examined MDA levels to assess the evolution of oxidative stress, providing as a quantitative indication of lipid peroxidation. The control group showed the highest levels of MDA, reflecting the highest level of oxidation. The group supplied 200 ppm BHT and those obtaining 400 ppm Satureja montana essential oil exhibited the lowest MDA levels. This condition suggests that the antioxidant efficacy of the essential oil is comparable to that of BHT. It has been shown that MDA levels increased at the 800 ppm dosage, corroborating the concept that elevated doses may demonstrate a pro-oxidant impact. Some research in the literature indicate that excessive antioxidant use can negatively impact specific oxidative stress conditions (Wang et al., 2011).
This study's results demonstrate that Satureja montana essential oil serves as an excellent natural antioxidant in inhibiting the oxidative degradation of fish oil. The lowest values of peroxide and MDA were observed, particularly at a concentration of 400 ppm. In comparison to the commercial antioxidant BHT, Satureja montana essential oil offered an equivalent level of oxidative protection, suggesting that natural antioxidants may serve as viable alternatives to manufactured ones. Natural antioxidants present a substantial alternative to the deleterious effects of synthetic chemicals and confer benefits for food safety. In conclusion, Satureja montana essential oil demonstrates potent antioxidant activities attributed to its constituents carvacrol, γ-terpinene, and p-cymene, which are thought to augment oxidative stability. Research indicates that plant essential oils abundant in phenolic

components may serve as natural antioxidants in aquaculture diets. Further research is required to examine the long-term stability of these natural components under varying temperature and storage conditions.

Table 1. Peroxide values (meq O_2/kg) of experimental groups after rapid oxidation procces.

Experimental Groups	N	Mean	Standart Deviation	Grouping Information	
BHT 200	3	25,480	1,426	B	
Control	3	75,48	2,94	A	
Fresh Fish Oil	3	5,212	1,091	E	
SMont EO0.1	3	26,595	1,308	B	
SMont EO0.2	3	18,876	1,016	CD	
SMont EO0.4	3	16,081	1,155	D	
SMont EO0.8	3	22,410	1,384	BC	
Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Control - BHT200	50,00	1,31	(45,54; 54,46)	38,31	0,000
Fresh Fish O - BHT200	-20,27	1,31	(-24,72; -15,81)	-15,53	0,000
SMont EO0.1 - BHT200	1,12	1,31	(-3,34; 5,57)	0,85	0,974
SMont EO0.2 - BHT200	-6,60	1,31	(-11,06; -2,15)	-5,06	0,003
SMont EO0.4 - BHT200	-9,40	1,31	(-13,86; -4,94)	-7,20	0,000
SMont EO0.8 - BHT200	-3,07	1,31	(-7,53; 1,39)	-2,35	0,286
Fresh Fish O - Control	-70,27	1,31	(-74,72; -65,81)	-53,84	0,000
SMont EO0.1 - Control	-48,88	1,31	(-53,34; -44,43)	-37,46	0,000
SMont EO0.2 - Control	-56,60	1,31	(-61,06; -52,14)	-43,37	0,000
SMont EO0.4 - Control	-59,40	1,31	(-63,85; -54,94)	-45,51	0,000
SMont EO0.8 - Control	-53,07	1,31	(-57,53; -48,61)	-40,66	0,000
SMont EO0.1 - Fresh Fish O	21,38	1,31	(16,93; 25,84)	16,38	0,000
SMont EO0.2 - Fresh Fish O	13,66	1,31	(9,21; 18,12)	10,47	0,000
SMont EO0.4 - Fresh Fish O	10,87	1,31	(6,41; 15,33)	8,33	0,000
SMont EO0.8 - Fresh Fish O	17,20	1,31	(12,74; 21,65)	13,18	0,000
SMont EO0.2 - SMont EO0.1	-7,72	1,31	(-12,18; -3,26)	-5,91	0,001
SMont EO0.4 - SMont EO0.1	-10,51	1,31	(-14,97; -6,06)	-8,06	0,000
SMont EO0.8 - SMont EO0.1	-4,19	1,31	(-8,64; 0,27)	-3,21	0,072
SMont EO0.4 - SMont EO0.2	-2,80	1,31	(-7,25; 1,66)	-2,14	0,382
SMont EO0.8 - SMont EO0.2	3,53	1,31	(-0,92; 7,99)	2,71	0,167
SMont EO0.8 - SMont EO0.4	6,33	1,31	(1,87; 10,79)	4,85	0,004

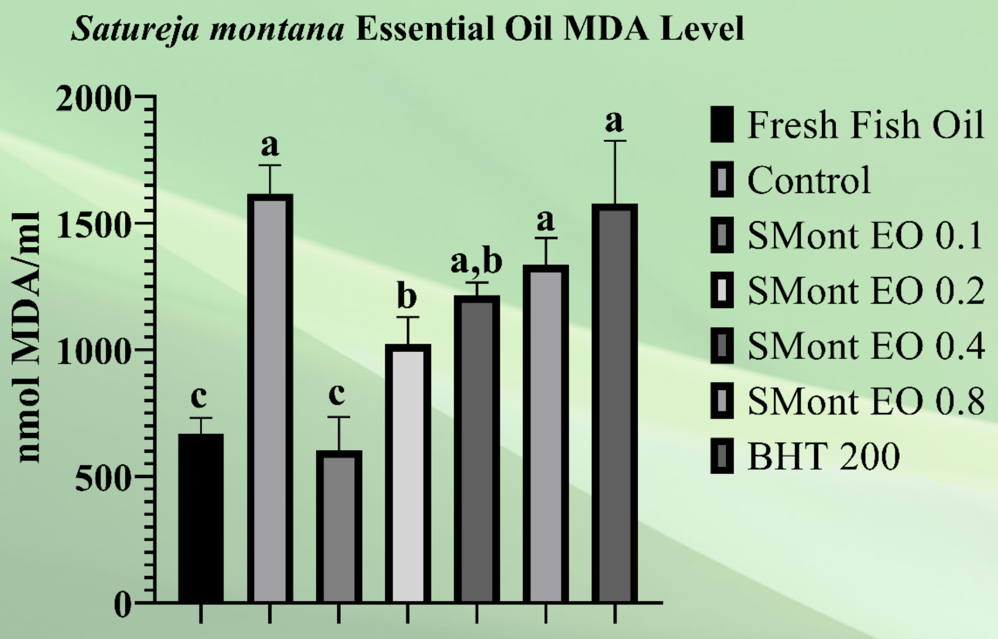

Figure 1. MDA levels of experimental fish oils after oxidation.

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