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Prevention of Fish Oil Oxidation by Melaleuca quinquenervia Essential Oil: Dose Dependent Antioxidant Effects

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Abstract

Fish oil, rich in omega-3 polyunsaturated fatty acids (PUFAs), is widely recognised for its health benefits. However, its susceptibility to oxidation poses a significant challenge and leads to reduced nutritional quality and potential health risks. This study investigates the antioxidant activity of Melaleuca quinquenervia essential oil (MQEO) in fish oil and compares it with the synthetic antioxidant butylated hydroxytoluene (BHT). Experimental groups were formed by adding different concentrations of MQEO (100-800 ppm) to fish oil and oxidation levels were evaluated by peroxide value (PV) and malondialdehyde (MDA) measurements. The results showed that higher concentrations (≥400 ppm) of MQEO showed significant antioxidant activity by effectively reducing peroxide formation and lipid peroxidation compared to BHT. The major active components of MQEO, such as eucalyptol, viridifluorol and α-terpineol, contributed to its protective effect by inhibiting lipid oxidation. Given the growing concerns about the safety of synthetic antioxidants, MQEO offers a promising natural alternative to protect lipid-based products susceptible to oxidation. The findings suggest that MQEO may improve fish oil stability and extend shelf life while reducing potential health risks associated with synthetic preservatives.

Key Words: *Fish oil, Melaleuca quinquenervia, oxidation, antioxidant, lipid peroxidation*

Introduction

Fish oil, rich in omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is a widely recognized dietary supplement due to its anti-inflammatory, neuroprotective, and cardioprotective properties. EPA and DHA contribute to cardiovascular health by regulating lipid metabolism and improving endothelial function (Harris, 2004). DHA is crucial in neuronal membrane integrity and cognitive function (Das et al., 2009). Additionally, omega-3 fatty acids' anti-inflammatory effects suggest their potential in managing metabolic and autoimmune diseases.

As the human body cannot synthesize omega-3 fatty acids endogenously, dietary intake or supplementation is necessary. Fish oil, derived from species like anchovies, sardines, mackerel, and tuna, has gained global popularity, particularly among individuals prioritizing preventive healthcare (Pike & Jackson, 2010; Tocher, 2010). However, the rising demand for fish oil raises concerns regarding marine resource sustainability. Overfishing, habitat degradation, and climate change threaten fish stocks, with reports indicating severe depletion of global fish populations (Barange et al., 2018; FAO, 2022). Given these challenges, alternative omega-3 sources are essential to mitigate environmental risks while maintaining health benefits (Jackson, 2006).

Fish oil easily oxidizes because of its high content of unsaturated fatty acids (PUFAs) (Kazuo, 2019). PUFAs attract free radicals and oxygen by the typical mechanism of its multiple double bonds reacting, leading to the oxidative degradation of PUFAs. As they get oxidized, secondary oxidation products involving hydroperoxides, aldehydes, and ketones are produced, and such substances are catalyzed by fish meal. Besides, they may be potential health hazards to the consumer (Min & Boff, 2002). Both taste and odor disorders are caused by fish oil oxidation and decreased nutritional value. In addition, oxidized lipids cause cellular damage by oxidative stress and threaten human health (Shahidi & Zhong, 2010). For this reason, it is a must to add antioxidant compounds to fish oil products to avoid oxidation.

Nowadays, most fish oils take advantage of synthetic antioxidants in the production process. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are frequently chosen as antioxidants for the food industry to prevent fat oxidation. However, new research has found that synthetic antioxidants may have potentially toxic effects and long-term health risks (Arneson & Roberts, 2007). The result is growing interest in natural sources of these compounds. Among these, the rule to contain oxidizes know-how to do so and are known as natural and, therefore, safe alternatives in food products (Ambrosio et al., 2021) are those derived from plants.

The study aimed to assess the antioxidant activity of Melaleuca quinquenervia essential oil in fish oil and to compare it with BH, a synthetic antioxidant. Experimental groups were formed by adding various proportions of Melaleuca quinquenervia essential oil to fish oil samples, and antioxidant activity was evaluated by intermittent measurement of the peroxide values. The study is predicted to bring significant outputs to the industry of natural antioxidants in the food sector.

Materials and Methods

Oxidation Experiments

The fish oil for which this research was obtained is from a Turkish company, a company that is professionally engaged in the production of fish oil for human consumption. Additionally, various concentrations of MQEO (100ppm, 200ppm, 400ppm, 800ppm) were dissolved in fish oil; having formed distinct experimental groups that were established to analyze its protective effect against the thermal oxidation of fish oil. Moreover, Butylated hydroxytoluene (BHT200) was used as a positive control at a concentration of 200 ppm. The treatments of the experimental afore-mentioned groups were implemented over a period of 96 hours, keeping them at a twofold climate of 55±0.5 °C that can be sustained by temperature-resistant containers and 70% humidity. Light was turned on to maintain the 7000 luxs level as cited in the paper (Kesbiç et al., 2023).

Peroxide Value

The peroxide value determination method (Cd 8b-90) established by the American Oil Chemists' Society (AOCS) was used to assess the protective efficacy of MQEO against oxidation under accelerated conditions. Unoxidized fish oil and 0.5 g samples from the oxidized experimental groups were dissolved in 5 mL of chloroform. The dissolved samples were then incubated with 15 mL of acetic acid and 1 mL of saturated potassium iodide for 10 minutes at ambient temperature and in the absence of light. Following incubation, titration was performed using 0.01 N sodium thiosulfate and a few drops of 1% starch in 75 mL of deionized water as the indicator. The peroxide value was calculated using a formula based on the color change that indicated the result of the titration.

$$PV\ (\%) = [(V1 - V0)\ N] / V$$

V1 and V0 denote the titrant volumes employed for the sample and blank, respectively; N represents the normality of the titration solution, and M indicates the sample weight. The peroxide value assay findings were assessed in triplicate as meq O_2 kg^{-1} oil.

Measurement of the MDA levels

The quantity of malondialdehyde (MDA) generated during lipid peroxidation has been determined using the method developed by Ohkawa et al. (1979), which relies on the reaction between thiobarbituric acid (TBA) and MDA. The intensity of the colorful reaction complex generated by MDA and TBA was quantified spectrophotometrically at a wavelength of 532 nm. Results are expressed as nmol MDA/mL (Ohkawa et al., 1979).

Volatile Components of Neroli Oil by GC-MS

Different MQEO volatile components based on GC-MS analysis were achieved using RTX-5MS capillary column. The process was carried out at 40°C oven temperature, and the injection volume was 1µL. This was compared to peaks in the W9N11 library. Thus, the volatile component profile of the oil was ascertained.

Statistical Analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analyses were conducted using IBM SPSS Statistics version 20. A one-way ANOVA test was performed to assess the significance of differences among the sample groups, followed by group comparisons using Tukey's HSD (Honest Significant Difference) test. A significance threshold of $p < 0.05$ was applied in all analyses.

Results and Discussion

The peroxide values of the experimental groups after rapid oxidation showed significant variation ($P < 0.001$), indicating differing levels of oxidative stability among the treatments (Table 1). The oxidized fish oil group exhibited the highest peroxide value (75.18±0.99 meq O_2 kg^{-1}), confirming extensive lipid oxidation. In contrast, the fresh fish oil group had the lowest peroxide value (4.59±1.19 meq O_2 kg^{-1}), reflecting its unoxidized state. The synthetic antioxidant BHT (200 ppm) significantly reduced peroxide formation (26.48±0.75 meq O_2 kg^{-1}), showing a strong protective effect. Among the Melaleuca quinquenervia essential oil (MQEO) treatments, MQEO8 demonstrated the greatest antioxidant activity, resulting in the lowest peroxide value (21.12±0.87 meq O_2 kg^{-1}), followed by MQEO4 (24.24±0.72 meq O_2 kg^{-1}) and MQEO2 (24.62±0.65 meq O_2 kg^{-1}), which were statistically

similar to BHT. MQEO1 exhibited a slightly higher peroxide value (32.84±1.35 meq O_2 kg^{-1}), but still significantly lower than oxidized fish oil. These findings suggest that MQEO, particularly at higher concentrations, effectively mitigates oxidative degradation in fish oil, comparable to the synthetic antioxidant BHT.

Table 1. Peroxide amounts of experimental groups after rapid oxidation.

Experimental Groups	meq O_2 kg^{-1}	P value
Oxidized Fish Oil	75.18±0.99[a]	< 0.001
BHT 200	26.48±0.75[c]	
MQEO1	32.84±1.35[b]	
MQEO2	24.62±0.65[c]	
MQEO4	24.24±0.72[c]	
MQEO8	21.12±0.87[d]	
Fresh Fish Oil	4.59±1.19[e]	

Melaleuca quinquenervia essential oil (MQEO)

As a result of MDA analysis in the samples, it was determined that the levels in oxidised oil and MQEO4, MQEO8 and BHT 200 groups were significantly higher than fresh fish oil group ($P<0.05$). While the MDA level in MQEO1 group was significantly lower than the oxidised oil group ($P<0.05$), there was no significant difference between MQEO2 group and fresh fish oil group ($P>0.05$). The levels of the experimental groups are presented in Figure 1.

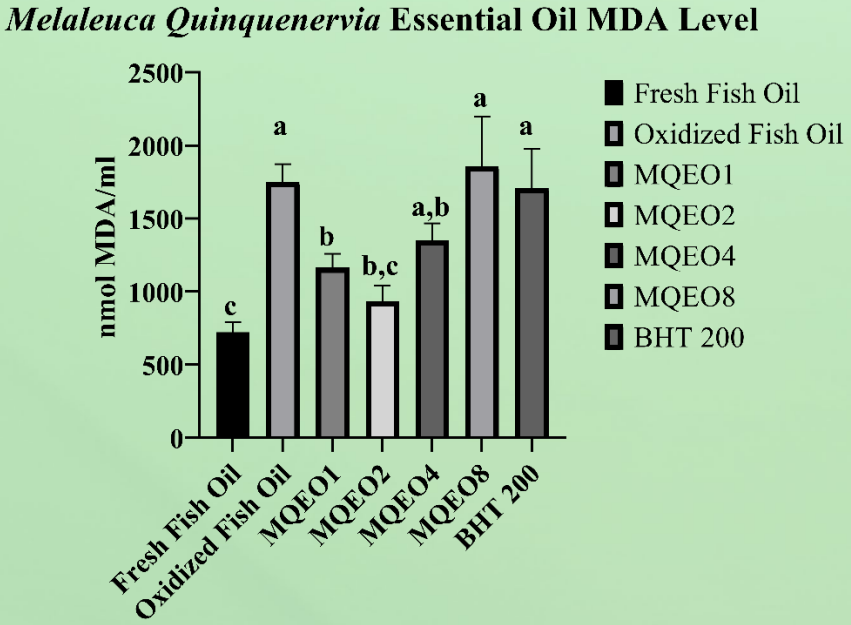

Figure 1. The MDA levels of the Experimental Groups.
Different letters indicate statistical differences between groups.

As a result of the analysis of the volatile components of *Melaleuca quinquenervia* essential oil, eucalyptol with a content of approximately 56%, viridifluorol and alpha terpineol with a ratio of 10% were determined. 1,8-Cineole (eucalyptol) is the main ingredient of *Melaleuca quinquenervia* essential oil and is, therefore, a monoterpene with beneficial antioxidant and antimicrobial properties. It has been evidenced that 1,8-Cineole, as an antioxidant, inhibits the peroxidation of lipids and is a source to neutralize the oxidative stress caused by the free radicals (Slamenova & Horvathova, 2013). Still, not only does it protect the cellular tubulin, but, also, 1,8-cineole acts in the DNA molecule thus attributing to the stability of the DNA and reduces the power of the inflammatory process (Mohammed et al., 2023). This element is also used in food preservation processes as a natural antioxidant which eliminates the necessity of the incorporation of synthetic antioxidants (Chrysargyris et al., 2020).
Virdifluorol, however, is a bioactive sesquiterpene with a mechanism to inhibit lipid oxidation by neutralizing free radicals. Studies have revealed that the essential oils containing viridiflorol represented remarkable DPPH radical scavenging activity and this compound presented a high antioxidant capacity which is known to be critical in shielding against oxidative stress (Mohammed et al., 2023). In addition, Viridifluorol has also been reported to stabilise biological systems by protecting cell membranes against oxidative stress (Macêdo et al., 2022). The use of essential oils containing viridifluorol is growing in the food industry, especially in fat-containing food products with long shelf life (Cutillas et al., 2017).

Another important component α-Terpineol is known for its strong antioxidant and anti-inflammatory properties. It has been shown that α-Terpineol reduces oxidative stress levels by reducing reactive oxygen species (ROS) and exhibits cell protective effects (Rodríguez et al., 2013). However, studies have shown that α-Terpineol inhibits lipid peroxidation and slows down oxidative deterioration in fat-based foods (Singh et al., 2009). Furthermore, α-Terpineol has been reported to reduce oxidative stress-induced inflammation by acting on anti-inflammatory mechanisms (Farhat & Jordán, 2009). Due to these properties, α-Terpineol is an important component that can be used as a natural antioxidant in the food industry.

Melaleuca quinquenervia essential oil exhibits a strong antioxidant activity thanks to the synergistic effect of these three components and provides a potential contribution to extend the shelf life of food products by preventing oxidative deterioration. As an alternative to synthetic antioxidants, the advantages of using these compounds obtained from natural sources, especially in oxidation-sensitive lipid-based foods such as fish oil, are becoming increasingly important.

In conclusion, the use of *Melaleuca quinquenervia* essential oil 200 ppm and above provided as effective protection as the commercially used synthetic antioxidant and the maximum application dose to suppress the oxidation process of fish oil accelerated by temperature, humidity and light.

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