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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2024; 10 (3): 201-210 Araştırma Makalesi/Research Article

Portakal Kabuğu Uçucu Yağının Sürdürülebilir Kullanımı: Balık Yağı Oksidasyonuyla Mücadelede Doğal Bir Antioksidan

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- Lipid Peroksidasyonu
- Sürdürülebilir Antioksidanlar

Öz

Bu çalışma, portakal kabuğu esansiyel yağının (OEo) balık yağı oksidasyonuna karşı koruyucu etkilerini ve potansiyel bir antioksidan olarak kullanımını araştırmaktadır. Çalışmada, balık yağına farklı oranlarda OEo (100 ppm-1600 ppm) ilave edilmiş ve oksidasyona karşı koruyucu etkileri, peroksit değeri (PV) ve malondialdehit (MDA) oluşumu üzerinden değerlendirilmiştir. Hızlandırılmış oksidasyon testi, 55°C sıcaklık, %70 nem ve sürekli ışık koşullarında 120 saat boyunca uygulanmıştır. Deney grupları arasındaki istatistiksel farklar da analiz edilmiştir. Bulgular, OEo ile desteklenmiş balık yağlarında PV ve MDA seviyelerinin kontrol grubuna kıyasla anlamlı derecede azaldığını göstermektedir ($p<0.05$). Özellikle 400 ppm OEo içeren grup, en düşük PV değerini (20 meq O2/kg) göstermiş olup bu değer, taze balık yağına yakın bulunmuştur. İstatistiksel analizler, OEo'nun oksidasyon engelleyici etkisinin doz bağımlı olduğunu, OEo16 grubunda serbest yağ asitleri (FFA) oranının kontrol grubuna göre azaldığını ortaya koymaktadır. Elde edilen bulgular, OEo'nun sentetik antioksidanlara doğal ve çevre dostu bir alternatif olarak kullanılabileceğini ve sürdürülebilir gıda üretiminde katkı sağlayabileceğini göstermektedir. Sonuç olarak, OEo'nun farklı konsantrasyonlarda balık yağı oksidasyonunu engelleme kapasitesi istatistiksel olarak doğrulanmış, 400 ppm OEo'nun oksidatif bozulmaya karşı en etkili korumayı sağladığı tespit edilmiştir.

Sustainable Use of Orange Peel Essential Oil: A Natural Antioxidant to Combat Fish Oil Oxidation

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- Orange Peel Essential Oil
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- Lipid Peroxidation
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Abstract

This study examines the protective effects of orange peel essential oil (OEo) against the oxidation of fish oil and its prospective application as an antioxidant. The investigation involved the addition of varying ratios of OEo (100 ppm-1600 ppm) to fish oil, and the protective effects against oxidation were assessed using measurements of peroxide value (PV) and malondialdehyde (MDA) generation. The accelerated oxidation test was conducted at a temperature of 55°C, 70% humidity, and continuous light for a duration of 120 hours. Furthermore, statistical disparities among the experimental groups were examined. The results indicated considerably reduced levels of PV and MDA ($p<0.05$) in fish oils supplemented with OEo compared to the control group. The group with 400 ppm OEo exhibited the lowest PV value (20 meq O2/kg), which was determined to be nearly equivalent to that of fresh fish oil. Statistical studies indicated that the oxidation-suppressing impact of OEo was dose-dependent, and the proportion of free fatty acids (FFA) was reduced in the OEo16 group relative to the control group. The findings indicate that OEo serves as a natural and eco-friendly substitute for synthetic antioxidants, potentially enhancing sustainable food production. In conclusion, statistical analyses validated the capacity of OEo to impede oxidation in fish oil at various concentrations, revealing that 400 ppm OEo was the most efficacious concentration, offering substantial protection against oxidative deterioration.

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INTRODUCTION

The orange occupies a pivotal role in global citrus production owing to its nutritional and health benefits, in addition to being the most favored variety in the juice sector (Jiménez-Castro et al., 2020). The orange peel comprises oil sacs and glands measuring between 0.4 and 0.6 mm in diameter. The sacs and glands are asymmetrically distributed in the flavedo, the outermost layer of the orange peel, and house essential oils (Sharifi et al., 2007). Approximately 5,436 kg of oil is extracted from every 1,000 kg of oranges, with 90% of this oil consisting of D-limonene. D-limonene is a hydrocarbon categorized as a cyclic terpene and has antioxidant characteristics (El-Ishaq et al., 2011; Ferrer et al., 2022). Consequently, it is important to extract the essential oils from orange peel in an effective and environmentally sustainable way. Essential oil extraction is conducted by hydro and steam distillation processes, both of which are devoid of solvents. This closed system model is not only a safer and more environmentally friendly method for the ecosystem due to the use of water vapor instead of chemical solvents, but it is also a sustainable method in terms of the efficient use of natural resources thanks to the water cycle within the system (Bozova et al., 2024; Felicia et al., 2024).

Fish oil is rich in PUFA, particularly alpha-linolenic acid (ALA), which the human body cannot produce and must acquire from dietary sources. Fish oil is also rich in eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA) (Rizliya & Mendis, 2013; Lembke & Schubert, 2014). Omega-3 PUFAs, characterized by five or more double bonds and classified as PUFAs, are referred to as highly unsaturated fatty acids (HUFA). The primary source of HUFAs ingested by humans is fish (Velasco et al., 2004; Pike & Jackson, 2010). The fatty acid composition of fish is strongly correlated with their feed ration, resulting in a high HUFA concentration in farmed fish that are fed diets rich in w-3 fatty acids (Taşbozan & Gökçe, 2017). Nonetheless, fish feed requires a greater quantity of PUFAs compared to human feed. This is due to the varied essential fatty acid requirements of fish. Consequently, fish oil serves as the primary lipid source in aquaculture feeds, and its incorporation into the diet enhances feed efficiency, quality, and growth rate in fish (Nasopoulou & Zabetakis, 2012; Oliva-Teles et al., 2022).

The presence of double bonds in polyunsaturated fatty acids renders unsaturated lipids susceptible to oxidation. Oxidation is a multifaceted reaction that typically commences with the generation of free radicals (Frankel, 1984; Min & Boff, 2002). Environmental factors like heat, light, metal ions, and humidity serve as effective initiators and accelerators in the oxidation of lipids. In the presence of these initiators, omega-3 fatty acids may relinquish a hydrogen radical, resulting in the formation of a lipid free radical. These free radicals interact with oxygen to produce hydroperoxides. Hydroperoxides subsequently result in the synthesis of substances including aldehydes, ketones, and short-chain carboxylic acids, which are prone to decomposing into undesirable small molecules (Kazuo, 2019; Lembke & Schubert, 2014). During the oxidation of fats, there is a preliminary exponential rise in the concentration of lipid peroxides. As deterioration progresses, the concentration of lipid peroxides diminishes but the concentration of potentially deleterious secondary oxidation products escalates (Albert et al., 2013). Consequently, the quantification of lipid peroxide species and secondary oxidation products through techniques such as gas chromatography-mass spectrometry (GC-MS), peroxide value (PV), and MDA assay is of paramount importance (Packer, 1999; Arneson & Roberts, 2007).

The consumption of lipid peroxides and secondary oxidation products hastens the oxidation of more fatty acids in living organisms, resulting in a chain process that produces more lipid peroxides. This is thought to end up in membrane peroxidation, cellular damage, and oxidative stress, which are recognized as pathogenic mechanisms in living organisms (Shahidi & Zhong, 2010). Consequently, unoxidized oils ought to be ingested as dietary supplements, and the oxidation of oils should be mitigated through the use of antioxidants. Various synthetic antioxidants can be used to protect fish oil against oxidation, but they are not as safe as natural antioxidants. Chain-breaking antioxidants such as BHT, which was used as a reference substance in this study, may be preferable. The utilization of orange peel essential oil as an antioxidant is significant for environmental conservation by reducing orange peel waste and promoting sustainable food production (Da Tan et al., 2023; Lucky et al., 2023).

The primary aim of this study was to clarify the oxidative mechanisms contributing to the expedited oxidation of fish oil with varying concentrations of orange peel essential oil under conditions of temperature, humidity, and continuous light, as well as to explore the feasibility of utilizing orange peel essential oil as an antioxidant sustainably.

MATERIAL AND METHOD

Extraction of Essential Oil from Orange Peel

The Clevenger apparatus, utilizing hydro distillation, was employed for the extraction of essential oils from orange peel. Fresh oranges were peeled, and the peels were diced into small parts. Orange peels were placed in a 1000 mL round-bottom flask, to which 300 mL of distilled water was added for every 100 g of peels, and the total volume was adjusted to two-thirds. The heater was maintained at boiling temperature, and during the 8.0±1.0 hour boiling period of the flask, the condenser was integrated into the system with a continuous water flow ensured. The essential oil molecules, transported by the water vapor, were condensed in the chiller and subsequently separated from the water based on their density within the Clevenger collector (Adams, 1997).

Analysis of Volatile Compounds in Orange Peel

The constituents of the essential oil were identified using GC-MS (Shimadzu GC-MS QP 2010 Ultra, Kyoto, Japan). The oil sample was diluted with n-hexane, and 1 µl of the sample was placed into the RXI-5MS injection port. Helium with a purity of 99.99% was utilized as the carrier gas at a flow rate of 1 mL/min. The column temperature was originally set at 50 °C for 5 minutes, thereafter, increased by 5 °C every minute until reaching 270 °C, and then held at 270 °C for an additional 5 minutes. The components, separated from the column based on their boiling points after a steady temperature increase, were introduced into the mass spectrometer for ionization and fragmentation. The ions were segregated based on their mass-to-charge ratios, recorded by the detector, and the resultant peaks were compared with the spectra in the Wiley Data Library (Wiley W9N11) to identify the compounds (Kesbiç & Gültepe, 2023).

Assessment of the Antioxidant Efficacy of Orange Essential Oil

To assess the scavenging action of orange peel essential oil constituents on 2,2-difenil-1-pikrilhidrazil (DPPH) radicals, a 0.06 mM DPPH stock solution was prepared using 100 ml of methanol. From the stock solution, 10 ml was taken out and diluted to 100 ml with methanol, resulting in the desired DPPH concentration for analysis. A mixture was made with a total volume of 250 µl, including a sample volume (µl), 50 µl of DPPH solution, and ethanol, depending upon the concentration. The 96-well plate was loaded with 250 µL per well for eight duplicates. The absorbance of the wells in the 96-well plates was measured at 515 nm using a UV-VIS spectrophotometer (Epoch 2 Microplate Spectrophotometer, BioTek, USA) at 0 and 30 minutes.

Oxidation Experiments Set Up

Commercial feed grade fish oil was used in experiment. Various amounts of extracted orange peel essential oil (OEo1 (100 ppm); OEo2 (200 ppm); OEo4 (400 ppm); OEo8 (800 ppm); OEo16 (1600 ppm)) were incorporated into fish oil to designed distinct experimental groups aimed at examining its protective impact against heat oxidation of fish oil. Butylated hydroxytoluene (BHT200) was employed as a positive control at a dosage of 200 mg/kg. The experimental groups were subjected to treatment for 120 hours in temperature-resistant containers under ambient conditions of 55.0±0.5 °C temperature, 70.0±0.5% humidity, and constant illumination (7000 lux).

Determination of Peroxide Value

The peroxide value determination method (Cd 8b-90) established by the American Oil Chemists' Society (AOCS) was employed to assess the protective efficacy of OEo against oxidation under accelerated conditions (Firestone 1989). Unoxidized fish oil and 0.5 g samples from oxidized experimental groups were dissolved in 5 mL of chloroform. The dissolved samples were incubated with 15 mL of acetic acid and 1 mL of saturated potassium iodide for 10 minutes at room temperature in the absence of light. Following incubation, titration was performed using 0.01 N sodium thiosulfate and few drops of 1% starch in 75 mL of deionized water as the indicator. The peroxide value was ascertained utilizing the subsequent formula, predicated on the color shift that signifies the outcome of the titration. The peroxide value assay results were evaluated in triplicate as meq O2/kg oil.

Quantification of Malondialdehyde

The quantity of 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 of fish oil (Ohkawa et al., 1979).

Determination of Lipid Profile Oxidated Fish Oils

After 120 h of rapid oxidation, 5 µL of the oils were loaded 1.5 cm from the bottom edge of a thin layer chromatography TLC plate (Silica gel 60, MERCK) using a micropipette. The plate was immersed at the bottom edge of the TLC tank containing a 1 cm high solvent mixture (n-hexane:diethylether:acetic acid (70:30:1 v/v)). The lipid classes were separated from each other by continuing the chromatographic development until at least 10 cm away from the application point (Fig. 1.). After the separation process was completed, the density values (%) of the free fatty acid (FFA) classes on the TLC plates were calculated according to Kaynar et al. (2013).

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Statistical analyses were conducted with IBM SPSS Statistics software. A one-way ANOVA was conducted to ascertain the statistical significance of differences across the sample groups, followed by group comparisons using Tukey's Honest Significant Difference test. In all analyses, $p<0.05$ was accepted as the significance level.

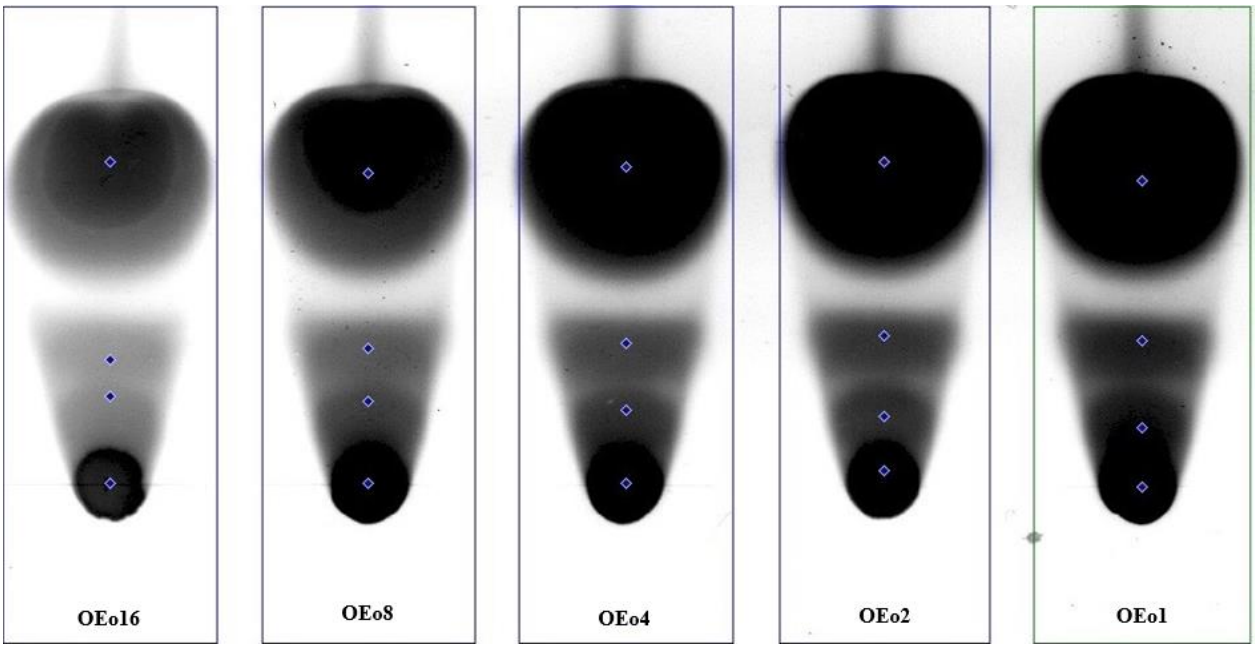

Figure 1. Lipid profiles of experimental fish oil after oxidation process.

RESULTS

Volatile profile of OEo was showed in Table 1. The results of the quantitative analysis shown that the major component of EO was D-Limonene (92.94%) in comparison to the W9N11 library.

Table 1. Volatile profile of orange peel essential oil

S.N	Compound	Retention Time (min.)	%
1	Myrcene	11.915	1.88
2	D-Limonene	13.383	92.94
3	LINALOOL	16.364	1.21
4	Capraldehyde	20.346	1.14
5	Others	-	2.84
6	Total		100

The standard curve presented in Fig. 2 was used to determine the antioxidant performance of OEo. The R^2 of the standard curve is 0.96 and according to curve equation, OEo IC_{50} concentration was calculated as 151.83 ppm.

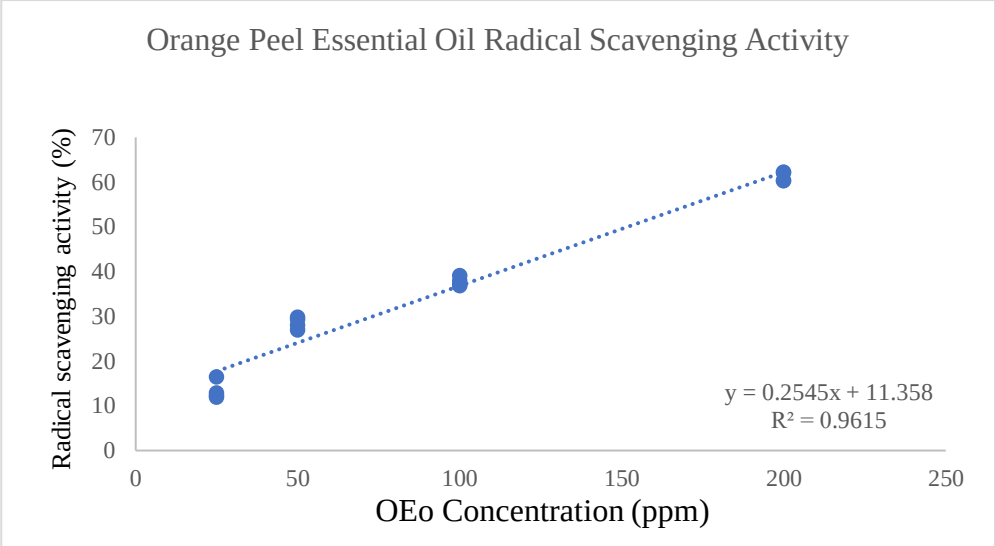

Figure 2. Radical scavenging activity of orange peel essential oil

After rapid oxidation treatment, the PV values of fish oils with varying ratios of OEo addition, as well as those without addition and subjected to thermal oxidation (fresh fish oil), were measured, as shown in Fig. 3. The markedly lowest PV value was seen in the fresh fish oil and the highest one is no OEo supplemented group (control) ($p < 0.05$). At the conclusion of the rapid oxidation procedure, which included continuous illumination for 120 hours at a constant temperature of 55.0°C and 70% humidity, it was revealed that the formation of PV should be restricted by the addition of OEo. Significant variations in PV values were observed in the experimental groups ($p < 0.05$), with the lowest PV values recorded in fish oils supplemented with 400 ppm OEo ($p < 0.05$).

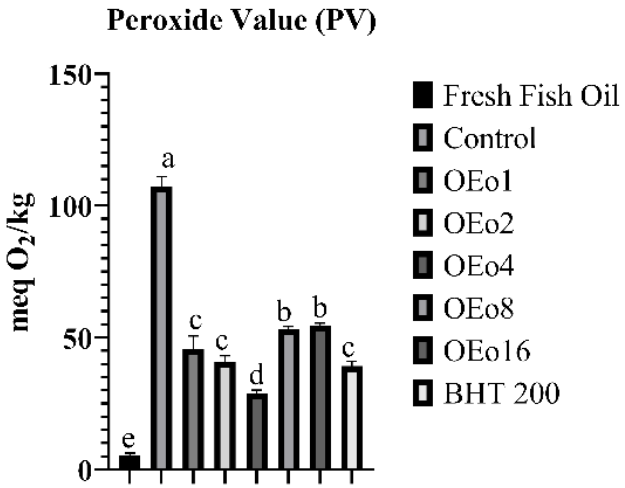

Figure 3. Effect of OEo addition at different ratios to fish oils on peroxide formation (n = 3). Values with different letters indicate significant differences between their group ($p < 0.05$).

Fig. 4 demonstrates that rapid oxidation treatment markedly elevated the MDA level ($p<0.05$). The MDA level was elevated in the control and BHT 200 groups, but it was diminished in the OEo4 and fresh fish oil groups in comparison ($p<0.05$). The MDA levels in the OEo16 and OEo8 groups were comparable to those in the fresh fish oil group ($p>0.05$). The OEo16 group was elevated compared to the OEo1 and OEo4 groups, although it was considerably inferior to the BHT 200 and control groups ($p<0.05$).

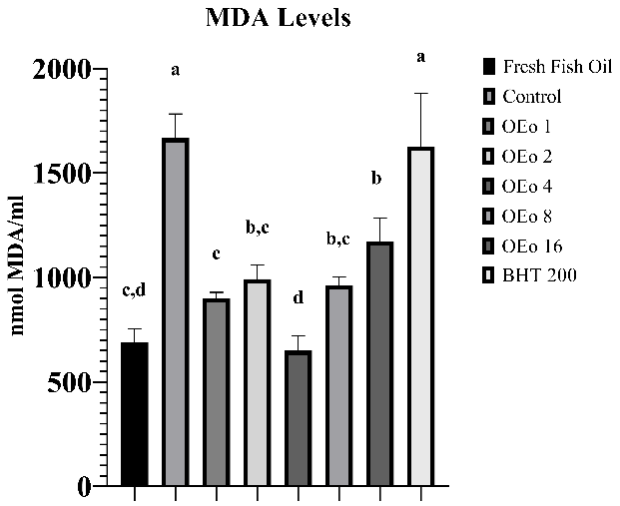

Figure 4. Malondialdehyde formation of experimental groups after rapid oxidation process.

After the rapid oxidation study, the proportional distributions of free fatty acids of the groups were shown in Fig. 5.

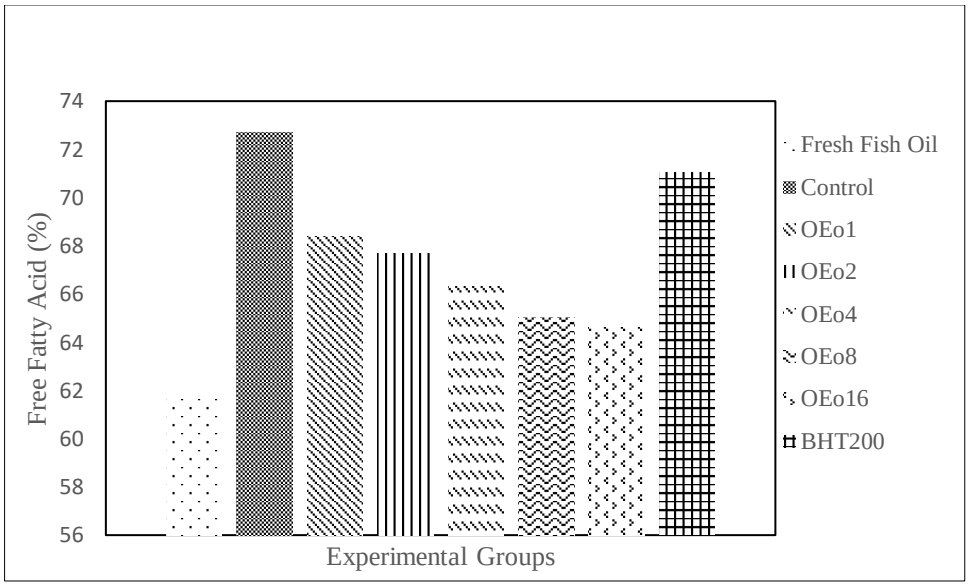

Figure 5. Free fatty acid percentage of experimental groups after rapid oxidation.

DISCUSSION

Zero-waste programs are widely acknowledged as essential elements of sustainability in industrial operations. Although organic waste from food processing is often seen as household trash and manageable, its disposal may become expensive and ecologically detrimental when generated in substantial volumes (Sharma et al., 2019). Orange peel, a byproduct of the orange juice sector, is an organic waste material (Cypriano et al., 2018). Owing to its elevated moisture content, low pH, and the presence of essential oils possessing recognized antioxidant and antibacterial qualities, it is inappropriate for fuel use and cannot be readily composted or immediately discarded in soil or water (Suzuki et al., 2033; Knudsen et al., 2011). Moreover, its strong aromatic components emit unpleasant scents in goods such as meat, milk, and eggs, restricting its incorporation into animal feed (Andrianou et al., 2023).

Orange essential oil (OEO), extracted from orange peel by hydro-distillation or steam distillation, is a volatile chemical combination. Prior research has shown that the use of OEO in animal feed may enhance shelf life (Kanat and Kesbiç 2024) and augment growth and health metrics in fish (Acar et al., 2015). Given that OEO has been shown to be non-toxic to fish in vitro, its impact on fish feed and fish oil (FO) has garnered considerable attention. Fish oil, a major source of PUFAs, is particularly prone to oxidation, which might diminish its quality. Besides its nutritional benefits, fish oil acts as an attractant in aquaculture feeds, improving palatability and augmenting feed consumption (Taecon 2004). Consequently, antioxidants are often included into fish oil diets to inhibit oxidation (O'Sullivan et al., 2005).

Antioxidants are generally classified as either natural or synthetic. Although synthetic antioxidants are often used in industrial applications, new research indicates that they may have specific health hazards (Wang et al., 2021). As a result, regulatory bodies have imposed restrictions or prohibitions on many synthetic antioxidants, hence increasing the need for natural substitutes. This transition has forced researchers to investigate natural substances with antioxidant capabilities (Alnahdi et al., 2011; Alu'datt et al., 2013). A prevalent technique for evaluating antioxidant activity involves measuring radical scavenging activity (RSA), which assesses an antioxidant's capacity to destroy free radicals (Gulcin., 2020). Numerous essential oils have shown RSA action (Saleh et al., 2010). The IC50 value of OEo for RSA was determined to be 151.83 ppm in the current investigation. In contrast, other citrus essential oils, including lemon, bergamot, and grapefruit, have documented IC50 values of 150.56 ppm (Ben Hsouna et al., 2017), 239.0 ppm (Sicari et al., 2016), and 220.60 ppm (Deng et al., 2020), respectively. Other investigations indicate that orange peel essential oil has IC50 values 150 ppm (Huynh et al., 2022), 235 ppm (İnan et al., 2028) and the previous study reported orange peel essential oil has no antioxidant activity (Magalhães et al., 2020). Discrepancies in RSA values across studies are likely attributable to variations in the bioactive chemicals contained in each oil and their respective amounts, potentially resulting in antagonistic or synergistic effects.

In the OEO used for this study, the predominant volatile component found was D-limonene, comprising 92.94% of the total volatile content. Furthermore, the oil was shown to have several other volatile chemicals. D-limonene is generally identified as the predominant component in OEO, however the exact composition of essential oils may fluctuate based on variables such as extraction technique and source material. Multiple elements, including as oxygen exposure, light intensity, and temperature, are recognized to expedite lipid oxidation (Gordon, 2020). Under experimental circumstances, these factors are often modified to replicate rapid oxidation. This research conducted oxidation experiments under continuous light exposure at 7000 lux, with relative humidity at 70% at a temperature of 55°C. Fish oil samples with differing concentrations of OEo addition were exposed to these settings to promote oxidation.

Lipid oxidation generates both primary and secondary oxidation products, with peroxide value (PV) serving as the predominant indication of primary oxidation (Gray 1978). This investigation revealed the PV of fresh fish oil to be 20 meq O2/kg, which is within acceptable limits for fish feed applications (Korkut et al., 2007). Subsequent to the oxidation test, it was noted that OEo supplementation inhibited peroxide generation in the oil. These results correspond with prior studies indicating that essential oils help mitigate lipid oxidation. In research using celery essential oil, fish oil samples were subjected to heating at 70°C for 24 hours, revealing that the peroxide value of samples containing essential oil was much lower than that of those devoid of it (Kesbic 2023). It is crucial to recognize that whereas essential oils might impede PV production at moderate supplementation levels, excessive quantities may exhibit pro-oxidant effects, as seen in the present research. This transpires when antioxidants engage with oxygen, facilitating the creation of peroxides under certain circumstances.

MDA is an aldehyde formed through the oxidation process of lipids and serves as a marker for lipid peroxidation. Oxidation transpires when polyunsaturated fatty acids interact with free radicals, resulting in reduced quality and pronounced oxidation of lipids. MDA is a significant by-product of this procedure and is extensively utilized to evaluate the oxidation level of oils (Gray 1978). The present study examined the effects of orange essential oil on MDA generation in crude fish oil subjected to oxidative stress through continuous light, humidity, and heating. The use of orange essential oil in varying quantities markedly reduced MDA production and demonstrated that the oil's antioxidant qualities effectively mitigated the oxidative degradation of fish oil. Fish oils supplemented with carnosic acid (CA), an established antioxidant, were stored at 30 and 4 degrees Celsius, resulting in increased MDA levels over time and temperature. The addition of CA to the oils significantly suppressed MDA formation (Wang et al., 2011). A study investigated the shelf life of fish oil supplemented with curcumin, the active component of turmeric recognized for its antioxidant properties. The fish oil was stored at temperatures of 14 and 25 degrees Celsius for a duration of 70 days. After 70 days, it was noted that the MDA levels in the curcumin-treated groups were lower under both temperature conditions compared to the control group (Huang et al., 2017). The current study yielded similar findings. Fish oils with varying ratios of OEo exhibited reduced MDA levels relative to the control group following a rapid oxidation process. Free fatty acids (FFA) serve as a significant marker of the oxidation process and signify the quality degradation of oils rich in polyunsaturated fatty acids, such as fish oil. In our study, FFA levels were dramatically reduced in the groups supplemented with orange essential oil (OEo). The OEo16 group exhibited the lowest FFA value (64.63), signifying that orange essential oil efficiently mitigates oxidative degradation. Numerous studies (Huang et al., 2017; Wang et al., 2011) in the literature substantiate the capacity of antioxidants to mitigate oxidative degradation and it showed that the decreasing the FFA formation. Moreover, studies on the effectiveness of natural substances in inhibiting lipid oxidation indicate that OEo is comparable to other antioxidants (Barrett et al., 2011). These findings underscore the capacity of orange essential oil to safeguard against FFA generation and enhance overall oil quality.

CONCLUSION

As a conclusion, it was determined that the use of OEo in fish oil limits fish oil oxidation and 400 ppm fish oil additive protects fish oil against oxidation by preventing PV and MDA formation.

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COMPLIANCE WITH ETHICAL STANDARDS

a) Authors' Contributions

1. HSE: Conducted the laboratory work, interpreted the data and prepared the manuscript.

2. HM: Conducted the lab work and prepared the manuscript.

3. ÖK: Conducted the laboratory study and interpreted the data.

4. ÜA: Interpreted the data and prepared the manuscript.

5. OSK: Conducted the laboratory work, interpreted the data and prepared the manuscript.

b) Conflict of Interest

The authors declare that there is no conflict of interest.

c) Declaration on Animal Welfare

This study does not involve animal testing.

d) Declaration of Human Rights

This study does not include human participants.

e) Destekleyen Kurum

Kastamonu Üniversitesi Bilimsel Araştırma Projeleri. Proje numarası: KÜBAP-01/2023/8

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