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Protective Effect of Celeriac (*Apium graveolens*) Leaf Essential Oil on Temperature and Oxygen-Induced Fish Oil Oxidation

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Abstract: The purpose of this work was to identify the volatile components of essential oil extracted from Celeriac (*Apium graveolens*) leaves (CEO) and assess its antioxidant performance during the thermal oxidation of fish oil. Steam distillation method and Clevenger apparatus was used to extract of CEO from fresh leafs. The volatile component analysis revealed that 98.81% of the volatile components in the resulting product could be recognized. Following examination, the principal components of the product were discovered to be Phthalide (3-isobutylidene) and Fenipentol with a concentration of 49.42% and 28.45% respectively. The product's antioxidant activity was tested using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) study. The 50% inhibitory concentration value (IC50) for CEO was discovered to be 30.52 ppm by the study. To test the product's ability to protect fish oil from oxidation, CEO ratios of 0% (CEO0), 0.1% (CEO0.1), 0.5% (CEO0.5), 1% (CEO1), and 3% (CEO3) were added to fish oil, and the experimental groups were subjected to 24 hours of oxidation at 70 °C with continuous ventilation. According to the oxidation investigation, the addition of CEO suppressed fish oil oxidation and significantly reduced the product's oxidation radicals ($p<0.05$) depending on the CEO concentration. According to the study's results, the group with 3% CEO had the lowest oxidation of fish oil caused by temperature and oxygen contamination.

Keywords: *Apium graveolens*, Essential oil, Volatile compounds, Natural antioxidant

Öz: Bu çalışmanın amacı, Kereviz (*Apium graveolens*) yapraklarından (CEO) elde edilen uçucu yağın uçucu bileşenlerini tanımlamak ve balık yağının termal oksidasyonu sırasında antioksidan performansını değerlendirmektir. Taze yağraklardan CEO saflaştırılması için buhardistilasyonu metodu ve Clavenger düzeneği kullanılmıştır. Uçucu bileşen analizi, elde edilen üründeki uçucu bileşenlerin %98,81'inin tanınabildiğini ortaya koymuştur. İncelemenin ardından, ürünün ana bileşenlerinin sırasıyla %49,42 ve %28,45 konsantrasyonla Ftalit (3-izobütiliden) ve Fenipentol olduğu tespit edilmiştir. Ürünün antioksidan aktivitesi 2,2-difenil-1-pikrilhidrazil (DPPH) çalışması kullanılarak test edilmiştir. Çalışmada CEO için %50 inhibitör konsantrasyon değeri (IC50) 30.52 ppm olarak bulunmuştur. Ürünün balık yağını oksidasyondan koruma kabiliyetini test etmek için balık yağına %0 (CEO0), %0,1 (CEO0,1), %0,5 (CEO0,5), %1 (CEO1) ve %3 (CEO3) oranlarında CEO eklenmiş ve deney grupları sürekli havalandırma ile 70 °C'de 24 saat oksidasyona tabi tutulmuştur. Oksidasyon araştırmasına göre, CEO ilavesi balık yağı oksidasyonunu baskılamış ve CEO konsantrasyonuna bağlı olarak ürünün oksidasyon radikallerini önemli ölçüde azaltmıştır ($p<0.05$). Çalışmanın sonuçlarına göre, %3 CEO içeren grup, sıcaklık ve oksijen kontaminasyonunun neden olduğu en düşük balık yağı oksidasyonuna sahipti.

Anahtar Kelimeler: *Apium graveolens*, Esansiyel yağ, Uçucu bileşen, Doğal antioksidan

1. Introduction

The exponential growth of the global population is progressively diminishing the probability of individuals accessing high-quality sustenance on a daily basis. Aquaculture, particularly the practice of fish farming, is widely regarded as a highly advantageous alternative due to its potential to offer a resolution to this issue [1]. According to data from 2017, the total fish production amounted to 179 million tons, of which approximately 88% was allocated for human consumption, while the remaining 12% was utilized for non-food purposes. The utilization of fish processing by-products and/or surplus catch, which is estimated to account for 25-35% of the overall fish proportion, is reportedly directed toward the production of fishmeal and fish oil [2]. Fishmeal and fish oil are widely recognized as highly nutritious and easily digestible fundamental sources of protein and lipids in the context of aquaculture [3]. The utilization proportions of fishmeal and fish oil in feed formulations for aquaculture are exhibiting a distinct declining pattern, as per the Food and Agriculture Organization's report of 2022 [4]. The utilization of nutritious and superior feed in fish nutrition is crucial for enhancing the quality of products and promoting sustainable development in the aquaculture sector. The quality of fish feed has a direct correlation with the growth, reproduction, and meat quality of fish. Therefore, in order to ensure sustainable aquaculture, it is imperative to reduce the amount of fish meal and fish oil added to fish feeds and to improve their quality [5, 6].

Fish oil is a secondary product that is derived from the pressing procedure of fish meals. It is obtained through the process of separating and purifying fish and its particles from the press liquid. According to Korkut, et al. [7], the oil content of fish is influenced by various factors such as their feeding habits, species, seawater temperature, and geographical location where they are caught. Fish oil serves as the primary origin of polyunsaturated fatty acids Omega-3 (ω-3) and Omega-6 (ω-6). The presence of polyunsaturated fatty acids (PUFA) in fish oil is known to render it susceptible to swift oxidation, thereby exerting a detrimental impact on its quality during the storage phase. Consumption of such oxidized fish oil can lead to severe health complications [8]. Fish oils are prone to oxidation due to their high content of polyunsaturated fatty acids (PUFA). The formation of unpleasant odor and quality deterioration significantly diminishes the quality of the product, and in advanced stages, can cause a conversion of fish oil from a nutritious substance to a hazardous one [9]. In addition, the existence of specific pigments namely myoglobin and hemoglobin, alongside minute quantities of metallic ions such as iron and copper, renders fish oil more vulnerable to oil oxidation as per the findings of Hsieh and Kinsella [10]. The process of oxidation has the potential to result in adverse consequences, including but not limited to the impairment of vitamins, alteration of color, and depletion of crucial fatty acids. These outcomes can have a detrimental impact on sensory perception and lead to a reduction in nutritional value [9]. The shelf life of fish oil and fish oil-enriched products is notably diminished by the process of oxidation. The presence of oxygen has been observed to cause oxidation of polyunsaturated fatty acids (PUFA) in fish oil. Environmental factors such as enzymes, light, metal ions, and temperature have the potential to induce degradation. The oxidation process causes the breakdown of hydroperoxides, which in turn leads to the loss of nutrients, sourness, and undesirable flavors [11, 12].

Apium graveolens, commonly known as celeriac, is an herbaceous plant that has gained recognition for its culinary and medicinal properties. The leaves of celeriac have been traditionally used in various cuisines and folk medicine. Recent studies have revealed that celeriac leaf essential oil (CEO) possesses a rich composition of volatile compounds with potential antioxidant properties, making it a promising natural antioxidant for lipid protection. The lipid protective activity of CEO can be attributed to its bioactive constituents, including phthalides, phenolic compounds, monoterpenes, and sesquiterpenes. These compounds exhibit diverse mechanisms of action that contribute to their antioxidant efficacy in lipid systems. Phthalides, such as 3-n-butylphthalide, have been reported to effectively inhibit lipid oxidation by scavenging lipid-derived free radicals and chelating pro-oxidative transition metal ions [13]. Phenolic compounds, another important class of constituents in CEO, act as potent antioxidants by donating hydrogen atoms or electrons to neutralize lipid radicals and break the free radical chain reactions. Furthermore, the monoterpenes and sesquiterpenes found in CEO, such as limonene, α-pinene, and β-caryophyllene, contribute to its lipid protective activity through their radical scavenging and metal chelation abilities [14]. These volatile compounds possess lipophilic properties that enable them to effectively interact with lipid substrates, thereby exerting their antioxidant effects directly within the lipid matrix. Moreover, the underlying mechanisms by which CEO protects lipids can be investigated by assessing its impact on key oxidative markers, including reactive oxygen species (ROS) generation, lipid hydroperoxides, and antioxidant enzyme activities. Understanding these mechanisms will provide valuable insights into the mode of action of CEO in lipid protection and contribute to the development of optimized antioxidant strategies [15].

The employment of antioxidants for the purpose of inhibiting oxidation in fish oil and fish oil-fortified commodities is a prevalent technique in the food sector, aimed at prolonging their period of preservation. Nevertheless, studies have indicated that the utilization of artificial antioxidants may result in enduring issues and buildup within the human body. Consequently, an increasing inclination is observed toward the creation of natural substances possessing antioxidant characteristics. Research has indicated that Celeriac (*Apium graveolens*), a plant that is utilized for both culinary and medicinal purposes, displays robust antioxidant characteristics. The primary aim of this research is to explore the potential application of Celeriac Leaf Essential Oil (CEO) in preserving the oxidative stability of fish oil, a crucial lipid source in the aquaculture feed sector.

2. Material and Method

Preparation of Celery Leaves and Essential Oil Extraction

Celeriac leaves to be used in the study were purchased from Kastamonu local market. The leaves were washed with tap water and dried with a clean paper towel before the oil extraction process. After that, the leaves were cut into small pieces with laboratory scissors and placed in a round bottom flask with distilled water (100g 300 mL^{-1}) [16] and subjected to hydrodistillation with Clevenger apparatus. The upper phase of the distillate accumulated in the collector was taken into amber glass vials. Afterward, the collected essential oil was filled with nitrogen gas and stored at -20°C to be used in the analysis and study.

Determination of Volatile Compound of Celeriac Leaves Essential Oil

The essential oil analysis of volatile components was conducted using GC/MS (Shimadzu GCMS QP 2010 ULTRA). In the course of the analysis, a capillary column of the RTX-5MS brand measuring 30 meters in length, 0.25 millimeters in diameter, and 0.25 micrometers in particle size was employed, with helium serving as the carrier gas. The experimental setup involved setting the column oven temperature 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 utilized in the experiment was 1µL, and the split (1/5) method was employed for injection. The analysis involved a temperature regimen that consisted of heating the sample to 40°C for 3 minutes, followed by a gradual increase from 40°C to 240°C at a rate of 4°C per minute, holding the temperature at 240°C for 10 minutes, and then increasing the temperature from 240°C to 260°C at a rate of 4°C per minute, holding the temperature at 260°C for 10 minutes. The entire oven program lasted for a total of 78 minutes. The determined peaks were subjected to comparison with the W9N11 library, resulting in the identification of volatile compounds [17].

Evaluation of Essential Oil's Antioxidant Activity

The combination of 0.2 mL essential oil, 0.5 mL DPPH solution, and 4 mL 80% ethanol was combined in a 15" vortex and left at room temperature for 15 minutes to assess the scavenging action of the produced essential oil on DPPH radical. The combination was read in a UV-VIS spectrophotometer at 517 nm at the conclusion of the experiment. Absorbances were recorded, and values for the percentage scavenging effect were calculated using the equation below [18]. The same process was done using antioxidants derived from industrial Vitamin-C and antioxidant activity was compared in this manner.

% scavenging activity= [1- (AS/A0)] x100.

The study of Thermal Oxidation

The aim of the study was to investigate the potential protective effect of celery leaf essential oil against the thermal oxidation of fish oil. The experimental design involved the addition of the essential oil to the fish oil at varying ratios of 0% (CEO0), 0.1% (CEO0.1), 0.5% (CEO0.5), 1% (CEO1), and 3% (CEO3), resulting in the formation of distinct experimental groups. The experimental groups were subjected to thermal oxidation at a constant temperature of 70±0.5 °C for a duration of 24 hours while being placed in heat-resistant bottles and provided with continuous ventilation.

Determination of Peroxide Value

The peroxide determination method (Cd 8b-90) published by the American Oil Chemistry Society AOCS [19] was used to determine the effect of thermal oxidation and the preservation of celery leaf essential oil. For this purpose, fish oil without thermal oxidation (Control) and samples taken from the experimental groups were dissolved with g 5 mL^{-1} chloroform, 15 mL acetic acid and 1 mL saturated potassium iodide were added and kept in the dark for 10 min at room temperature. After waiting, it was subjected to titration with 75 mL deionized water and 0.01 N adjusted sodium thiosulfate in the presence of a few drops of starch (1%) indicator. The amount of consumption obtained at the endpoint of the titration, which is the clear color formation, was calculated using the following formula and the peroxide value was calculated.

Peroxide value (PV)= [(V1-V0) x N] / M

V1 and V0 are the amounts spent for the sample and blind respectively, N is the normality of the titration solution and M is the sample weight. The results of the PV analysis performed in three replicates were calculated as meqO_2 kg^{-1} oil.

Statistical Analysis

The data obtained from the experiment were subjected to statistical analysis using the MiniTab software. The data underwent One-way analysis of variance (ANOVA) and was subsequently subjected to the Tukey multiple comparison test. Statistical significance was determined for intergroup differences at a significance level of $p<0.05$.

3. Result

Table 1 displays the profile of the volatile components found in the CEO. The findings indicate that a significant proportion of 98.81% of the volatile component content of the extract was identifiable. The primary constituents of this composition are Phthalide (49.42%), Fenipentol (28.45%), and D-Limonene (6.54%).

Table 1. Volatile profile of Celeriac leaf essential oil (CEO).

Sn	Compound	Retention Time (min.)	Concentration (%)
1	D-Limonene	12.660	6.54
2	1-Propanone,1-(2,4-dimethylphenyl)	34.413	4.04
3	Valeric acid	35.344	4.31

4	Fenipentol	36.442	28.45
5	Phthalide	36.622	49.42
6	3 n butyl phthalide	36.815	6.05
		TOTAL	**98.81**

The study found a correlation between the concentrations of CEO and Vit-C samples in DPPH solution and their respective inhibition percentages, as indicated by correlation factors. A linear relationship was observed between the aforementioned variables. The IC50 values were calculated using the linear equations provided, and the resultant data is illustrated in Figure 1. As demonstrated in Figure 1, the IC50 values for CEO and Vit-C were determined to be 30.52 and 29.93 ppm, respectively.

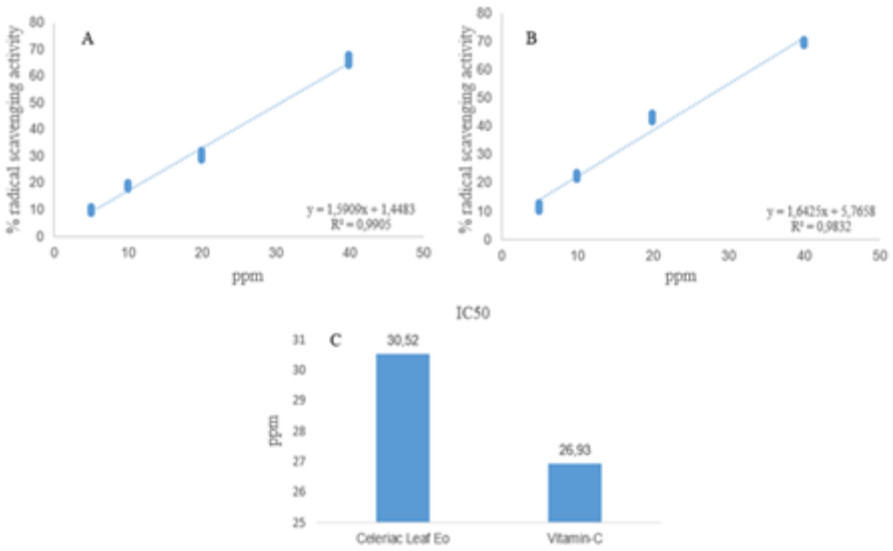

Figure 1. % radical scavenging activity of CEO (A), % radical scavenging activity of Vitamin-C (B) and the minimal concentration of CEO and Vitamin C for 50% radical scavenging (C).

The PV of fish oils subjected to thermal treatment, with varying ratios of CEO addition, as well as fish oil without any addition and thermal oxidation (Control), is depicted in Figure 2.

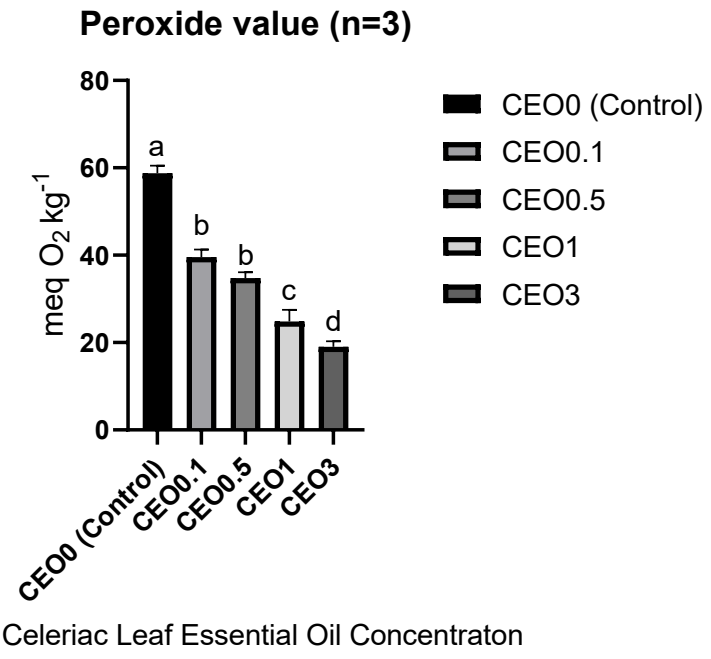

Figure 2. Peroxide values of fish oils containing different ratios of CEO after thermal oxidation

4. Discussion and Conclusion

Essential oils are highly concentrated plant extracts that are widely used in aromatherapy, cosmetics, and as natural remedies for various ailments. They are known for their pleasant aroma and many beneficial properties, including antioxidant and antimicrobial effects [20-22]. One potential application of essential oils is in protecting oil sources from oxidation [23]. Oxidation is a natural process that occurs when oils are exposed to air and light, causing them to break down and lose their nutritional value [24]. This can lead to rancidity, which not only affects the taste and smell of the oil but also reduces its health benefits [25]. Research has shown that certain essential oils, such as oregano, rosemary, and thyme, have strong antioxidant properties that can help protect oils from oxidation. These oils contain compounds such as carvacrol, thymol, and rosmarinic acid, which have been shown to inhibit the oxidation process and prevent the formation of harmful free radicals [26]. While the use of essential oils to protect oil sources from oxidation shows promise, more research is needed to fully understand their effectiveness and determine the optimal conditions for their use. It is also important to note that some essential oils may have adverse effects on certain individuals, so caution should be taken when using them [27]. Overall, the use of essential oils as natural preservatives for oil sources is an intriguing area of research that has the potential to provide a more sustainable and healthful alternative to synthetic preservatives.

Numerous research endeavors have been undertaken to explore the safeguarding properties of plant-based sources on marine oils derived from diverse origins [28-30]. The majority of the conducted studies employed PV as a metric to assess the safeguarding potential of botanical commodities on marine lipid sources. PV is considered to be a highly dependable indicator for identifying the onset of primary oxidation in oils [31]. Oil sources derived from marine organisms, such as fish oil, possess a high concentration of polyunsaturated fatty acids (PUFAs), rendering them vulnerable to oxidative degradation under various environmental stressors, including elevated temperature, light exposure, and humidity. As per good manufacturing practice (GMP), natural antioxidants like vitamin C are incorporated into fish oils produced for feed or food quality, owing to their reported benefits [32]. The present study involved a comparison between the CEO antioxidant activity and vitamin C.

Celeriac (*Apium graveolens*) is a biennial plant belonging to the Apiaceae family, which is widely used as a food and medicinal herb in many countries [33]. Celeriac leaf essential oil is a volatile oil obtained by steam distillation of the leaves of the celeriac plant. CEO is rich in many bioactive compounds, including phenolic compounds, terpenes, and flavonoids, which have been reported to have strong antioxidant activity [34]. As in previous studies, phthalide isomers and numerous bioactive molecules responsible for the distinctive aroma of CEO were identified in the present study. Antioxidants are compounds that protect cells from oxidative stress caused by free radicals, which are highly reactive molecules that can damage cellular components and contribute to the development of many chronic diseases such as cancer, diabetes, and cardiovascular disease. Antioxidants can neutralize free radicals and prevent oxidative damage, thereby protecting cells and tissues from damage [35]. The efficacy of natural antioxidants has been established in various studies [36, 37] like the present study, indicating their potential benefits not only for metabolic processes but also for safeguarding fats against rancidity.

To conclude, it has been demonstrated that the CEO exhibits potent antioxidant properties owing to its elevated levels of bioactive constituents. The potential natural antioxidant properties of CEO render it a promising candidate for employment in the food and feed industries.

Competing Interest / Conflict of Interest

The authors declare that they have no competing interests.

Author Contribution

We declare that all Authors equally contribute.

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