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Antioxidant Activity of Rosemary Oil and Olive Oil and its Impact on the Shelf Life of Fish Fillets

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ABSTRACT

Background. Microbial spoilage is causing losses to food production at a large scale. This research aims to use oil-based natural preservatives with excellent antioxidant and antimicrobial properties. It provides updated information regarding the protein, fats, ash, and moisture contents of fish fillets after preservation with rosemary and olive oils, with glycerin and gelatin powder-based nanoemulsions.

Method. To check the effect of oil-based preservation, proximate analysis and antioxidant activity tests were performed. Statistical analysis was performed using one-way ANOVA.

Result. The experimental groups showed significant ($p < 0.05$) differences in different parameters as compared to the control group.

Conclusion. The study found that rosemary and olive oil-based nanoemulsions significantly improved the nutritive value of fish fillets and extended their shelf life due to their strong antioxidant and antimicrobial properties.

Keywords: antioxidant, fish fillets, nanoemulsions, natural preservatives, seafood, shelf life

Highlights

- The study reports on the antioxidant activity of natural oils.
- Natural nanoemulsions are a better alternative to synthetic chemicals and have potential health benefits.
- Natural nanoemulsions are good for enhancing the shelf life of fish fillets.

1. INTRODUCTION

The food industry, particularly seafood industry, is facing major issues regarding food quality and safety [1–3]. It is a fact that the quality of food and its nutritional value are being compromised. However, low temperature, careful packing, and coating with natural preservatives and antioxidants are utilized to increase the shelf life of fish and its products [4–7].

Fish is considered a vital aspect of human nutrition and a rich source of polyunsaturated fatty acids (PUFAs), particularly those in the omega-3 family. The oxidation of these unsaturated fatty acids can pose significant risks [8, 9]. Moreover, fish and other seafood are highly perishable due to chemical and microbial degradation during processing and storage. As a result, to maintain the safety, quality, and shelf life of these products, appropriate

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preservation procedures are necessary [10–13].

Antioxidants prevent free radical generation, stabilize hydroperoxides, and reduce the oxidation and rancidity processes. Due to the potential negative side-effects of artificial antioxidants, the demand for natural antioxidants is increasing [14–16].

Essential oils, also called natural oils, are volatile odoriferous oils [17]. Although essential oils were originally used to enhance or improve the flavor of foods, their antioxidant and antibacterial properties make them excellent candidates for replacing chemical preservatives [18].

Rosemary, which is a real essence from the Lamiaceae family, has been investigated extensively because of its bioactive qualities. Rosemary extracts perform bioactivities as hepatoprotective, insecticide, antifungal, antioxidant, and antibacterial activities. This research examines the importance of rosemary in food preservation and its outcomes in terms of protective activities considering the increasing application of its real essence in preservers [19]. Similarly, olive oil is a significant part of the human diet. Indeed, olive oil has garnered attention in different countries primarily due to the certainty that it forms a beneficial part of the diet for the inhibition of many non-communicable and chronic diseases [20, 21].

There are different methods used for food preservation. Vacuum packing is one method used for reducing lipid oxidation (auto oxidation) and other chemical changes because of the limited number of oxygen molecules. The use of vacuum packing extends the shelf life of fish fillets [22–24]. Similarly, nanoemulsions are also considered as self-preservatives, antioxidants, and antimicrobials. They are utilized in food processing technology to maintain color, flavor, antimicrobial, and antioxidant qualities [25–27]. Rohu (*Labeo rohita*) is one of the most significant fishes consumed in Pakistan. This study aimed to utilize olive oil and rosemary nanoemulsions (natural antioxidants) to prevent inappropriate changes that shorten the shelf life of frozen fillets.

2. MATERIAL AND METHODS

2.1. Selection of Fish Sample and Study Site

The current study was carried out at the Food and Biotechnology Research Centre, PCSIR, Lahore. Experimental fish was collected from the fish pond complex of the Manawa Fisheries Research and Training Institute, Lahore. *Labeo rohita* fillets were selected as experimental fish for this study.

2.2. Nano emulsion Preparation

Nanoemulsions, based on natural oils, were prepared in a two-step oil and water phase by using the methods of [28] and [29]. A 20% oil phase of nano emulsion was prepared using olive and rosemary oils, separately. For this purpose, 15% of total emulsion, 3% gelatin, and 2% glycerin were added. Later on, 20% (v/v) of the emulsion was prepared by adding 80% water. A 25% emulsion of each oil was prepared using 20% of each oil, 3% gelatin, and 2% glycerin. Afterwards, each oil phase was mixed with 75% distilled water. A homogenizer was used for 5 minutes at 72 amplitudes to prepare the emulsion.

2.3. Preparation of Fish Samples in Nano emulsion

Labeo rohita fillets were taken and thoroughly washed, cut into 12 pieces, and washed again with tap and distilled water. Fish fillets were immersed for 15 mins in 20% and 25% nano emulsion of each oil.

Afterwards, the fillets were vacuum packed and stored in the refrigerator at 4ºC for 0 to 30 days [29, 30].

2.4. Proximate Analysis

The proximate analysis of fish samples was carried out in terms of moisture, ash content, crude protein, and crude fat to examine the nutritive value of the fish. For this purpose, fish fillets were dried in oven at 60ºC for 24 hours and grinded to obtain their powder, which was then analyzed for proximate composition in fish nutrition [30].

2.5. Determination of Antioxidant Activity

Samples (5 g each) were taken in the flask and mixed with 20 ml of 70% methanol. Then, 0.04% DPPH was added in the 50 ml flask to make a dilute solution. It was then set on a shaker until it acquired a dark purple color. Afterwards, samples were placed in a spectrophotometer to check the absorbance of oils and antioxidant activity was determined by using the following formula:

%Antioxidant activity= (Absorbance of blank-absorbance of sample) x100\Absorbance of blank

2.6. Statistical Analysis

Data analysis was done by using one-way ANOVA and SPSS (version 22.00) to observe the significant differences between the experimental and control groups.

3. RESULTS

In this study, samples were treated with 20% and 25% rosemary and olive oil nanoemulsions, respectively. It was noticed that olive oil reduced the water content of fish fillets by 7.8% and 7.7%, respectively. Whereas, rosemary oil showed a decrease of 6% and 7% as compared to the control (with 8.3% and 8.1% water contents), as shown in Figure 1.

While the effects of nano emulsion were recorded for the shelf life of fish and meat, the protein percentage was estimated to have increased as compared to the control group, as shown in Figure 2.

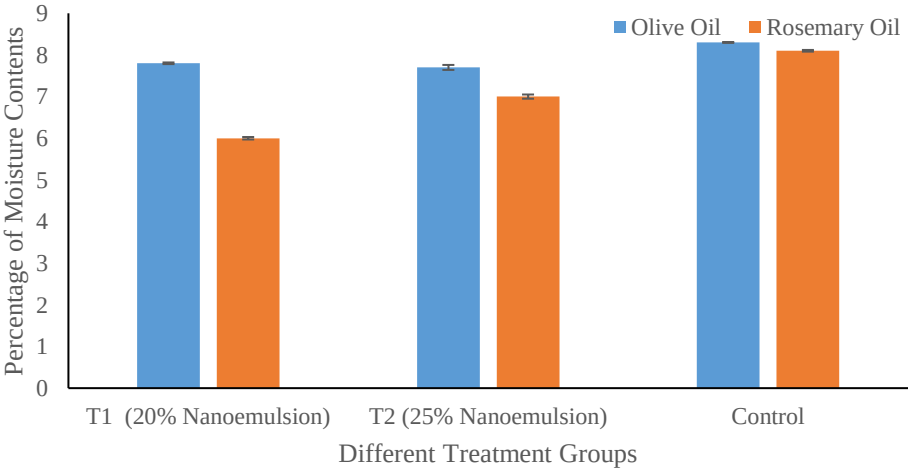

Figure 1. Percentage of Moisture Contents with Nanoemulsions of Olive Oil and Rosemary Oil

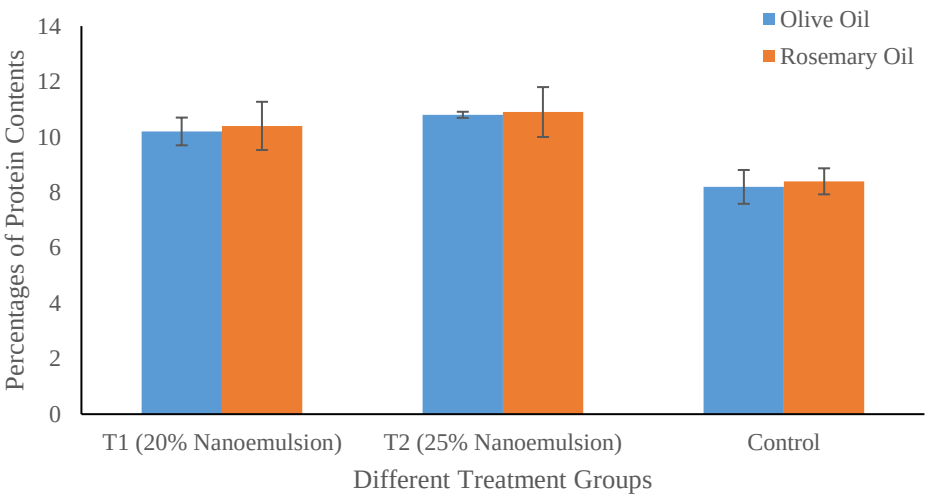

Figure 2. Percentages of Protein Contents with Nanoemulsions of Olive Oil and Rosemary Oil

Fat is an important nutrient in food. It is used as fuel by the body which stores energy in the form of fat. Hence, fat is a major reservoir of energy in the body. Coating retention of nanoemulsions based on olive oil and rosemary oil showed an increase in fat contents as compared to the control group, as shown in Figure 3.

The analysis of ash content in fish samples was also conducted and a significant increase was noticed in nanoemulsion samples as compared to the control group, as shown in Figure 4.

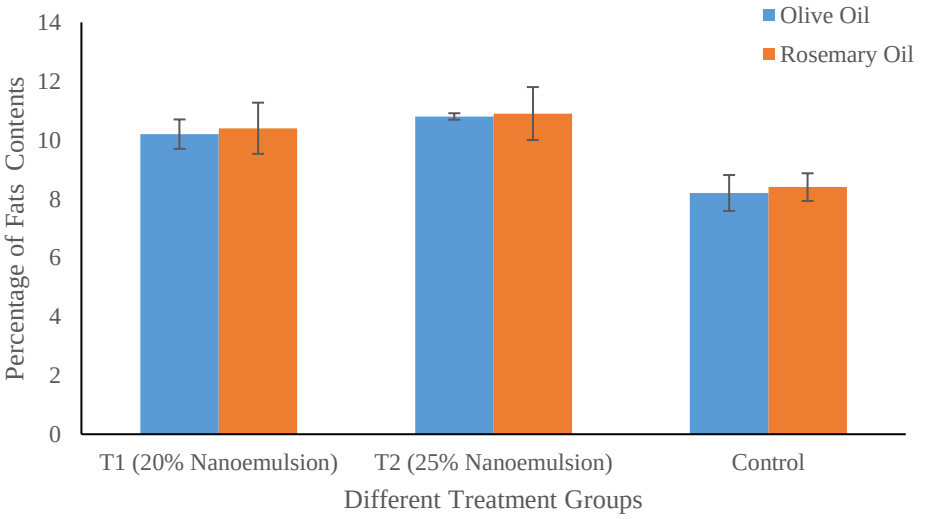

Figure 3. Percentage of Fat Contents with Nanoemulsions of Olive Oil and Rosemary Oil

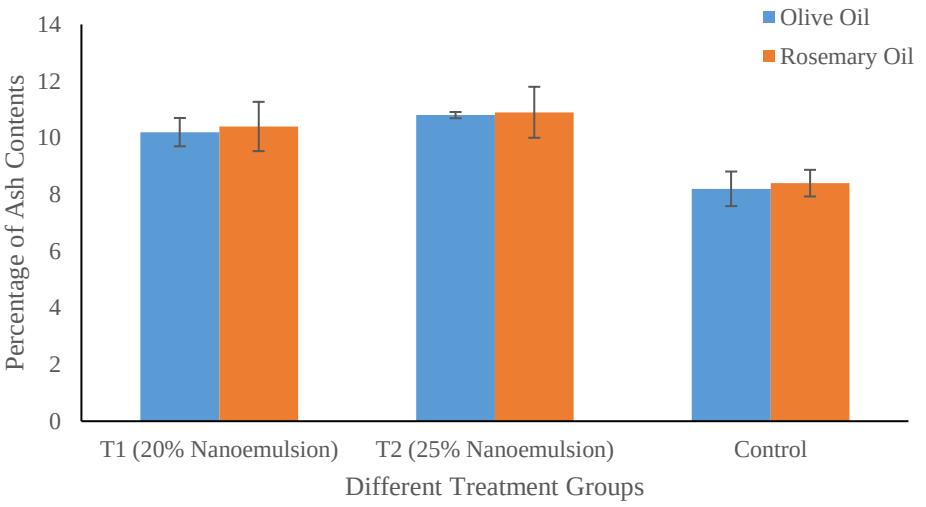

Figure 4. Percentage of Ash Contents with Nanoemulsions of Olive Oil and Rosemary Oil

There is a high risk of quality loss in meat due to lipid oxidation in meat and fish products which leads to a rancid taste and an off flavor. It also limits storage time. After storage for 30 days, the results of rosemary oil-based nanoemulsion showed 62% and 77% antioxidant activity, while olive oil based nanoemulsion showed 61% and 70% antioxidant activity, as compared to the control group which showed 50% and 52% antioxidant activity, as shown in Table 1.

Table 1. Antioxidant Activity of Rosemary Oil and Olive Oil in Experimental and Control Groups

Essential Oils	T1 20% nanoemulsion	T2 25% nanoemulsion	Control Group
Olive oil	61.9±0.05	70.9±0.05	50.2±0.05
Rosemary	62.5±0.05	77.8±0.05	52.6±0.05

4. DISCUSSION

Natural oils, such as rosemary oil and olive oil, are some of the best preservatives available to control any chemical change in fish meat. The current study was directed to check the antioxidant activity and shelf life of fish fillets after using oil-based natural preservatives. For this purpose, the fillets of *Labeo rohita* were used to check the efficacy of nanoemulsion. It was determined that rosemary and olive oils reduced lipid oxidation among fish fillets and helped to increase their storage time by maintaining the nutrient value of the fish. While, crude proteins (CP), crude fats (CF), moisture, and ash values showed significant results with $p<0.05$ as compared to the control group. These findings coincide with the studies conducted by [29, 31, 32].

Ozogul et al. [33] investigated the effects of rosemary extract at different levels (1% R1 and 2% R2) on the quality of vacuum-packed sardine by conducting

sensory, biochemical, and microbiological analyses (total viable counts). The samples were stored in the refrigerator (4±1ºC) over a storage period of 20 days. The results showed that the use of 1% rosemary extract improved the sensory quality of both raw and cooked sardine. While 2% rosemary extract was found to be the most effective (p<0.05) in various biochemical tests, such as lipid oxidation. These findings coincide with the findings of the current study in which rosemary essential oil was applied on fish fillets as a preservative. It increased their shelf life by decreasing the moisture content, maintaining crude protein, fat, and ash values from 0-30 days and helped to maintain their nutritive value.

According to a study conducted by [34] and Sayyari et al. [35], edible coatings of essential oils improve fish quality by acting as a barrier against moisture transfer and oxygen uptake and reducing the moisture content. Controlling quality deterioration and extending shelf life is easier with an edible covering comprised of biodegradable ingredients. The foregoing study described the impact of essential oils, vacuum packing on oxidative constancy, and qualitative attributes of Rohu (*Labeo rohita*). The maximum values of crude protein, crude fats, and ash content were detected in treated samples (52%, 10%, and 12%), whereas lower values were identified in control. Further, essential oils (oregano, cinnamon, and rosemary) showed high antioxidant activity (70%, 65%, and 77%) as compared to the control group. This finding coincides with the current study in which fish samples were vacuum packed with edible coatings of oils, glycerin, and gelatin powder, which performed a strong role in blocking moisture transmission and acceptance of oxygen to enhance the worth and shelf life of the fish. Moreover, vacuum packing also helped to maintain the meat as a safe food. The current study also coincides with a previous study conducted by Yazgan et al. [36]. El-Sayed et al. [37] investigated the influence of oil-in water nanoemulsion on the sensory, chemical, and microbiological qualities of sea bream and sea bass fillets stored at 2°C. The results showed that the use of nanoemulsion extended the shelf life of fish when compared with the control group. Treatment with nanoemulsion significantly (p<0.05) maintained the values of chemical parameters as compared to the control group throughout storage. Similarly, in the current study, treatment group of natural preservatives significantly (p<0.05) maintained the nutritive value as compared to the control group. Various studies reported on commercial and natural oil applications supported the idea that oils enhance the shelf life of fish fillets [38–40]. The studies determined that olive oil and rosemary-based nanoemulsions extend the shelf life of fish. Indeed, rosemary acts as a strong antioxidant and is recommended as the best natural preservative.

4.1. Conclusion

It was concluded that natural oils are one of the best natural preservatives that help to maintain the nutritional value of fish meat. It is a low-cost method that is easy to adopt and has a strong positive effect on increasing the shelf life and storage of fish meat. Rosemary and olive oil-based nanoemulsions showed a strong antioxidant activity and resisted chemical changes in fish meat. They also maintained crude fat, ash values, and produced a strong impact as good antioxidants. Therefore, natural preservatives are a good and safe way to replace synthetic and costly artificial preservatives.

CONFLICT OF INTEREST

The author of the manuscript has no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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