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Effects of Habitats and Feeding Patterns on Fatty Acid Profile of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

In this study, fatty acid profiles of rainbow trout, which has been bred in farms, and the trouts escaping from the farm to the nature (fish living in the canal between the farm and the Körkün Brook) and, which escaped from the farm and reached the Körkün Brook and fed here with natural nutrients have been compared. The rainbow trout obtained from the culture medium and the trout escaping from the farms to the nature were found to be significantly different in the fatty acid profile. The basic fatty acids of the samples taken from our research groups are; myristic acid (C14: O-), palmitic acid (C16: 0), palmitoleic acid (C16: 1), heptadecenoic acid (C17: 1), stearic acid (C18: 0), oleic acid (C18: 1 n9), linoleic acid (C18: 2 n6), vaccenic acid (C18: 1 n7), linolenic acid (C18: 3 n3), arashidic acid (C20: 0), arashidonic acid (C20: 4n6), eicosapentaenoic acid (C20: 5 n3), eruric acid (C22: 1 n9), docosahexaenoic acid (C22: 6 n3). In our study, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids were determined respectively; 29.389-21.451%, 25.36-59.49% and 34.17-18.56%. As a result, the habitats and feeding of the fish affected the fatty acid profile of rainbow trout.

Key words: Culture trout, wild trout, fatty acid composition

Research article

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INTRODUCTION

Rainbow trout is one of the widely held cultured fish farming in the world and Turkey. According to FAO data, rainbow trout ranks 17th in 2016 among the fish species grown in the world and it ranks 2nd among the salmonidae family members after the Atlantic salmon. World rainbow trout production, which was 752 thousand tons / year in 2010, reached 814 thousand tons in 2016 (FAO, 2018). The most cultivated fish species in Turkey is the rainbow trout. When fisheries statistics are examined in Turkey it is easily seen that the farming of trout increased very quickly. Our trout production which was 44,533 tons / year in 2000 was 114,497 tons / year in 2018. 104,887 tons of this cultivation is produced in inland waters and 9,610 tons is bred in marine environments (TurkStat, 2019). Turkey meets 14.06% of the world production of rainbow trout which is 814 thousand tons with this production amounts, and this amount of production makes Turkey one of the world's largest trout producer.

Trout farming in Turkey first began in the enterprises established in terrestrial environments where the natural water resources exist. With the growth of the sector, trout farms have been moved to some rivers and dam lakes and even to marine environment. With the spread of trout farms in such areas, the number of trout escapes from farms to nature have increased considerably. Fish escaping from farms have become source of income for fishermen in many regions. According to TUIK data, 738 tons of trout fishing was carried out in 2010 and 282 tons in 2018. Among the provinces where hunting is made, some provinces such as Kayseri, Elazığ and Muğla where trout farms are intense attract attention.

In aquaculture, many studies have been carried out in order to increase growth, change the nutrient content and fatty acid profile of fish and extend shelf life (Acar et al., 2019; Öz, 2018a; Dikel et al., 2019; Öz, 2018b; Büyükdeveci et al., 2018; Öz et al.). The fatty acid profile of fish determines the quality of fish meat, especially the taste of fish meat (Dönmez and Tatar, 2001). The main components that determine the quality of fish meat are proteins and lipids. Also, The tasty of fish meat results from cardiovascular diseases, brain, nervous system and important pharmacological effects against cancer, lipid and unsaturated fatty acids in their body compositions (Shapiro, 1999). Many studies have been conducted to examine seasonal and habitual changes in the fatty acid profile of fish (Durmuş et al., 2014; Taşbozan et al., 2016). Fish meats, especially rainbow trout (*Oncorhynchus mykiss*), which are widely cultivated, contain highly unsaturated fatty acids. These high rates are largely related to the nutritional properties of fish (Boggio et al., 1985). In recent years, demand for aquaculture, which is the main source of ω-3 PUFAs, has been increasing dramatically. The most important natural source of EPA and DHA is fatty fish (Uçak et al., 2019).

In this study, it was aimed to determine the fatty acid profiles of rainbow trout living in different parts of the same water system and fed differently.

MATERIALS AND METHODS

Material

The fish used in the study were obtained from Öz Trout Production Enterprise located on the shore of the Körkün Brook, which is located in the Pozantı district of Adana province, from the canal providing water to this enterprise and from the Körkün Brook near the enterprise. The fish escaping from the farm were caught with the throw net from the Körkün Brook and the canal providing water to the enterprise and those of the same size group (300-350 grams) were analyzed. 300-350 grams of trout were brought from a special trout farm on the shore of the Körkün Brook and analyzed for fatty acid profiles.

Fatty Acid Analysis

Fatty acid methyl esters of extracted lipids have been made according to Ichibara et al. method (1996). 4ml of 2M KOH and 2ml of n heptane were added to 25mg of the extracted oil sample. The mixture was then vortexed at room temperature for 2 minutes and centrifuged at 4000 rpm for 10 minutes and the heptane layer was taken for analysis at GC. Fatty acid composition was performed using flame ionization detector (FID) and GC (Gas Chromatographic, Figure 3.4) with 30m x 0.32mm ID x 0.25µm film thickness SGE column auto sampling (Perkin Elmer, USA). The injector and detector temperatures were set to 220 ° C, then to 280 ° C, respectively. The oven temperature was maintained at 140 ° C for 5 minutes. Subsequently, it was brought to 200 ° C by increasing 4 ° C per minute and from 200 ° C to 220 by increasing 1 ° C per minute. The sample amount was 1ml and the carrier gas control was provided at 16ps. Split application was performed at 1:50 ratio. Fatty acids were identified by comparison of the FAME mixture of standard 37 components based on their arrival times. The results of the two GC analyze performed in the same way are expressed in% GC with ± standard deviation values.

RESULTS AND DISCUSSION

The fatty acid profile of rainbow trout from three different environments used in our research is shown in Table 1. As a result of the research, the highest total SFA ratio (29.38%) was found in the samples taken from the Körkün Brook, while the lowest total SFA value (21.45%) was found in rainbow trout from the cultural medium. Similar to the total SFA values, the highest PUFA ratio (34.17%) was found in the fish caught from the Körkün Brook, while the lowest PUFA ratio (18.56%) was found in the farmed trout. Contrary to the total SFA and PUFA amounts, the highest total MUFA ratio (59.49%) was found in rainbow trout fed by commercial feeds from the cultural medium, while the lowest MUFA ratio (25.36%) was observed in rainbow trout hunted from the Körkün Brook.

In a previous study, the total SFA, MUFA and PUFA ratios of rainbow trout hunted from nature were as follows; 28.04 ± 0.54, 24.69 ± 0.73 and 35.07 ± 0.95 were found to be the same (Öz, and Dikel, 2015a). In a study conducted in the same region, total SFA, MUFA and PUFA ratios of brown trout (*Salmo trutta*) were as follows; 29.167, 21.583 and 31.213 (Öz, and Dikel, 2015b).

One of the most important reasons for the different fatty acid profiles in fish is their diet. The fatty acid profile of the feed on which the fish are fed is very important in shaping the fatty acid profile of the fish. Feeding patterns of fish and their environment are important for fatty acid profile and body content (Öz and Dikel 2015a; Yıldız et al. 2006; Öz, 2017; Şener et al. 2005).

In our study, EPA (C20: 5n3) values of the studied fish were found to be between 6,798 ± 0,31-1,926 ± 0,41 and DHA (C22: 6 n3) values ranged between 9,197 ± 0,71-10,151 ± 0,44. In a previous study, the EPA rate of trout from production farms was 1.93 ± 0.11 and the DHA rate was 5.69 ± 0.21 (Özyılmaz, 2019). In another study, similar to our study, the fatty acids of rainbow trout living in different environments were examined and the highest EPA ratio (8.74 ± 0.52) was found in the rainbow trout that escaped from the farms and was hunted from Seyhan dame lake while the lowest EPA ratio (3.14 ± 0.24) was found in the samples taken from the net cages. When DHA levels were examined, the highest rate (18.49 ± 1.66) was found in trout taken from net cages and the lowest rate (5.66 ± 0.87) was found in trout hunted from Seyhan dame lake (Taşbozan et al., 2016).

There are studies on the habitat of fish and especially the feed acid contents that change the fatty acid profile. In a study conducted in 2018, black cumin oil was added to trout feeds and their effects on growth performance and fatty acid profile after feeding period were investigated. In the study, total PUFA amounts of rainbow trout were found between 32.09 ± 2.02 and 38.56 ± 2.78 (Öz et al., 2018b).

As a result, in this study, fatty acid profiles of rainbow trout from three different environments were compared. In the analysis, it was found that the fatty acid profile of rainbow trout varied depending on the habitat and diet.

Table 1. Fatty acid profile of rainbow trout (*Oncorhynchus mykiss*) from three different environments

Fatty acids	The environment where fish are taken		
	The Körkün Brook	**Earth Lined Canal**	**Aquaculture**
C11:0	0,035±0,01	0,023±0,01	0,011±0,00
C12:0	0,208±0,03	0,145±0,01	0,029±0,00
C13:0	0,023±0,00	0,184±0,02	0,011±0,00
C14:0	2,287±0,10	1,871±0,12	1,271±0,21
C14:1	0,034±00	0,024±0,01	0,010±00
C15:0	0,291±0,03	0,223±0,08	0,165±0,02
C16:0	18,691±0,64	16,391±0,21	14,323±0,23
C16:1	7,312±0,39	4,801±0,28	2,021±0,26
C17:0	0,383±0,05	0,189±0,04	0,214±0,02
C17:1	0,321±0,12	0,228±0,08	0,131±0,02
C18:0	4,987±0,44	4,015±0,42	4,378±0,34
C18:1 n9	16,210±0,44	19,161±0,39	22,31±0,51
C18:1 n7	8,917±0,35	6,823±0,14	3,194±0,14
C18:2 n6	6,590±0,40	18,145±1,25	33,691±0,46
C18:3 n6	0,597±0,06	0,384±0,11	0,139±0,07
C18:4 n6	0,655±0,17	0,329±0,21	0,129±0,02
C20:0	0,137±0,01	0,129±0,07	0,146±0,01
C20:1	0,598±0,09	0,511±0,06	0,693±0,09
C20:2 cis	0,439±0,19	0,891±0,17	1,201±0,11
C20:3 n6	0,368±0,12	0,448±0,14	0,914±0,11
C20:4n6	1,14±0,18	0,892±0,11	0,794±0,18
C20:5 n3	6,798±0,31	4,341±0,47	1,926±0,41
C22:0	0,093±0,01	0,116±0,03	0,249±0,01
C22:1 n9	0,157±0,05	0,245±0,09	0,392±0,05
C22:2 cis	0,069±0,02	0,087±0,02	0,117±0,03
C23:0	0,110±0,02	0,095±0,04	0,063±0,02
C24:0	2,144±0,18	3,78±0,31	0,591±0,02
C22:6 n3	9,197±0,71	9,226±0,12	10,151±0,44
C24:1	0,131±0,03	0,164±0,04	0,243±0,01
∑SFA	29,389	27,161	21,451
∑MUFA	25,36	43,279	59,491
∑PUFA	34,173	23,421	18,565

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Effects of Gelatin Edible Films Containing Onion Peel Extract on the Quality of Rainbow Trout Fillets

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Abstract

In this study, quality changes of rainbow trout fillets covered with gelatin films prepared by adding onion peel extract (OPE) in different concentrations (2.5%, 5% and 10%) were examined during storage at 4±1°C. For this purpose, trout fillets are divided into five groups: the group covered with gelatin film (GF), the groups covered with gelatin film containing different concentrations of OPE (O2.5, O5, O10), and the group without coating (control, C). According to the results obtained, peroxide values increased until end the of storage, but the highest values were found to be in the C and GF groups. The lowest TBARS values were observed in the groups covered with gelatin films enriched with OPE. Results of the study showed that lipid oxidation was delayed in the groups covered with gelatin films prepared by adding OPE. Microbial growth increased in all groups by the end of storage, and the highest values were observed in the C and GF groups at the end of storage. As a result, it can be concluded that the addition of OPE increased the effectiveness of gelatin films and delayed microbiological spoilage and lipid oxidation in rainbow trout fillets during refrigerated storage.

Keywords: Rainbow trout, gelatin film, onion peel extract, lipid oxidation, fish quality

Research article

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INTRODUCTION

Fish contains high amount of long chain omega-3 polyunsaturated fatty acids (PUFAs) which are essential for the growth and development of human. These long-chain fatty acids, especially eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) reduce the risk of cardiovascular diseases and some types of cancer, contributing to the development of the body (Shahidi, 2015). However, due to neutral pH, high level of PUFAs and free amino acids fish is very perishable. The main causes of deterioration in fresh fish are the lipid oxidation and presence of microorganisms. Various preservation techniques have been developed in order to prevent spoilage, maintain quality and extend the shelf life. Traditional processing methods such as drying, salting, smoking, marination, fermentation have been used for long years in order to maintain quality in seafood products. (Sampels, 2015). In addition, temperature-based conservation techniques such as cooling and freezing and chemical preservatives are also used in the food industry to control water activity, enzymatic, oxidative and microbial degradation. Packaging technology is widely used to extend shelf life, maintain hygiene and quality in foods sensitive to microbial and oxidative spoilage (Ghaly et al., 2010; Ahmad et al., 2012). Recently edible films and coatings based on proteins, polysaccharides and lipids have been gaining importance in food preservation. Edible films and coatings are thin layers formed on the product which can be eaten together with the food (Dursun and Erkan, 2009). Edible films and coatings can prevent lipid oxidation, color deterioration and can enhance the product quality (Gennadios et al., 1997) by acting as moisture, oxygen, carbon dioxide or vapour barriers (Ojagh et al., 2010). At the same time, the film should be durable and flexible and completely should cover the product. There is not a single coating material with all these properties. For this reason, edible film and coating material is obtained from many sources, including polysaccharides, lipids and proteins, and formulations are prepared by adding plasticizing agents. Edible films combined with plant extracts, essential oils, antioxidants, colourants, flavourings and spices can provide some benefits such as improving the organoleptic and nutritional properties of the product they applied (Bourtoom, 2008; Falguera et al., 2011).

Onions are versatile vegetables that can be consumed as fresh and processed. It is also a principal source of biologically active compounds such as phenolic acids, flavanoids and anthocyanins (Singh et al., 2009). The onion shell contains twenty times flavanoids (especially quarcetin) more than the onion itself. In addition, a lot of waste is obtained during the processing and consumption of the onion. In recent years, evaluation of food by-products as a natural antioxidant and antimicrobial agent is gaining importance due to their inexpensiveness and simple extraction processes (Uçak, 2019).

Therefore, in this study it was aimed to investigate the effects of the gelatin films enriched with different concentrations of onion peel extract on the quality of rainbow trout fillets during cold storage (4°C).

MATERIAL AND METHODS

Materials

Rainbow trout (*Oncorhynchus mykiss*) were provided from a fish farm in Niğde and transported to the laboratory in ice boxes as freshly. They were washed after gutted, beheaded and filletted. Onion peels were collected from local markets.

Onion peel extraction

Onion peels (OPs) were dried at 45^{o}C for 48 h after washed twice in tap water and ground into powder with a blender. For the extraction procedure, 100 mL OPs powder and 100 mL ethanol (70%) were put into a flask and sonicated with ultrasonic bath for 1 h at 25^{o}C.

After extraction procedure, the onion peel extracts (OPE) were filtered and concentrated by using rotary evaporator (IKA, HB-10 digital, Germany) at 45°C under vacuum.

Gelatin film and fish samples preperation

Gelatin films were prepared according to method of Gomez-Estaca et al. (2009) with slight modifications. Gelatin (food grade, Zag kimya, Turkey) dissolved in distilled water (8 g/100 mL) at room temperature. Then glycerol (0.1 mL per g of gelatin) and D-sorbitol (0.15 g per g of gelatin) were added to the solution and kept at 45^{o}C for 15 min. Onion peel extract (OPE) was added to the film solution in concentration of 2.5%, 5% and 10% (by volume per mass of gelatin). 40 mL of the film solutions were poured into square polystyrene foam dishes in order to obtain films. All the film solutions were put into cabin for drying at room temperature for 48 h at 50% relative humidity. The fish fillets were wrapped according to Ahmad et al. (2012) method with slight modifications. Dried gelatin films were peeled from the foam dishes and both sides of films were sterilized under UV for 10 min. First fish group was coated with gelatin film without OPE, second group was coated with gelatin film containing 2.5% OPE, third group was coated with gelatin film containing 5% OPE, fourth group was coated with gelatin film containing 10% OPE, and the last group left as control without wrapping. Each fillet was coated on both sides. Then, each sample wrapped with strech film and stored at refrigerator (4±1^{o}C).

Analyses

For the determination of pH value, the probe of the pH-meter (Thermo Scientific Orion 2-star, Germany) was dipped into the fish homogenatas prepared with distelled water (1:1, w:v).

Total volatile basic nitrogen (TVB-N) was determined according to Schormüller (1968). 10 g homogenized fish sample was washed into the distillation flask, and 1 mg magnesium oxide was added. Samples were boiled and distilled into 10 mL of 0.1 mol equi/L HCl solution in a conical flask with addition of tashiro-indicator. After distillation, the flask were titrated with 0.1 mol equi/L NaOH. TVB-N results were expressed as mg nitrogen/100 g sample.

Peroxide value (PV) was determined according to method of AOAC (1990). Approximately 2 g sample was stirred with 30 mL of solution including 3chloroform:2glacial acetic acid (v/v). After then 1 mL of saturated potassium iodide (KI) solution was added. The mixture was stored in a dark place for 5 min. Later on, 75 mL of distilled water was added and the mixture was titrated with sodium thiosulfate ($Na_2S_2O_3$) (0.1M) with the addition of starch solution as an indicator. The results were calculated as meq O_2/kg.

Thiobarbituric acid (TBARS) was determined using the method of AOCS (1998). Thiobarbituric acid content determinations were conducted depending on the principle of colorization of malondialdhyde present in the lipids with TBARS reagent. After addition of the same amount of TBARS reagent in the samples solved in n-butanol, the mixture was put in the water bath at 95°C for 120 min. Results were calculated as;

TBARS (mg MDA/kg) = 50 x (The absorbance of lipid- The absorbance of blank) / sample weight (mg).

For the microbiological analyses fish sample (10 g) was mixed with 90 mL pre-chilled sterile ringer solution. Further decimal serial dilutions were used from this homogenate. For the determination of total psychrophilic bacteria and total viable counts Plate Count Agar (PCA) was used. Then the plates were incubated at 8^{o}C for 7 days and 37^{o}C for 24-48 h, respectively. For the Enterobacteriaceae determination, pour plating method was used in Violet Red Bile Agar (VRBA) and the plates incubated at 37^{o}C for 36-48 h.

Statistical analysis performed in triplicate and analysis was carried out with SPSS (Statistical Analysis System, Cary, NC, USA) software and different applications were subjected to multiple comparison tests.

RESULTS AND DISCUSSION

Total phenolic and antioxidant activity values of onion peel

Phenolic compounds are noted for their nutritional and functional benefits, such as antioxidant and antimicrobial effects. Onion peel is the principal source of biologically active compounds such as phenolic acids, flavanoids and anthocyanins (Singh et al., 2009). In this study, the total phenolic compound of OPE was 656.50 mg GAE/g, while the antioxidant activity value was 964.23 µmol trolox/g. Similarly Ifesan (2017) found the total phenolic content of the onion peel extracted with 80% ethanol to be 664.30 mg GAE/g.

pH value

Changes in the pH value of rainbow trout fillets wrapped with gelatin films incorporated with different concentrations of OPE are given in Table 1. Initially, the pH value of fillet was found to be 6.53 and increaed in all groups during the storage period. Significant differences ($P<0.05$) were found between groups C and CF and groups wrapped with gelatin films prepared with OPE during storage. At the end of storage, the highest pH value was 7.14 and 7.00 in the C and CF groups, respectively, while the lowest pH was observed as 6.70 in the O10 group. Ludorf and Mayer (1973) reported that the pH limit for fresh fish was 6.80-7.00. According to Baygar et al. (2012) the pH value of fresh fish is between 6.00-6.50. Alparslan et al. (2014) found that the pH values of rainbow trout fillets coated with gelatin films prepared with laurel essential oil were lower than the control samples.

Peroxide value

The peroxide value, one of the primary oxidation products, is used to measure the initial level of oxidation in oils (Iqbal et al., 2008). The changes in peroxide values of trout fillets covered with gelatin films containing different concentrations of OPE are given in Table 1. At the beginning, the peroxide value was found to be 2.00 meq O_2/kg, and reached the highest values in the C and CF groups (14.00 and 9.99 meq O_2/kg). The lowest peroxide values ($P<0.05$) were observed in O5 and O10 groups as 8.00 and 6.00 meq O_2/kg. Hamilton et al. (1998) reported that the peroxide value of a good quality fish should be lower than 5 meq O_2/kg. Control group reached this value on the 4th day of storage, while the gelatin film coated group reached at 8th day. Peroxide values of rainbow trout fillets coated with films containing OPE exceeded the limit value on the 12th day of storage. Alparslan et al. (2014) reported that the peroxide values of trout fillets coated with gelatin films prepared by adding laurel essential oil were lower than those of the control group. Similarly, Fadıloğlu and Çoban (2018) found lower peroxide values in trout fillets coated with chitosan films enriched with sumac than the control group.

Thiobarbituric acid (TBARS) value

Thiobarbituric acid (TBARS) has been widely used in determining secondary oxidation products such as aldehydes or carbonyls (Shahidi and Wanasundara, 1998). Changes in the TBARS values of trout fillets wrapped with films incorporated with different concentrations of OPE are shown in the Table 1. TBARS value of trout fillets was initially determined as 0.63 mg MA/kg. This value increased in all groups during the storage period and reached the highest values ($P<0.05$) in C and CF groups as 2.70 and 2.07, respectively, at the end of the storage. Significantly lowest ($P<0.05$) TBARS values were observed in the O10 group and were found to be 0.60 mg MA/kg at the end of storage. Martinez et al. (2017)

observed TBARS value of sea bass fillets coated with chitosan and alginate films incorporated with resveratrol as 0.62 mg MA/kg initially and reported that films are effective in preventing lipid oxidation. Similarly Alsaggaf et al. (2017) found that TBARS values of Nile tilapia coated with chitosan films enriched with pomegranate seeds were lower than those of the control group during 30 days of storage. In the present study, it can be concluded that gelatin films enriched with OPE show less oxygen permeability and retarded lipid oxidation due to their antioxidant properties.

Microbiological analysis

Changes in microbiological quality of trout fillets coated with gelatin films enriched with OPE during refrigerated storage are presented in Table 2. Initially, the total number of mesophilic and psychophilic bacteria of trout fillets was found to be 1.77 and 1.54 log cfu/g, respectively, while the total coliform bacteria number was 1.71 log cfu/g. Total yeast and mold was not observed at the beginning of the storage. At the end of the storage, the total mesophilic bacteria count in the C and CF groups was observed as 6.22 and 6.19 log cfu/g, respectively. These values were found as 6.06, 5.81 and 5.34 log cfu/g in O2.5, O5 and O10 groups, respectively, at the end of the storage. The total mesophilic bacteria count was observed as 6.22 and 6.19 log cfu/g in the C and CF groups, respectively. These values were found to be 6.06, 5.81 and 5.34 log cfu/g in the O2.5, O5 and O10 groups. In terms of the total number of psychrophilic organisms, the highest value was observed in C samples (7.43 log cfu/g) at the end of storage, while the lowest value was observed in O10 samples (6.88 log cfu/g). The total coliform bacteria count increased in all groups untill at the end of storage and significantly ($P<0.05$) lowest values were found in fillets wrapped with films enriched with OPE. The total yeast and mold counts increased in all groups, but at the end of the storage significantly($P<0.05$) lowest value was found in the O10 group (3.33 log cfu/g). In accordance with the microbiological data obtained, it is concluded that an increase in microbial growth was observed in all groups during storage, but the lowest values and slower growth were determined in trout fillets wrapped with films containing OPE. This shows that OPE inhibit the microbial growth with the antimicrobial activity. Uçak (2019) found the initial total viable count of trout fillets wrapped with gelatin films incorporated with garlic peel extract as 2.27 lof cfu/g and it was reported that the microbial growth was slower than in this samples than the control group. According to Jouki et al. (2014) reported that microbial growth of trout fillets coated with chitosan films prepared with thyme essential oil was lower than those of the control group.

CONCLUSION

Based on the results of this study, GPE could inhibit bacterial growth and maintain sensory and chemical quality of rainbow trout fillets during refrigerated storage. Gelatin film without GPE has 2 days shelf-life extention effect on the rainbow trout fillets, while application of gelatin film enriched with GPE extended the shelf-life of fillets 5 days. Results showed that, addition of 4% concentration of GPE into gelatin film was much more effective, since the lowest microbiological and chemical scores were obtained from this group. Thus, GPE can be an effective antioxidant and antimicrobial agent in the gelatin based edible films and it can be used for the extention of shelf-life of fish and fish products

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Table 1. Changes in chamical quality of rainbow trout fillets coated with gelatin films containing OPE during storage at 4°C

	Storage (day)	C	CF	O2.5	O5	O10
pH	**0**	6.53 ± 0.21^{Ab}	6.53 ± 0.21^{Ac}	6.53 ± 0.21^{Ab}	6.53 ± 0.21^{Aab}	6.53 ± 0.21^{Aab}
	4	6.64 ± 0.00^{Ab}	6.42 ± 0.13^{Cc}	6.30 ± 0.06^{Ec}	6.56 ± 0.19^{Bab}	6.36 ± 0.01^{Dc}
	8	6.51 ± 0.04^{Ab}	6.48 ± 0.13^{Ac}	6.31 ± 0.07^{Bc}	6.38 ± 0.01^{ABbc}	6.37 ± 0.10^{ABbc}
	12	6.56 ± 0.15^{Ab}	6.49 ± 0.16^{Ac}	6.29 ± 0.06^{Bc}	6.22 ± 0.01^{Bc}	6.20 ± 0.02^{Bc}
	14	6.78 ± 0.24^{Ab}	6.76 ± 0.03^{Ab}	6.72 ± 0.02^{Aab}	6.60 ± 0.17^{Aab}	6.58 ± 0.08^{Aa}
	16	7.14 ± 0.24^{Aa}	7.00 ± 0.09^{Aa}	6.74 ± 0.12^{Ba}	6.73 ± 0.17^{Ba}	6.70 ± 0.04^{Ba}
PV	**0**	2.00 ± 0.00^{Ad}	2.00 ± 0.00^{Ad}	2.00 ± 0.00^{Ac}	2.00 ± 0.00^{Ac}	2.00 ± 0.00^{Ad}
	4	5.00 ± 0.00^{Ac}	4.99 ± 0.00^{Ac}	4.99 ± 0.00^{Abc}	4.00 ± 0.00^{Bbc}	2.50 ± 0.71^{Ccd}
	8	6.50 ± 0.17^{Ac}	5.00 ± 0.00^{Ac}	4.99 ± 0.00^{Abc}	4.48 ± 2.11^{Ab}	3.98 ± 1.40^{Abc}
	12	9.00 ± 0.00^{Ab}	8.50 ± 0.70^{Ab}	6.50 ± 0.71^{Bab}	6.00 ± 0.00^{Bab}	5.50 ± 0.71^{Bab}
	14	12.50 ± 0.71^{Aa}	9.00 ± 0.00^{Bb}	7.00 ± 0.00^{Cab}	6.99 ± 0.00^{Ca}	5.98 ± 0.00^{Da}
	16	14.00 ± 1.41^{Aa}	9.99 ± 0.01^{Ba}	8.50 ± 0.71^{ABa}	8.00 ± 0.00^{Ca}	6.00 ± 0.00^{Da}
TBARS	**0**	0.63 ± 0.04^{Ae}	0.63 ± 0.04^{Aa}	0.63 ± 0.04^{Ab}	0.63 ± 0.04^{Aa}	0.63 ± 0.04^{Aa}
	4	0.73 ± 0.00^{Ade}	0.60 ± 0.14^{Aa}	0.60 ± 0.14^{Ab}	0.56 ± 0.15^{Aa}	0.47 ± 0.02^{Aa}
	8	0.86 ± 0.00^{Acd}	0.60 ± 0.01^{Ba}	0.58 ± 0.05^{Bb}	0.56 ± 0.11^{Ba}	0.47 ± 0.05^{Ba}
	12	0.95 ± 0.03^{Ac}	0.89 ± 0.02^{Aa}	0.82 ± 0.11^{Aab}	0.56 ± 0.01^{Ba}	0.55 ± 0.14^{Ba}
	14	1.41 ± 0.05^{Ab}	0.86 ± 0.49^{ABa}	0.77 ± 0.19^{ABab}	0.73 ± 0.25^{Aba}	0.48 ± 0.04^{Ba}
	16	2.70 ± 0.12^{Aa}	2.07 ± 0.06^{Ba}	0.96 ± 0.02^{Ca}	0.87 ± 0.08^{Ca}	0.60 ± 0.05^{Da}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF: samples wrapped with gelatin film, O2.5: samples wrapped with gelatin film incorporated with 2.5% OPE, O5 film: samples wrapped with gelatin film incorporated with 5% OPE, O10: samples wrapped with gelatin film incorporated with 10% OPE.

Table 2. Changes in microbiological quality of rainbow trout fillets coated with gelatin films containing OPE during storage at 4°C (log CFU/g)

	Storage (day)	C	CF	O2.5	O5	O10
Total mesophilic bacteria counts	**0**	1.77 ± 0.03^{Ad}	1.77 ± 0.03^{Ae}	1.77 ± 0.03^{Ad}	1.77 ± 0.03^{Ae}	1.77 ± 0.03^{Ae}
	4	1.53 ± 0.07^{Ad}	1.54 ± 0.04^{Af}	1.56 ± 0.09^{Ad}	1.49 ± 0.02^{Af}	1.48 ± 0.00^{Af}
	8	2.63 ± 0.53^{Ac}	2.88 ± 0.17^{Ad}	2.50 ± 0.03^{Ac}	2.58 ± 0.14^{Ad}	2.88 ± 0.17^{Ad}
	12	5.00 ± 0.23^{Ab}	4.46 ± 0.02^{Bc}	4.36 ± 0.16^{Bb}	4.23 ± 0.06^{Bc}	3.19 ± 0.17^{Cc}
	14	5.46 ± 0.01^{Ab}	5.35 ± 0.04^{Ab}	5.22 ± 0.21^{Aa}	4.96 ± 0.03^{Bb}	4.67 ± 0.05^{Cb}
	16	6.22 ± 0.01^{Aa}	6.19 ± 0.01^{Aa}	6.06 ± 0.07^{Aa}	5.81 ± 0.14^{Ba}	5.34 ± 0.05^{Ca}
Total psychrophilic bacteria counts	**0**	1.54 ± 0.06^{Af}	1.54 ± 0.06^{Ae}	1.54 ± 0.06^{Ae}	1.54 ± 0.06^{Ae}	1.54 ± 0.06^{Ae}
	4	1.82 ± 0.04^{Ae}	1.62 ± 0.14^{ABe}	1.54 ± 0.05^{ABe}	1.24 ± 0.23^{Bf}	1.20 ± 0.35^{Be}
	8	3.46 ± 0.02^{Ad}	3.04 ± 0.04^{Bd}	2.76 ± 0.20^{Bd}	2.83 ± 0.02^{Bd}	2.79 ± 0.29^{Bd}
	12	5.43 ± 0.02^{Ac}	5.32 ± 0.00^{Ac}	5.15 ± 0.21^{ABc}	4.90 ± 0.02^{BCc}	4.71 ± 0.12^{Cc}
	14	6.44 ± 0.02^{Ab}	6.43 ± 0.01^{Ab}	6.35 ± 0.02^{Ab}	6.38 ± 0.03^{Ab}	6.14 ± 0.14^{Bb}
	16	7.43 ± 0.01^{Aa}	7.38 ± 0.02^{Aa}	7.31 ± 0.01^{Aa}	7.30 ± 0.00^{Aa}	6.88 ± 0.16^{Ba}
Total yeast and mold counts	**0**	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ad}
	4	1.43 ± 0.21^{Ad}	0.00 ± 0.00^{Bd}	0.00 ± 0.00^{Be}	0.00 ± 0.00^{Bd}	0.00 ± 0.00^{Bd}
	8	1.84 ± 0.02^{Ac}	1.84 ± 0.09^{Ac}	1.68 ± 0.08^{Ad}	1.72 ± 0.06^{Ac}	1.79 ± 0.01^{Ac}
	12	2.01 ± 0.03^{Ac}	1.99 ± 0.30^{Ac}	1.87 ± 0.06^{Ac}	1.97 ± 0.03^{Ac}	1.89 ± 0.04^{Ac}
	14	4.02 ± 0.02^{Ab}	3.94 ± 0.04^{Ab}	3.82 ± 0.05^{ABb}	3.64 ± 0.17^{Bb}	2.82 ± 0.01^{Cb}
	16	4.94 ± 0.03^{Aa}	4.92 ± 0.09^{Aa}	4.83 ± 0.05^{Aa}	4.62 ± 0.31^{Aa}	3.33 ± 0.10^{Ba}
Total Enterobacteriacaea	**0**	1.71 ± 0.16^{Ae}	1.71 ± 0.16^{Ae}	1.71 ± 0.16^{Af}	1.71 ± 0.16^{Af}	1.71 ± 0.16^{Ae}
	4	1.46 ± 0.17^{Ae}	1.50 ± 0.03^{Ad}	0.00 ± 0.00^{Be}	0.00 ± 0.00^{Be}	0.00 ± 0.00^{Bd}
	8	3.28 ± 0.03^{Ac}	3.30 ± 0.05^{Ac}	3.00 ± 0.00^{Bd}	2.85 ± 0.11^{Cd}	3.01 ± 0.01^{Bc}
	12	5.34 ± 0.06^{Ab}	5.29 ± 0.03^{Ab}	5.11 ± 0.04^{Bc}	5.03 ± 0.03^{Bc}	4.84 ± 0.11^{Cb}
	14	5.45 ± 0.00^{Ab}	5.39 ± 0.03^{ABb}	5.39 ± 0.06^{ABb}	5.34 ± 0.04^{ABb}	5.02 ± 0.30^{Bb}
	16	6.48 ± 0.00^{Aa}	6.45 ± 0.02^{ABa}	6.40 ± 0.04^{Ba}	6.33 ± 0.03^{Ca}	6.32 ± 0.00^{Ca}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF: samples wrapped with gelatin film, O2.5: samples wrapped with gelatin film incorporated with 2.5% OPE, O5 film: samples wrapped with gelatin film incorporated with 5% OPE, O10: samples wrapped with gelatin film incorporated with 10% OPE.

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Effects of Feed Additives in Fish Feed for Improvement of Aquaculture

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Abstracts

Nutrition is the major crucial factor determining the potential of cultured fish to exhibit its genetic capability for growth and reproduction. The increased in costs and short quantity of fish feed have given reason to the need to increase research for substitutes .Therefore, the fish feeds require to be enriched with additives. Feed additives are eatable substances that are included in fish feeds in very little quantity to improve the feeds which in returns improve the growth performance and decrease mortality rate in fish. Feed additives could be widely categorized into two classes which are living and non-living. Utilization of inexpensive live feed supplements as feed additives is broadly accepted and embraced as a result of its eco-friendly nature. Hence, just few options are accessible in this class. The living organisms applied as feed are probiotics, Plants and some algae. Probiotics are applicable microorganism in feeding the host. Inclusion of probiotics to the feeds improves the feed conversion ratio and reduces the death rate. Probiotics are proven to have potential to enhance the immunity of fish to response and improve the immune system in fish There are strict laws on the usage of antibiotics and chemotherapeutics in thin the aqua feed industry as a result of bioaccumulation (Lim, et al., 2013). More importance is accorded to feed additives. Recently, many researchers proved the positive potentials of medicinal plants as feed additives. The herbs increased the growth and usage of feed in the fish and likewise decreased diseases through regulation of pathogens in gastrointestinal tract A combination of medicinal plants is applied and found to be effective and have potentials to combat diseases problem, and could supplement insufficiency of nutrients and phytochemicals. Also, treatments of fish with medicinal plants before cooking could improve the taste of the cooked fish. There is urgent need to research more on novel feed additives like inclusion of herbs on fish feeds which reduce feed costs, maximum digestibility and prevention of residual effects of hormones and antibiotics on fish muscles which in return have effects on the human that consumes them.

Key words: Aquaculture, Fish feeds, Feed additives, Probiotics, Plant antioxidant

Review article

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INTRODUCTION

Fishes are one of the excellent and inexpensive source of lean meat and more than half of the population in the world relied on fish for dietary protein source. In the past decade, high importance has been accorded to fish production and their nutrition. Nutrition is the major crucial factor determining the potential of cultured fish to exhibit its genetic capability for growth and reproduction. Live food is the best feed for fish, as it is natural and healthy (Oramary et al., 2016). The most commonly applied fish feed constitutes of fish meals as source of protein, which is between 10-50% of the running costs (Mellen, 1927). Moreso, the most accepted edible fish food habits have been strongly established (Gupta and Banerjee, 2016).The increased in costs and short quantity of fish feed have given reason to the need to increase research for substitutes (Bimbo and Crowther, 1992).Therefore, the fish feeds require to be enriched with additives. Feed additives are eatable substances that are included in fish feeds in very little quantity to improve the feeds which in returns improve the growth performance and decrease mortality rate in fish (Dada, 2015). In aquaculture, enhancement of growth performance of the fish is one of the major crucial goals. Several studies have been performed on the fish feed development. Few of the research examine the effect of natural immune-stimulant additives to fish feed. The inclusion of oils into feeds of aquatic animals, especially fish, enhance the growth performance of the species being cultured, as well as feed consumption and protein utilisation rates (Bell et al., 2000; Montero et al., 2005). There are strict laws on the usage of antibiotics and chemotherapeutics in thin the aqua feed industry as a result of bioaccumulation (Lim, et al., 2013). More Importance is accorded to feed additives. Feed additives could be widely categorized into two classes which are living and non-living. Utilization of inexpensive live feed supplements as feed additives is broadly accepted and embraced as a result of its eco-friendly nature. Hence, just few options are accessible in this class. The living organisms applied as feed are probiotics, Plants and some algae.

Probiotics in fish feeds

Probiotics are applicable microorganism in feeding the host. Inclusion of probiotics to the feeds improves the feed conversion ratio and reduces the death rate. Probiotics are proven to have potential to enhance the immunity of fish to response and improve the immune system in fish (Kumar, et al., 2016).Supplements that includes the host source probiotics such as *B. mycoides* significantly increase stress, tolerance which could assist in live transportation (Ambers, et al., 2015).Recently many of the probiotics supplements are fortified with prebiotics to improve the probiotics actions. Prebiotics are categorized as non-digestible to the host that improve the growth and metabolism of probiotics in the animal gastrointestinal tract. The generally applied prebiotics is referred to as Fructooligosaccharide (FOS) (Ye et al., 2011; Akrami et al., 2013).

Application of Herbs in fish feeds

Nowadays, many researchers proved the positive potentials of medicinal plants as feed additives. The herbs increased the growth and usage of feed in the fish and likewise decreased diseases through regulation of pathogens in gastrointestinal tract (Farahi et al., 2012; Manaf et al., 2016). A combination of medicinal plants is applied and found to be effective and have potentials to combat diseases problem, and could supplement insufficiency of nutrients and phytochemicals. Also, treatments of fish with medicinal plants before cooking could improve the taste of the cooked fish (Agbabiaka et al., 2016). Many researchers established that the usage of

plant based protein in fish feeds increased growth performance (Kumar et al., 2016). Meanwhile some draw their conclusion that soya beans based protein substitute in fish feeds improve fish growth. Hence, the constituents of plant sources of protein includes enzymes inhibitors, toxins such as gossypol tannins, saponins, alaectins, Phytic acid and so on which could affect the fish growth performance negatively (Ali, et al., 2016). The effective ways to overpower the adverse effect of toxins or antin-minerals or anti-vitamins such as phytase could be substituted in the feed. Phytase, have potentials to enhance the availability of phytate-phosphorus in fish feeds (Cain and Garling, 1995; Nwanna, 2007). Moreover the ratio of dietary calcium and phosphorus are essential for the activity of phytatse (Li et al., 2016). The application of probiotics in fish diets could also reduce the adverse effects of anti-nutrients ((Mellen, 1927). Some researchers found positive outcomes on vegetables and other food wastes in fish diets (Akpoilih et al., 2016).Though, some scientists established the application of poultry waste meals in replacement of fish meal due to the fact that poultry waste is cheaper and affordable than fish meals (Yones and Metwalli, 2016). Few studies established that salt could be utilize as additive in fish feeds as growth enhancers (Kumar, et al., 2016). But the utilization of such additives is specific to some particular species and depends on location of the river. Antibiotics and many other chemicals have been tried as growth enhancers, antibacterial and for other several functions in fish and shellfish (Jayaprakas and Sambhu, 1996). The application of theses hormones, antibiotics and other chemicals is discouraged in aquaculture field as a result of the residual effects in fish muscle also in prawns. Plants are of nature source and are safe and affordable, they are proved to promote several activities such as anti-stress, growth enhancer, stimulate appetite and immunostimulation in aquaculture activities (Citarasu and Babu, 2002; Sivaram, et al., 2004). Several extracts from herbs and spices are established to increase animal performance through stimulation of action on gut secretions or through a direct bactericidal effect on gut microflora and moreso, the plants active principles in the diets induce the flow of the digestive enzyme and the growth enhancement in herbs leads to high protein synthesis (Citarasu, 2010).

Uses of Garlic in Fish feed

Garlic is one of the important medicinal herb broadly cultivated in many countries and has played a crucial dietary function as well as medicinal purpose for centuries. Garlic (*Allium sativum*) is a perennial bulb-growing plant which belongs to the genus Allium in the family *Liliaceae* that has been used for decades as a flavouring agent, traditional medicine, and a functional food for improvement of physical and mental health. Garlic has been researched in several forms of extracts: aqueous, ethanol and dried powder (Shin and Kim, 2004). It consists a variety of organosulfur compounds like allicin, ajoene, S-allylcysteine, diallyl disulfide, S-methylcysteine sulfoxide and S-allylcysteine (Chi et al., 1982). The inclusion of the garlic extract proved a significant increment in weight gain and feed efficiency of sterlet sturgeon in 10wks trial. Nonetheless, the parameters are not significantly different between 0.5 and 1% GE groups, while condition factor was highest in 0.5% GE group among treatments. From these results, it was concluded that the addition of 0.5% GE to commercial diet was optimal for growth performance of sterlet sturgeon. In second trial, fish were fed diets with (0.5% GE) and without GE for 5 wk. In all parameters investigated, fish fed diet 0.5% GE present a significant increment, (Shalaby et al., 2006) reported significant increased weight gain, feed efficiency, protein efficiency ratio (PER) and specific growth rate (SGR) in Nile tilapia which were fed diet containing garlic powder of 30.0 g/kg diet. Also, Diab et al. (2002) indicated feeding diet with 2.5% garlic/kg diet yield in the highest growth performance in *O. niloticus*. In the same

species, Abou-Zeid (2002) reported a positive increment in biomass and specific growth rate with garlic inclusion. Metwally (2009) also indicated that the best performance was obtained in Nile tilapia fed diet with garlic powder of 32 g/kg diet. A significant improvement in growth, feed conversion and protein efficiency was seen in rainbow trout fish when fed with diet containing 1.0% garlic (Nya and Austin, 2009). Dietary garlic extract could lead to excessive lipid accumulation in fish flesh as a result of increase in protein utilization for fish fed garlic extract diet can decrease the function of lipid as an energy source for growth, therefore, deposition of lipid was high in garlic extract group than in control. Although there is constant controversy concerning the effect of garlic as growth enhancer for fish, the report suggested that dietary garlic for juvenile sterlet sturgeon (60 to 250 g) can greatly affect growth performance and feed utilization. Protein efficiency ratio and feed efficiency are applied as quality indicator for fish diet and its amino acid balance. Wherefore these factors are applied for evaluation protein utilization and turnover (Shalaby et al., 2006).

Garlic paste

Garlic paste is applied as antibiotic and antibacterial properties, garlic prohibits the multiplication of bacteria, hence, there is properties of antibacterial in garlic. Nutritional content of garlic/ 100g, Moisture: 62.8%, protein: 6.3%, carborhydrates: 29%, Fiber 0.8%,Total fat : 0.1%, Total ash 1.%, calcium: 0.03%, phosphorus: 0.31%, Iron : 0.001%, vitamins C:13mg and Nicotinic acid 0.4mg.The caloric value is 142 per 100gm(Bhosale et al., 2010).

Uses of Rosemary in Fish feed

Rosemary extract is a positive dietary additive to induce effective technical and economical growth of catfish reared. (Turan and Yiğitarslan, 2016) proved that the inclusion of rosemary extracts increased growth and enhanced few nonspecific immunity indicators of tilapia, *Oreochromis mossambicus*. Likewise, rosemary extract improved feed conversion, efficiency of broilers fed diet supplemented with such herb (Ghazalah and Ali, 2008). Cristea et al. (2012) indicated that fish of feed additives improve the digestibility and utilization efficiency of nutrients in aquaculture.

Cumin Powder

Animal scientist found out that cumin stimulates the secretion of pancreatic enzymes, crucial factors in nutrient digestibility and assimilation. Nutritional content of cumin /100gms , carborhydrates 44.24gm, proteins 17.819gm, fats 1.535, dietary fibers 10.5gm. iron 66.36mg, sodium 168mg, zinc 4.8mg,ncalcium 931mg, vitamins like vit . A 64mg, thiamine B_1 0.628mg, riboflavin B_2 0.327mg, niacin B_3 4.579mg, foliate B_9 10mg, vitamin C 7.7mg and E 3.38mg (USDA, 2008).

Black cumin Oil

Black cumin is a cultivated seed that has been known since acient time. The nutrient level of the black seed consists of 20.8% crude protein, 3.7% ash, 7.0% moisture, 34.8% lipids, and 33.7% carbohydrate (Atta, 2003). Black cumin's substances have potent to act as antibacterial, antifungal, antiviral, antiprotozoal, antihistaminic, anti-oxidant, anti-inflammatory, and immunostimulant. Specifically, it is apply to treat several health issues such as asthma, hypertension, inflammation, cough, bronchitis, headache, eczema, flu fever, and dizziness (Altınrterim, 2010). Supplement of black cumin added to fish feed yield positive effects on the

growth rate of fish and resulted in lower microbial activity during storage period (Öz, 2013). Black cumin oil is also very active in some pathogenic Gram-positive and Gram-negative bacteria (Hanafy and Hatem, 1991). According to Öz et al. (2017) in a research on the effects of black cumin oil (*Nigella sativa*) on sensory, chemical and microbiological properties of rainbow trout during 23 days of storage at 2±1 ºC. The result of the study showed the total sensory scores of the cooked rainbow trout reduced throughout the storage period. There was no significant differences between all groups in terms of sensory parameters ($p>0.05$). The sensory result shows that addition of black cumin to rainbow trout feed had no influence on fish in terms of flavor, aroma, or color; the control group and the other groups showed similar in overall acceptability by the panelists. Also changes in TVB-N values of rainbow trout kept in ice, during storage, the result showed significant differences in the TVB-N content of rainbow trout, at the end of storage, TVB-N did not reach the limit of 35 mg/100 g treated with black cumin Oil (Öz, et al., 2017). In the study carried out by Öz et al. (2018) on the Effect of black cumin oil (*Nigella sativa*) on the growth performance, body composition and fatty acid profile of rainbow trout (*Oncorhynchus mykiss*) The results showed that black cumin oil affected protein content and the differences between groups were statistically significant ($p<0.05$). Also, from the same work, on the results of lipid, the quantity of black cumin oil in the feed increased the total amount of lipids in the fish. The crude ash level in the study were also statistically significant differences. The study of (Öz, et al., 2018) showed that rainbow trout fed with black cumin oil supplements have higher raw protein, raw cinder, lipid and proportion of dry matter when compared to the results given by others in several studies. Also, in another research carried out by Öz (2018) on the effects of black cumin (*Nigella sativa*) oil on ammonia and biogenic amine production in rainbow trout, In the study, the author found that initial histamine level at the beginning of the storage period was 0.01–0.06 mg/100 g and it remained under 2 mg/100 g throughout the storage period. Histamine levels of 5 mg/100 g, is the legal limit determined by the FDA in trout muscle (FDA, 1995), was not seen in any of the groups throughout the study period, the conclusion on the effect of black cumin on histamine is that , black cumin oil has potent to reduce histamine in trout muscle. The results of the study of Öz (2018) suggest that the inclusion of black cumin oil decreased ammonia and biogenic amine production in rainbow trout.

CONCLUSION

Fish and shellfish are valuable and inexpensive sources of Omega fatty acids and several other important nutrients for human consumption. There is constant need to increase aquaculture production and management. There is urgent need to research more on novel feed additives like inclusion of herbs on fish feeds which reduce feed costs, maximum digestibility and prevention of residual effects of hormones and antibiotics on fish muscles which in returns have effects on human that consumes them.

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Essential Oils for Applications in Fish and Other Seafood Products

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Abstract

The global concern is to produce products more natural and fresh, less processed and less ready to eat processed food. Due to their nutritional value, fish and other seafood products have been considered among the most important food in human diets and their consumption increased substantially over the past few decades. Seafood is highly perishable food products due basically to microbiological growth and lipid oxidation, which are known to be the principal causes of quality deterioration of such products. Recently, there is an extensive focus on antioxidant and antimicrobial effects of natural preservatives such as essential oils (EOs), as an effective alternative to synthetic additives, to enhance oxidative and microbial stability of foods and extend their shelf life. The antioxidative and antimicrobial activities of some common EOs, either alone or in combination with other preservative systems, in fish and other seafood are mentioned. The main chemical components and principal sources, According to the biological properties of EOs, the major compounds in EOs are present 85% of the oil. As chemical properties, the EOs are consist of a differing family of organic compounds with low molecular weight and they can be divided into different groups depending on their chemical structure: terpenes, terpenoids, aromatic (phenylpropanoids) and other compounds. In the Application of EOs to fish preservation, the effectiveness of a wide range of EOs against lipid oxidation and microbial growth was extensively documented by many researchers. It was reported that oregano EO is the most frequently used for applications as fish preservatives, followed by rosemary and thyme EOs.

Keywords: Essential oils, fish, microbial stability, preservation, oxidation, quality, shelf life, natural additives

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INTRODUCTION

Nowadays, the global concern is to produce products more natural and fresh, less processed and less ready to eat processed food. Due to their nutritional value, fish and other seafood products have been considered among the most important food in human diets and their consumption increased substantially over the past few decades (Ghanbari et al., 2013; Samples, 2015a).

Essential fatty acids (EFA) are critical aspects for animal tissues in energy production especially seafood. The major EFA in seafood is polyunsaturated fatty acids such as omega 3 and omega 6. Oxidative degradation of lipids produces off-flavor and off-odor which cause the low quality of seafood product (Ramanathan and Das, 1992). To prevent these results from losing product quality, preservation methods are required to apply on seafood products and they will help to extend the shelf life of the product.

Essential oils (EOs) are produced from different parts of plants and their mechanisms have been approved against microorganisms. EOs contain natural antioxidant and antimicrobial agents that are extracted from different parts of the plant as a complex mixture of hundreds of individual aromatic volatile oily compounds (Calo et al., 2015; Jayasena and Jo, 2013). There are more than 3000 types of EOs while 300 from them are commercial needs for applications in food or other industries (Bakkali et al., 2008; Burt, 2004).

Main chemical components and principal sources

According to the biological properties of EOs, the major compounds in EOs are present 85% of the oil while, the other compounds are minor compounds that are present in trace quantities and may have combined with other compounds (Bakkali et al., 2008; Burt, 2004).

As chemical properties, the EOs are consists of a differing family of organic compounds with low molecular weight and they can be divided to different groups depending on their chemical structure: terpenes, terpenoids, aromatic (phenylpropanoids) and other compounds (Bakkali et al., 2008; Hyldgaard et al., 2012). Terpenes are hydrocarbons contains several isoprene units, which have been classified by the number of isoprene units in the molecule (mono-, sesqui- and diterpenes). Terpenoids are different from terpens by containing oxygen and can be classified into alcohols, esters, aldehydes, ketones, ethers, and phenols. Famous terpenoids in EOs are thymol, carvacrol, linalool, linalyl acetate, citronellal, piperitone, menthol, and geraniol, while eugenol and cinnamaldehyde, which are the best-known phenylpropanoids (Hyldgaard et al., 2012; Jayasena and Jo, 2013). The main phenolic compounds are responsible for the preservative effects of EOs: thymol, carvacrol, and eugenol (Burt, 2004; Jayasena and Jo, 2014). In previously published research, they examined several types of EOs such as oregano, rosemary, thyme, laurel, sage, cinnamon, clove, and basil in seafood products used as antimicrobial and antioxidant agents.

Oregano leaves (*Origanum vulgare*) are commercial spices that belong to Mediterranean cuisine used in raw or cooked food because of their pleasant aroma and taste. The antioxidant and antimicrobial activities of oregano EO were examined in different commercial or model food (Goulas and Kontominas, 2007; Vatavali wt al., 2013). It had documented that the carvacrol and thymol are the main compounds responsible for the antimicrobial and antioxidant activity of oregano EO (Rodriguez-Garcia et al., 2016). Also, it was reported that the thyme EO has a high content of phenolic compounds. Thus, thyme (*Thymus vulgaris*) EO can be a potential natural antioxidant and antimicrobial agents (Hyldgaard et al., 2012; Kostaki et al., 2009).

Basil EO was used in flavoring food for several years. Also, basil EO has been reported that it has an effect against microorganisms from its antimicrobial activity (Suppakul et al., 2003). This activity has applied to the major active volatile components, including linalool, methylchavicol, eugenol, methyl eugenol, methyl cinnamate, 1,8-cineole, and caryophyllene (Kuorwel et al., 2011; Perricone et al., 2015).

Recently, many of EOs which are extracted from clove (*Eugenia caryophyllata*) (Emir Çoban & Patir, 2013), sage (Salvia officinalis L.) (Emir Çoban et al., 2016), Zataria multiflora Boiss (Emir

Çoban and Tuna Kelestemur, 2016), turmeric and lemongrass (Masniyom et al., 2012), and lemon (Alfonzo et al., 2017) have been applied on seafood products alone or combination with other preservation methods, was effective in extending the shelf life and improving the quality of the products.

Methods of application

Commonly in the seafood industry, EOs have been applied in seafood products by different methods:
One of them is the direct treatment which is added directly to the seafood products during manufacturing and processing. Also, EOs can be used as edible films and coatings and the addition of EOs to animal feed. Thus, some researchers have been reported the use of edible coating films enriched with EOs as alternative and interesting option in order to reduce the required doses (Dogan and Izci, 2017; Ojagh et al., 2010; Sanchez-Gonzalez et al., 2011; Yuan et al., 2016). in addition, recent research has been documented another technique to reduce the organoleptic effects of EOs using the preparation of micro- and nanoemulsions, which improves not only the antimicrobial and antioxidant stability, but also the functional properties and organoleptic quality of the product (Acevedo-Fani et al., 2016; Alfonzo et al., 2017; Calo et al., 2015; Ozogul et al., 2017; Perricone et al., 2015).

Mechanisms of action

1- Antimicrobial activities

EOs in high concentration can inhibit the bacteria growth (bacteriostatic), that means the microbial cells will recover their reproductive capacity after neutralization of the agent, or to kill Bacterial cells (bactericide) (Swamy et al., 2016). It was documented that lipoteichoic acids in the cell membrane of gram-positive bacteria may facilitate the penetration of hydrophobic compounds of EOs, while the presence of an extrinsic membrane, surrounding the cell wall of gram-negative bacteria limits the diffusion rate of hydrophobic compounds through the lipopolysaccharide layer. That is why gram-positive bacteria are slightly more susceptible to EOs than gram-negative ones (Rodriguez-Garcia et al., 2016; Tongnuanchan and Benjakul, 2014). Even though that the possible modes of action for EOs as antimicrobial agents have been widely reviewed, their exact mechanism of action is not yet clear (Calo et al., 2015; Maqsood et al., 2013;Tajkarimi et al., 2010). Numerous studies were described that the antimicrobial activity of EOs could be attributed to their major constituents mainly the phenolic constituents, also their interaction with minor constituents present in oils (Burt, 2004; Hyldgaard et al., 2012; Jayasena and Jo, 2013; Perricone et al., 2015). Because of the complexity of the chemical composition of EOs, it has been documented that the antimicrobial activity of EOs may not be attributable to a unique mechanism (Burt, 2004). Nonetheless, there was almost a universal agreement on the fact that the hydrophobicity of compounds present in EOs enabled them to pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. Additionally, EOs could inhibit various enzyme systems including the enzymes responsible for the regulation of energy and synthesis of structural components (Bakkali et al., 2008; Burt, 2004; Jayasena and Jo, 2013).

2- Antioxidant activities

Previously, synthetic additives such as BHA and BHT were used as antioxidants in food products. Recently, the food industries focused on natural sources for antioxidants due to the safety concern of synthetic chemical toxicity (Vareltzis et al., 1997). Because of that, the using of natural antioxidants has been increased in food processing especially in seafood products for controlling lipid oxidation

thus extending the shelf life of the products. Therefore, the effort of extracting natural antioxidants from natural sources has been increased such as phenolic compounds. The use of EOs could be considered as a good alternative since the majority of EOs are classified as generally recognized as safe (GRAS) (Kapetanakou and Skandamis, 2016; Maqsood et al., 2013; Ribeiro-Santos et al., 2017). The EOs application is getting interested as natural antioxidants because of the inherent ability of some of their components to stop or delay the oxidation of lipids and extend the shelf life of the food products (Amorati et al., 2013; Patel, 2015). Several studies demonstrated that the EOs, as antioxidants, have several modes of direct or indirect actions including, among other mechanisms, prevention of chain initiation and free-radical scavenging activity (Maqsood et al., 2013; Rodriguez-Garcia et al., 2016). Also, it was reported that phenolic compounds such as carvacrol, eugenol, and thymol are the main group responsible for the antioxidant activity of Eos (Amorati et al., 2013; Jayasena and Jo, 2014). The principle of phenolic compounds in the retardation of lipid oxidation in fish muscle is mainly because of their redox properties, allowing them to act as hydrogen donors, reducing agents, singlet oxygen quenchers as well as metal chelators (Maqsood et al., 2014; Tongnuanchan and Benjakul, 2014). Several methods have been used to determine the antioxidant performance of EOs.

Application of EOs to fish preservation

Recently, the effectiveness of a wide range of EOs against lipid oxidation and microbial growth was extensively documented by many researchers. It was reported that oregano EO is the most frequently used for applications as fish preservatives, followed by rosemary and thyme EOs (Patel, 2015). Different effects were observed depending on the EO used its concentration, as well as the characteristics of the raw material. However, it should be considered that EOs used as a natural food additive at high concentrations may lead to undesirable sensory properties on treated fish and may even cause allergic reactions. Indeed, some EOs are characterized by a strong odor and flavor which could leave a bad aftertaste, thus reducing the acceptance or liking degree for fish and seafood products (Atares and Chiralt, 2016; Ribeiro-Santos et al., 2017).

CONCLUSION

Different EOs incorporated directly into fish and other seafood, or applied indirectly by other methods, can effectively inhibit or reduce lipid oxidation and growth of various microorganisms. Many EOs could be used alone or in combination with other preservative treatments to further prevent or retard oxidation and microbial spoilage in food systems, especially in fish and fish products, thereby extending the shelf life of these products. Being the principal constituents of EOs, many authors reported that phenolic compounds are mainly responsible for their antimicrobial and antioxidant properties.

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