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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 (day)	C	CF	O2.5	
pH	**0**	6.53 ± 0.21^{Ab}	6.53 ± 0.21^{Ac}	6.53 ± 0.21^{Ab}	
	4	6.64 ± 0.00^{Ab}	6.42 ± 0.13^{Cc}	6.30 ± 0.06^{Ec}	
	8	6.51 ± 0.04^{Ab}	6.48 ± 0.13^{Ac}	6.31 ± 0.07^{Bc}	
	12	6.56 ± 0.15^{Ab}	6.49 ± 0.16^{Ac}	6.29 ± 0.06^{Bc}	
	14	6.78 ± 0.24^{Ab}	6.76 ± 0.03^{Ab}	6.72 ± 0.02^{Aab}	
	16	7.14 ± 0.24^{Aa}	7.00 ± 0.09^{Aa}	6.74 ± 0.12^{Ba}	
PV	**0**	2.00 ± 0.00^{Ad}	2.00 ± 0.00^{Ad}	2.00 ± 0.00^{Ac}	
	4	5.00 ± 0.00^{Ac}	4.99 ± 0.00^{Ac}	4.99 ± 0.00^{Abc}	
	8	6.50 ± 0.17^{Ac}	5.00 ± 0.00^{Ac}	4.99 ± 0.00^{Abc}	
	12	9.00 ± 0.00^{Ab}	8.50 ± 0.70^{Ab}	6.50 ± 0.71^{Bab}	
	14	12.50 ± 0.71^{Aa}	9.00 ± 0.00^{Bb}	7.00 ± 0.00^{Cab}	
	16	14.00 ± 1.41^{Aa}	9.99 ± 0.01^{Ba}	8.50 ± 0.71^{ABa}	
TBARS	**0**	0.63 ± 0.04^{Ae}	0.63 ± 0.04^{Aa}	0.63 ± 0.04^{Ab}	
	4	0.73 ± 0.00^{Ade}	0.60 ± 0.14^{Aa}	0.60 ± 0.14^{Ab}	
	8	0.86 ± 0.00^{Acd}	0.60 ± 0.01^{Ba}	0.58 ± 0.05^{Bb}	
	12	0.95 ± 0.03^{Ac}	0.89 ± 0.02^{Aa}	0.82 ± 0.11^{Aab}	
	14	1.41 ± 0.05^{Ab}	0.86 ± 0.49^{ABa}	0.77 ± 0.19^{ABab}	
	16	2.70 ± 0.12^{Aa}	2.07 ± 0.06^{Ba}	0.96 ± 0.02^{Ca}	

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

	Storage (day)	C	CF	O2.5	
Total mesophilic bacteria counts	**0**	1.77 ± 0.03^{Ad}	1.77 ± 0.03^{Ae}	1.77 ± 0.03^{Ad}	
	4	1.53 ± 0.07^{Ad}	1.54 ± 0.04^{Af}	1.56 ± 0.09^{Ad}	
	8	2.63 ± 0.53^{Ac}	2.88 ± 0.17^{Ad}	2.50 ± 0.03^{Ac}	
	12	5.00 ± 0.23^{Ab}	4.46 ± 0.02^{Bc}	4.36 ± 0.16^{Bb}	
	14	5.46 ± 0.01^{Ab}	5.35 ± 0.04^{Ab}	5.22 ± 0.21^{Aa}	
	16	6.22 ± 0.01^{Aa}	6.19 ± 0.01^{Aa}	6.06 ± 0.07^{Aa}	
Total psychrophilic bacteria counts	**0**	1.54 ± 0.06^{Af}	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}	
	8	3.46 ± 0.02^{Ad}	3.04 ± 0.04^{Bd}	2.76 ± 0.20^{Bd}	
	12	5.43 ± 0.02^{Ac}	5.32 ± 0.00^{Ac}	5.15 ± 0.21^{ABc}	
	14	6.44 ± 0.02^{Ab}	6.43 ± 0.01^{Ab}	6.35 ± 0.02^{Ab}	
	16	7.43 ± 0.01^{Aa}	7.38 ± 0.02^{Aa}	7.31 ± 0.01^{Aa}	
Total yeast and mold counts	**0**	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	
	4	1.43 ± 0.21^{Ad}	0.00 ± 0.00^{Bd}	0.00 ± 0.00^{Be}	
	8	1.84 ± 0.02^{Ac}	1.84 ± 0.09^{Ac}	1.68 ± 0.08^{Ad}	
	12	2.01 ± 0.03^{Ac}	1.99 ± 0.30^{Ac}	1.87 ± 0.06^{Ac}	
	14	4.02 ± 0.02^{Ab}	3.94 ± 0.04^{Ab}	3.82 ± 0.05^{ABb}	
	16	4.94 ± 0.03^{Aa}	4.92 ± 0.09^{Aa}	4.83 ± 0.05^{Aa}	
Total Enterobacteriacaea	**0**	1.71 ± 0.16^{Ae}	1.71 ± 0.16^{Ae}	1.71 ± 0.16^{Af}	
	4	1.46 ± 0.17^{Ae}	1.50 ± 0.03^{Ad}	0.00 ± 0.00^{Be}	
	8	3.28 ± 0.03^{Ac}	3.30 ± 0.05^{Ac}	3.00 ± 0.00^{Bd}	
	12	5.34 ± 0.06^{Ab}	5.29 ± 0.03^{Ab}	5.11 ± 0.04^{Bc}	
	14	5.45 ± 0.00^{Ab}	5.39 ± 0.03^{ABb}	5.39 ± 0.06^{ABb}	
	16	6.48 ± 0.00^{Aa}	6.45 ± 0.02^{ABa}	6.40 ± 0.04^{Ba}	

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