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Original article

Physicochemical and antimicrobial effects of gelatin-based edible films incorporated with garlic peel extract on the rainbow trout fillets

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Summary. Influence of gelatin-based edible films incorporated with garlic peel extract (GPE) with concentration of 4% and 8% (by volume per mass of gelatin) on the microbiological, sensorial and physicochemical quality of rainbow trout fillets during refrigerated storage at 4±1 °C were evaluated. Gelatin films enriched with GPE retarted the total mesophilic and total psychrophilic bacteria, and Enterobacteriaceae counts during the storage period. Compared to control samples, lipid oxidation was delayed in the samples wrapped with gelatin films incorporated with GPE, especially in concentration of 8%. The sensory results are correlated with physicochemical and microbiological results. The shelf-life of rainbow trout fillets was found 5 days for control and the fillets wrapped with gelatin film without GPE, respectively, while the shelf life of fish was 10 days for the fillets wrapped with gelatin film incorporated with GPE. According to results of the study, the incorporation of GPE into gelatin film could enhance the both antimicrobial and antioxidative characteristics of the film. Therefore, gelatin film enriched with GPE efficient in maintaining the qualities of the rainbow trout fillets during refrigerated storage.

Key words: garlic peel extract, edible films, gelatin, rainbow trout, shelf life

Introduction

Fresh fish is one of the most important product due to its high nutritional value such as high level of omega-3 fatty acids, proteins and vitamins. However, it is one of the most perishable product which is susceptible to both microbiological and chemical deterioration during storage (1). Therefore, maintenance the freshness of fish with favorable preservation techniques is required. Temperature-based preservation techniques such as chilling and freezing had beed used in fish and fish products (2). With the increasing demand for high quality product with the extended shelf-life and minimal processing has promoted the development of several innovative techniques (3).

Edible films and coatings are a promosing trend in the last years to improve the shelf-life of perishable food products and to answer reliable product demands of the consumers (4,5). In addition, edible films and coatings can prevent lipid oxidation, color deterioration and enhance the product quality (6) by acting as moisture, oxygen, carbon dioxide or vapour barriers (7). Nowadays, there is an increasing attention to use of edible films incorporating with essential oils and plant extracts with antioxidant and antimicrobial properties.

Garlic (*Allium sativum* L.) is a popular food ingredient and also has been used as a medicine to treat various human diseases from time immemorial (8,9). Additionally, garlic has higher concentrations of phenolic compounds compared with other vegetables (10). In general, garlic is consumed as fresh, however, dried garlic slices and powder find approval in recent years as well (11). Because of the discarded peels after process-

ing and the consumption of garlic, massive garlic peel become one of the promising by-product. 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. Ifesan et al. (12) reported that ethanolic extract of garlic peel extract (GPE) demonstrated both antioxidant and antibacterial activities in cooked beef. There is very limited study on the evaluation of GPE. Additionally, there is no information on the incorporation of gelatine based edible film and GPE. Therefore, the objective of this investigation was to evaluate the effects of gelatin-based film incorporated with GPE on the chemical, sensorial and microbiological properties of rainbow trout (*Oncorhynchus mykiss*) fillets during storage at 4 °C.

Material and Methods

Extraction of garlic peels

Garlic peels (GPs) were collected from the local markets in Nigde, Turkey. After washed twice in tap water, the peels were dried at 45 °C for 48 h and ground into powder with a blender. For the extraction procedure, the method of Ifesan et al. (12) was used. GPs powder and etanol solvent (80 %) were stirred (1:10, g:mL) in a flask for 24 h. After extraction procedure, the garlic peel extracts (GPE) were filtered and concentrated by using rotary evaporator (IKA, HB-10 digital, Germany) at 45 °C under vacuum.

Preperation of gelatin films

Gelatin films were prepared according to method of Gomez-Estaca et al. (13) 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 °C for 15 min. Garlic peel extract (GPE) was added to the film solution in concentration of 4 % and 8 % (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.

Preperation of samples

Rainbow trout (*Oncorhynchus mykiss*) fillets were provided from a fish farm in Niğde, Turkey and transported to the laboratory in ice boxes. They were washed after gutted, beheaded and filletted. The average weight and length of fish were 199.92±13.96 g and 17.44±1.29 cm, respectively. Two fillets were obtained from each fish. The fillets were divided into four groups. The fish fillets were wrapped according to Ahmad et al. (14) 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 0 % GPE (G0), second group was coated with gelatin film 4 % GPE (G4), third group was coated with gelatin film 8 % GPE (G8) 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 4±1 °C for 10 days.

Physicochemical analysis

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 distilled water (1:1, w:v) (15).

Total volatile basic nitrogen (TVB-N) was determined according to Schormüller (16). 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 500 mL conical flask with addition of tashiro-indicator. After distillation, the 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 (17). Approximately 2 g sample was stirred with 30 mL of solution including 3 chloroform:2 glacial 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 reactive substances (TBARS) analysis was conducted using the method of Tarladgis

et al. (18). 10 g of fish sample was steam distilled with 2.5 mL HCl: distilled water (1:2). 5 mL of the distillate was mixed with 5 mL thiobarbituric acid (TBA) solution (0.288 g/100 mL). Then the mixture put in the water bath at 110 ˚C for 35 min for the color reactions. The absorbance was measured with a UV–VIS spectrophotometer at 538 nm against a blank containing distilled water and TBA solution. The results were expressed as mg malonaldehyde/kg fish flesh.

Microbiological analyses

Fish sample (10 g) was mixed with 90 mL prechilled 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 °C for 7 days and 37 °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 °C for 36-48 h.

Sensory analysis

For the sensory evaluation the method of Amerina et al. (19) was used with slight modification. Eight experienced panellists were evaluated the sensory characteristics of fish samples in terms of odour, texture, color and general acceptance by use of a nine-point hedonic scale. A score of 9-7 indicated "very good", a score of 6.9-4.0 "good", a score of 3.9-1.0 denoted as spoiled.

Statistical analysis

All measurements were performed in triplicate and analysis was carried out using the SAS software (Statistical Analysis System, Cary, NC, USA). Variance analysis (ANOVA) was used to evaluate the data and 5% significance level of Duncan's test was based to compare the differences between means of parameters.

Results and Discussion

pH value

pH value of the rainbow trout fillets wrapped with gelatine films incorporated with GPE and without GPE during storage at 4 °C for 10 days is shown in Table 1. The initial pH of the rainbow trout fillets was determined as 6.35. pH of the control samples was significantly ($P<0.05$) higher than those of the samples wrapped with gelatin film and increased to 7.18 at the end of the storage. Baygar et al. (20) reported that the pH value is between 6.0-6.5 in fresh fish. Additionally, according to Ludorf and Mayer (21) the acceptable limit of pH value for fresh fish is between 6.8 and 7.0. Generally, pH value of the all samples showed increase at the end of the storage and reached 6.76, 6.88 and 6.67 in the group G0, G4 and G8, respectively. It was reported that the accumulation of alkaline compounds such as ammonia and TMA, etc. results in the increase in pH value (22). Alparslan et al. (23) reported an increase in pH of rainbow trout fillets wrapped with gelatin film enriched with laurel essential oil and reached 6.61 at the end of the storage (26th day). They observed lower pH values in the fillets wrapped with gelatin film containing laurel esseantial oil. Fadiloglu and Coban (24) determined the initial pH of rainbow trout fillets treated with chitosan coating as 7.07 and finally increased to 7.77 after 12 days. In the present study, the lower increase in the pH value of rainbow trout fillets wrapped with gelatin film incorporated with 8% GPE was observed compared with unwrapped groups ($P<0.05$).

Total volatile basic nitrogen (TVB-N)

TVB-N is widely used as an indicator of the quality of fresh fish. TVB-N content of rainbow trout fillets coated with and without gelatin film (gelatin film or GPE film) during storage is shown in Table 1. At the beginning of the storage, TVB-N value of fresh fish was 18.2 mg N/100 g and showed increase in all groups during the storage period. It was observed that the control group demonstrated significantly ($P<0.05$) higher values than those of the samples coated with gelatin film incorporated with GPE. At the end of the storage, TVB-N values reached 42.70, 39.90, 34.30 and 30.10 mg N/100 g in control, G0, G4 and G8 groups, respectively. It was suggested that TVB-N content in freshly caught fish is typically between 5 and 20 mg N/100 g, and TVB content of 30-35 mg N/100 g is usually regarded as the limit of acceptability for fish (25). Control and G0 groups exceeded the limit value at the 5th and 7th day of the storage, where-

as fillets wrapped with gelatin film containing GPE reached unacceptable limit at the end of the storage. During the storage period, significantly (P<0.05) lower TVB-N values were found in G4 and G8 groups compared to the control and G0 samples. The increase of TVB-N is related to the the activity of spoilage bacteria and endogenous enzymes (26). This shows that the TVB-N results are related to microbiological findings. Similar studies reported that, fish fillets coated with edible films containing different concentrations of extracts and essential oils showed lower TVB-N values compared to uncoated samples during storage period (14, 23, 24).

Peroxide value (PV)

Lipid oxidation is the main cause of fish spoilage after microbial growth. Yuan et al. (27) reported that the application of edible films and coatings with incorpation of antioxidants represents a new approach to solve oxidation problem in food products. Peroxide value (PV) is a measurement of peroxides and hydroperoxides which are the primary oxidation products (28). The effect of gelatin film on the changes of PV of rainbow trout fillets is represented in Table 1. At the beginning of the storage PV in the fillets was determined as 2.0 meq O_2/kg and increased with the storage time in all samples. At the end of the storage, PV of the rainbow trout fillets reached 8.99, 8.74, 6.99 and 5.49 meq O_2/kg in control, G0, G4 and G8 groups, respectively. Control group exhibited higher value of PV than those of the samples coated with gelatin film. Significantly (P<0.05) lower PV was observed in the rainbow trout fillets wrapped with gelatin film incorporated with GPE. Varlık et al. (29) reported that a PV of less than 2 meq O_2/kg fish as "very good," up to 5 meq O_2/kg as "good," and 8-10 meq O_2/kg as at acceptability limit. According to literature, control group and the samples wrapped with gelatin film without the addition of GPE exceeded this limit value at day 5, while the samples wrapped with gelatin film containing 4% and 8% exceeded limit value at day 7 and 10, respectively. Similar results were observed in rainbow trout fillets coated with chitosan and gelatin film (23,24). In this study, incorporation of 8% GPE and gelatin film was much more effective in retarding the lipid oxidation of rainbow trout fillets during refrigerated storage.

Thiobarbituric acid reactive substance (TBARS)

Thiobarbituric acid reactive substances (TBARS) is an index of lipid oxidation which is widely used for the evaluation of secondary lipid degree (30). The initial TBARS value of rainbow trout fillet was 0.45 mgMDA/kg and increased in all samples during the storage priod (Table 1). TBARS values of the control and the samples coated with gelatin film without GPE were higher than those of the samples wrapped with gelatin film incorporated with GPE. At the end of the storage TBARS values of the rainbow trout fillets were found as 1.38, 1.42, 1.25 and 1.11 mgMDA/kg in control, G0, G4 and G8 samples, respectively. During the storage, fish fillets wrapped with gelatin film containing 8% GPE showed significantly (P<0.05) lower TBARS value. The results of TBARS revelaed that lipid oxidation in rainbow trout fillets could be retarded with the use of gelatin film enriched with GPE. Similar results were observed by Ahmad et al. (14) who described the delay of lipid oxidation in sea bass slices coated with gelatin films combined with lemograss essential oil (LEO) as a result of the antioxidant property of LEO. In the present study, it can be suggested that gelatin film incorporated with GPE could show low oxygen permeability due to antoxidant characteristics of GPE. Alparslan et al. (23) observed the the initial TBARS value of rainbow trout fillets as 0.03 mgMDA/kg and they reported lower TBARS value in the samples coated with gelatin films containing laurel essential oil. Martinez et al. (31) reported 0.62 mg MDA/kg of TBARS value for seabass at the beginning. Alsaggaf et al. (28) found lower TBARS value of Nile tilapia coated with chitosan film incorporated with pomegranate peel extract and TBARS value of the coated fish ranged between 0.21 and 0.32 mgMDA/kg during 30 days storage. It was reported that edible films and coatings serve as barriers against water vapor, gases, and flavor compounds and improving structural integrity and mechanical-handling properties of foods (32). Jeon et al. (30) observed that chitosan-coating reduced moisture loss and lipid oxidation of Atlantic cod and herring. Addition of GPE into the gelatin film enhanced the antioxidant property of gelatin film and showed lower TBARS value in the rainbow trout fillets than that of the uncoated samples.

Table 1. Changes in physicochemical properties of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4°C

	Storage period (days)	C	G0	G4	G8
pH	0	6.35±0.18^{Ad}	6.35±0.18^{Ac}	6.35±0.18^{Ad}	6.35±0.18^{Ab}
	2	6.75±0.00^{Bc}	6.88±0.00^{Aa}	6.54±0.01^{Cc}	6.49±0.08^{Cb}
	5	6.92±0.00^{Bb}	6.75±0.00^{Cb}	6.94±0.00^{Aa}	6.74±0.01^{Da}
	7	7.07±0.00^{Aa}	6.65±0.01^{Db}	6.74±0.01^{Bb}	6.69±0.00^{Ca}
	10	7.18±0.01^{Aa}	6.76±0.01^{Dab}	6.88±0.02^{Bab}	6.67±0.04^{Ca}
TVB-N	0	18.20±3.96^{Ae}	18.20±3.96^{Ae}	18.20±3.96^{Ad}	18.20±3.96^{Ad}
	2	26.60±1.98^{Ad}	25.90±0.99^{Ad}	21.70±0.99^{Bcd}	19.60±1.98^{Bd}
	5	35.70±0.99^{Ac}	30.10±0.99^{Bc}	25.20±1.98^{Cc}	23.10±0.99^{Dc}
	7	39.90±0.99^{Ab}	37.10±0.99^{ABb}	28.00±1.98^{Cb}	25.90±0.99^{Db}
	10	42.70±0.99^{Aa}	39.90±0.99^{Ba}	34.30±0.99^{Ca}	30.10±0.99^{Da}
Peroxide value	0	2.00±0.00^{Ac}	2.00±0.00^{Ac}	2.00±0.00^{Ac}	2.00±0.00^{Ac}
	2	3.00±0.00^{Ac}	2.97±0.00^{Bc}	2.00±0.00^{Cc}	1.98±0.00^{Cc}
	5	5.49±0.71^{ABb}	6.49±0.71^{Ab}	4.50±0.71^{ABb}	3.49±0.70^{Bb}
	7	9.49±0.69^{Aa}	9.00±1.41^{Aa}	5.50±0.71^{Bb}	3.00±0.00^{Cbc}
	10	8.99±0.02^{Aa}	8.74±0.70^{Aa}	6.99±0.01^{Ba}	5.49±0.71^{Ca}
TBARS	0	0.45±0.14^{Ac}	0.45±0.14^{Ad}	0.45±0.14^{Ad}	0.45±0.14^{Ac}
	2	0.63±0.02^{Ac}	0.60±0.01^{Ad}	0.62±0.03^{Acd}	0.47±0.01^{Bc}
	5	0.97±0.13^{Ab}	0.81±0.05^{ABc}	0.71±0.02^{Bc}	0.65±0.02^{Bc}
	7	1.09±0.02^{Ab}	1.05±0.07^{Ab}	0.99±0.03^{ABb}	0.90±0.03^{Bb}
	10	1.38±0.02^{Aa}	1.42±0.02^{Aa}	1.25±0.04^{ABa}	1.11±0.11^{Ba}

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, G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

Microbiological analyses

Total viable count (TVC) of rainbow trout fillets wrapped without and with films (gelatin film or GPE film) during storage at 4°C is shown in Fig. 1. At the beginning, the number of bacteria in trout samples was found as 2.27 log CFU/g which is lower than the initial number of rainbow trout fillets reported by the other researchers (7,33-35). In the control and gelatin film wrapped without GPE samples TVC increased significantly (P<0.05) and finally reached 7.47 and 7.30 log CFU/g, respectively. It was observed that TVC showed significant (P<0.05) increase in all groups entire the storage. However, this value did not exceed 7.0 log CFU/g which is upper limit value (36) in the samples coated with gelatin film contatining GPE. Significantly (P<0.05) lower TVC was found in gelatin coated (containing GPE) rainbow trout fillets during the storage period. Bakri and Dougles (37) reported that antibacterial effect of garlic resulted from interaction of sulphur compounds, allicin, with sulphur groups of microbial enzymes, leading to an inhibition of microbial growth. Ifesan et al. (12) observed that garlic peel extract showed bacteriostatic activity in the beef samples. Additionally, they concluded that the bioactive compounds present in the garlic bulb are likely to be available in the peel. In the present study, incorporation of garlic peel extract in gelatin film resulted in microbial spoilage delay.

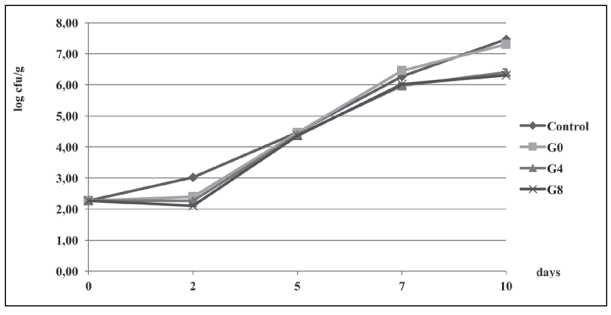

Figure 1. Changes in total viable counts of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4 °C. G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

It was reported that gram-negative psychrotrophic bacteria group are responsible from aerobic spoilage in chilled stored fresh fish (38). The changes in psychrophilic bacteria count (PBC) of rainbow trout fillets are presented in Fig. 2. A continuous increase of PBC was observed in all groups during the storage period. The PBC of control samples and gelatin wrapped without GPE samples were higher ($P<0.05$) than those of the samples coated with gelatin film containing GPE. Inititally, PBC was 2.59 log CFU/g in the rainbow trout fillets and reached 7.47, 7.38, 6.90 and 6.41 log CFU/g in control, G0, G4 and G8 groups, respectively. During the storage period, rainbow trout fillets coated with GPE gelatin film showed lowest PBC, in comparision with the control and gelatin film coated (without GPE) samples ($P<0.05$). Rezaei et al. (39) monitored the initial number of PBC of rainbow trout fillets as 2.3 log CFU/g and increased to 6.1 log CFU/g after 18 day of the ice storage. Ucak et al. (40) reported the initial PBC of rainbow trout fillets treated with onion peel extract (OPE) as 2.47 log CFU/g and PBC of the OPE treated fillets remanined lower than the control during the storage. Similarly, initial PBC was found as 3.1 log CFU/g in rainbow trout fillets wrapped with chitosan films incorporated with oregano or thyme essential oil (41).

Enterobacteriaceae is considered a hygiene indicator and one of the spoilage microorganisms of fresh rainbow trout (42). Papadopoulos et al. (44) reported that the contribution of Enterobacteriaceae to the microflora of fish should be considered during the assessment of fish spoilage. The initial number of Enterobacteriaceae was 1.53 log CFU/g which is lower than the initial number of rainbow trout fillets (2.27 log CFU/g) reported by Ozogul et al. (35), but this value reached 7.37 log CFU/g in the control group at the end of the storage (Fig. 3). The coating of fish fillets, either with gelatin-based film alone or with combinations of GPE, led to inhibition of microbial growth during the storage. However, the lowest Enterobacteriaceae was observed in gelatin coated samples incorporated with GPE ($P<0.05$). This indicated that gelatin film showed slight effect on the inhibition of Enterobacteriaceae without the addition of GPE. At the end of the storage, Enterobacteriaceae increased to 7.01, 6.93 and 6.48 log CFU/g in G0, G4 and G8 samples, respectively. Oz (43) concluded that the addition of garlic into rainbow trout diet reduced the number of Enterobacteriaceae in fish meat and kept it at a lower level during storage.

Sensory evaluation

The sensory results of the rainbow trout fillets coated without and with films are given in Table 2. The fillets coated with gelatin film incorporated with GPE received higher scores than those of the control and gelatin film wrapped without GPE samples ($P<0.05$). According to sensoy evaluation of color, odour, texture and overall acceptance of control samples was considered as unacceptable by the 5th day of the storage. However, the fillets wrapped with gelatin film containing GPE was reached unacceptable scores by the 10th

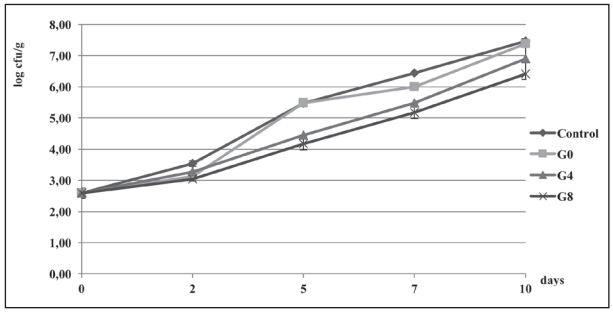

Figure 2. Changes in total psychrophilic bacteria count of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4 °C. G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

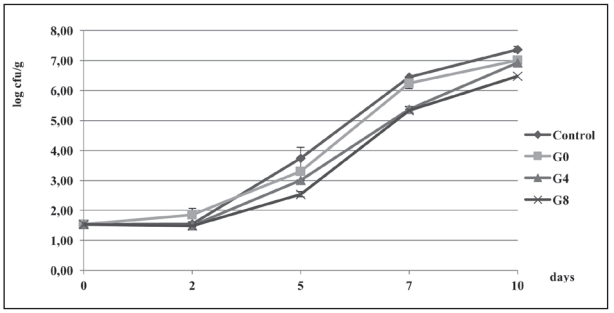

Figure 3. Changes in total Enterobacteriaceae count of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4 °C. G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

Table 2. Changes in sensory scores of rainbow trout fillets wrapped with gelatin films containing GPE during storage at 4 °C

	Storage period (days)	C	G0	G4	G8
Color	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	2	7.25±0.50^{Bb}	7.25±0.50^{Bb}	8.75±0.50^{Aa}	8.50±0.58^{Aa}
	5	3.75±0.96^{Cc}	6.00±0.00^{Bc}	7.50±0.58^{Ab}	7.50±0.58^{Ab}
	7	1.75±0.50^{Cd}	2.00±0.82^{Cd}	4.00±1.15^{Bc}	6.00±0.00^{Ac}
	10	1.25±0.50^{Ad}	1.50±0.58^{Ad}	2.00±0.00^{Ad}	2.00±0.82^{Ad}
Odour	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	2	6.75±0.50^{Bb}	6.50±0.58^{Bb}	9.00±0.00^{Aa}	8.75±0.50^{Aa}
	5	3.25±1.50^{Cc}	5.00±0.00^{Bc}	7.75±0.50^{Ab}	7.75±0.50^{Ab}
	7	1.00±0.00^{Cd}	2.25±0.96^{Bd}	4.00±1.15^{Ac}	4.50±0.58^{Ac}
	10	1.25±0.50^{Bd}	2.00±0.00^{Ad}	2.25±0.50^{Ad}	2.25±0.50^{Ad}
Texture	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	2	7.25±0.50^{Bb}	7.50±0.58^{Bb}	8.75±0.50^{Aa}	9.00±0.00^{Aa}
	5	3.75±2.06^{Bc}	6.00±0.00^{Ac}	7.75±0.50^{Aa}	7.75±0.50^{Ab}
	7	2.00±0.82^{Bd}	2.75±0.50^{Bd}	3.50±1.73^{ABb}	4.50±0.58^{Ac}
	10	1.25±0.50^{Bd}	1.50±0.58^{Bc}	3.00±0.00^{Ab}	3.00±0.00^{Ad}
Overall acceptance	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	2	6.50±0.58^{Bb}	7.00±1.15^{Bb}	8.75±0.50^{Aa}	8.50±0.58^{Aa}
	5	3.50±1.73^{Cc}	5.00±0.00^{Bc}	7.25±0.00^{Ab}	7.50±0.58^{Ab}
	7	1.75±0.96^{Bd}	2.25±0.96^{Bd}	4.50±0.58^{Ac}	5.50±0.58^{Ac}
	10	1.25±0.50^{Bd}	1.50±0.58^{Bd}	3.00±0.82^{Ad}	3.25±0.50^{Ad}

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, G0 film: samples wrapped with gelatin film, G4 film: samples wrapped with gelatin film incorporated with 4% GPE, G8 film: samples wrapped with gelatin film incorporated with 8% GPE.

day of the storage. This indicated that incorporation of gelatin film with GPE showed antioxidant and antimicrobial effect and delayed spoilage of rainbow trout fillets. Gelatin film without GPE retarded the spoilage in the fillets as well, but showed lesser extention in the shelf-life. The sensory a scores was correlated with microbiological and chemical results.

Similar results were observed by Jasour et al. (45) who reported 4 days shelf-life extension in the chitosan coated rainbow trout fillets compared with control samples. Jouki et al. (41) reported the shelf-life of rainbow trout fillets wrapped with edible film containing oregano or thyme essential oil as 18 days, while the shelf-life of control samples was 10 days. In many studies, it was found that the application of edible films enriched with essential oil or plant extract extended the shelf life of fish (7,23,24,28,46). The sensory results of this study showed that gelatin film containing GPE extended the shelf-life of rainbow trout fillets and the shelf-life was found as 5, 7 and 10 days for the control, G0 and GPE samples, respectively.

Conclusions

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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