Using of Quinoa Based Film to Extend the Shelf Life of Rainbow Trout Fillets under Cold Storage (4±1°C) Condition

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ABSTRACT

In this study, the structural features (thickness and light transmittance) of edible films that obtained from quinoa starch were analyzed, and the films were applied to rainbow trout fillets. These fillets were classified under two different groups without quinoa starch film (control) and coated with quinoa starch film (QSBF). Thereafter, these fillets were storage in the refrigerator at (4±1°C) for 12 days. Analyzes were performed on days 0, 3, 9, and 12 during storage to detect chemical and microbial changes in both groups. The film thickness was 0.195 ± 0.010 mm, and the light transmittance values were between 10–37%. At the end of storage, the highest values of chemical analysis were found in the control group 20.35±0.49 mg/100g in (TVB- N), 3.87±0.10 µmol MA/kg in (TBARS) and 6.27±0.00 in (pH), respectively. The highest values of microbial analysis were found in the control group 5.35±0.22 log cfu/g in (TAMB), 3.32±0.48 log cfu/g in (LAB), Pseudomonas 5.72±0.28 log cfu/g and Enterobacteriaceae 4.38±0.68 log cfu/g in the control group respectively, although (TPC) was found 6.84±0.11 log cfu/g in the QSBF group. The results of chemical and microbial analysis showed that statistical difference between control and treatment groups was significant (p<0.05). Quinoa starch-based biofilms (QSBF) had a chemically and microbiologically protective effect on trout fillets that were stored. Further studies are needed to optimize the use of (QSBF) as microbiological and chemical barriers for fish filet packaging.

Introduction

Rainbow trout (Oncorhynchus mykiss) is one of the most common aquaculture fish species in the world. In recent years, the rainbow trout breeding industry has become highly developed, and its production efficiency has increased due to newly designed technological systems (Crawford and Muir, 2008). Fish and fishery-related products have been recognized as important sources of human nutrition (Masniyom, 2011). Fish-related products provide nutritional benefits due to their valuable protein content and are a significant source of polyunsaturated fatty acids. However, the quality of spoilable products, like fish meat, must be protected to ensure a long term shelf life (Dursun and Erkan, 2009). Traditional preservation methods, such as vacuum and modified atmospheric packaging, have typically been used to extend fish shelf life, although these methods require large capital investments. As an economical alternative, research has
focused on the development of antimicrobial packaging systems, such as edible films or coatings that can be combined with active ingredients. Edible films and coatings can be produced from different sources including proteins, lipids and polysaccharides (Sayanjali et al., 2011). The shelf life of fish fillets has reportedly been extended from 6 to 13 days, depending on the species to which these active packaging materials have been applied (Socaciu et al., 2018). Quinoa (Chenopodium quinoa) was selected by FAO (2014) as a food safety crop for the 21st century because it is tolerant to salinity and drought stresses and can grow in marginal regions. Quinoa’s high antioxidant levels and protein content make it an excellent nutrient source. However, quinoa has rarely been studied as a substrate for edible coating materials. Quinoa protein is a good biopolymer for edible films due to its excellent elongation and adhesive properties, which can be achieved without the use of plasticizers (Abugoch et al., 2011). To the best of our knowledge, the application of edible film derived from quinoa seed has not been previously reported, particularly to extend the shelf life of rainbow trout fillets. Therefore, this study aimed to evaluate the efficacy of quinoa-based edible films for increasing the shelf life of rainbow trout fillets.

**Material and Methods**

**Materials**

Rainbow trout (Oncorhynchus mykiss L., 1758) that were obtained from the Atatürk University Faculty of Fisheries, Inland Fisheries Research and Production Center were used in this study. The trout had an average weight of 300 ± 15 g. White quinoa (Chenopodium quinoa) from a commercial company (Damietta Gold (500 g), Turkey) was used as the film base material.

**Methods**

**Obtaining of Quinoa Starch and Films**

Quinoa seeds were soaked in a 1:2 ratio in pure water for 8 hours and then milled using a blender. The supernatants were removed by passing the milled portions through fine mesh sieves. The remaining material was placed in centrifuge tubes and centrifuged three times at 1000 g for 15 minutes at 4°C, with the supernatants removed after each centrifuge. At the end of the process, the solution pH was adjusted to 10.5 with 0.20% aqueous NaOH to dissolve the remaining proteins. Then, 1 mol L⁻¹ HCL was added to neutralize the solution. After neutralization, the remaining material was washed five times with distilled water to remove the protein and salts from the solution, leaving the starch (Araujo-Farro et al., 2010). The films were prepared using the method reported by Araujo-Farro et al., (2010), with minor changes. The quinoa starch obtained in the sterile cabinet was placed in glassware at a concentration of 4 g/100 mL to form film solutions. After stirring for 5–10 minutes in the magnetic stirrer to achieve saturation, the solution was left in a water bath at 82°C for 30 minutes until the solution achieved a plastic consistency. After standing, 1% glycerol (glycerol approx. 87%) was added to the solution, and it was remixed with a magnetic stirrer, followed by incubation at 35°C for 16 hours to obtain films.

**2.2.2. Preparation and Wrapping of Fillets with Film and Trial Plan**

The fish were prepared in the laboratory using sterile scalpels to open and remove the internal organs and to clean the dorsal and abdominal muscles from the gill caps to the tail fins. The process was repeated by inverting the fish; the fillets were obtained by separating the bones, tail and gills together with their heads. For wrapping of fillets, care was taken to cover the entire surface of the fillets with films prepared under sterile conditions (Ahmad et al., 2012). Trout fillets coated under vacuum aspirator in sterile environment were placed on aluminum foils. Edible films were prepared from starches (4% quinoa-100 mL distilled water-1% glycerol) from Quinoa (C. quinoa) seeds. The prepared films were subjected to some structural (film thickness and light transmittance) analyzes. Fillets (120 pieces) are extracted from 60 trout, around 18 kg, 60 fillets were covered with films and the rest of them were accepted as control group without any treatment and the experiment was designed repetition. The treatment and control group fillets were subjected to chemical and microbiological analyzes on certain days (0, 3, 6, 9 and 12 days) of storage at 4±1 °C.

**Structural Analysis of Quinoa Films**

Thickness measurements of edible films

The film thickness was measured with a micrometer (Tronıc, model 1131-150/Japan) with sensitivity of 0.001 mm. Measurements were made from 10 different regions of the films and the overall average was calculated.

**UV-VIS absorption spectroscopy of edible films**

Light absorption measurement of edible film coatings was carried out with a UV-VIS spectrophotometer (Perkin-Elmer, model Lamda 35/USA) having wavelength range of 200-1100 nm. In our study, quinoa starch edible film sample was placed into solid sample chamber and absorption spectra between 200-1100 wavelengths were taken. Light absorption values were then calculated using the equation given below.

\[
Absorbance \ (A) = \log \frac{I_0}{I} = 10^{-\epsilon bc}
\]

where \(A\); absorbance, \(I_0\); intensity of light on the sample, \(I\); the intensity of the light leaving the sample, \(\epsilon\); molar absorption coefficient (L/mol, cm), \(b\); thickness of the sample cup (cm), \(c\); and the concentration (mol/L). The ratio between light and light intensities entering the sample cup is expressed as transmittance (T) and calculated by the following formula (Arık et al., 2005).

\[
Transmittance \ (T) = \frac{I_0}{I} = 10^{-\epsilon bc}
\]

**Chemical Analysis**

Total volatile basic nitrogen (TVB-N) analysis

TVB-N analysis of the samples was performed using the method reported by Malle and Tao (1987). The TVB-N value was determined by calculating formula by considering the spent H₂SO₄ solution (n) in the titration.
Thiobarbituric acid reactive substance (TBARS) analysis

The method given by Lemon (1978) was used to determine the TBARS value. TBARS value (µmol MA/kg) was calculated by substituting the absorbance values based on the square and the standard curve prepared with TEP (1, 1, 3, 3 tetraethoxypropane) in the following formula (Aras Hisar et al., 2004).

pH analysis

In order to measure the pH values, 10 g of paralleled samples were taken into small pieces and 100 mL of purified water was added. The obtained suspensions were homogenized in Ultra-Turrax for 1-2 minutes and then measured with pH meter (Schott, Lab Star) (Gökalp et al. 2001).

Microbiological Analysis

Microbial analysis was carried out from cold-stored fillets were done for each bacteria group (total aerobic mesophilic, psychrotrophic, lactic acid, *Pseudomonas* and *Enterobacteriaceae*). 10 g fillet samples was taken and diluted in 99 mL of 0.85% (w/v) sterile saline solution. To homogenise, a Stomacher (Lab. Stomacher Blander 400 BA 7021, Sward medical) was used the samples in a sterile polyethylene bag for 1.5 min and sterile 9 mL 0.85% (w/v) NaCl solution for dilution. The number of total aerobic mesophilic bacteria (TAMB; Merck, at 30±1°C for 48 h; Harrigan, 1998); lactic acid bacteria (in MRS; Merck, at 30°C for 48 h in anaerobic conditions; Papamanoli et al., 2003), *Pseudomonas* counts were determined using *Pseudomonas* agar base (Oxoid) supplemented with C-FC (Cetrimide-Fucidin-Cephloridine) selective supplement (Oxoid) incubated at 25°C for 48 h. PCA plates were then incubated for 7 days at 10°C for psychrotorophic bacteria count (González-Fandos et al. 2005). *Enterobacteriaceae* (Violet Red Bile Agar, Oxoid and Violet Red Bile Dextrose Agar, Merck 1.10275.0500, at 37°C for 24 h; Harrigan, 1998) were counted, respectively.

Statistical Analysis

The data obtained as a result of the experiments were subjected to variance analysis using SPSS package program (SPSS 20 Software, USA) and Duncan multiple comparison test was applied for the applications that were found to be significantly different according to the results of this analysis. Logarithmic transformation was applied to microbiological values.

Results

**Structural Results of Quinoa Films**

The thickness of the films obtained from the quinoa starch we used in our study was determined as 0.195±0.010 mm. The light transmittance values of the films obtained from the quinoa starch at different wavelengths were given in Figure 1. The light transmittance of the quinoa starch edible films was 10% at 220 nm, 26% at 400 nm, 30% at 600 nm, 34% at 800 nm and 37% at 1000 nm, respectively.

**Chemical Analysis**

**Total volatile basic nitrogen (TVB-N)**

TVB-N content of rainbow trout fillets coated with quinoa starch film (QSBF) and without quinoa starch film (control) during storage is shown in Figure 2A. At the beginning of storage, TVB-N results in the control and film coating groups were 13.85±0.63 mg/100g. Increases were recorded in both groups up to the end of storage depending on storage time and the highest value was found 20.35±0.49 mg / 100g in the control group and 18.65±0.21 mg / 100g in the QSBF group. In statistical analysis, day and group, which is one of the main sources of variation, were found very important at p<0.01 level, while the effect of group x day interaction was found significant at p<0.05 level.

**Thiobarbituric acid reactive substance (TBARS)**

TBARS results during the storage days are shown in Figure 2B. At the beginning of storage, TVB-N results in the control and film coating groups were 13.85±0.63 µmol MA/kg, respectively. Increases were observed in both groups up to the end of storage period, and the highest value was found to 3.87±0.10 µmol MA/kg in the control group at the end of storage period. According to the analysis of variance, the effect of group and day, which are the main sources of variation on TBARS value, was very important (p<0.01), and the effect of group x day interaction was insignificant (p>0.05).

**pH**

pH values of treatment and control group is shown Figure 2C. At the beginning of storage, pH values of control and QSBF groups were determined as 6.27±0.00 and 6.28±0.02, respectively. Increases were observed in both groups until the end of storage period, and the highest value was found to 7.66±0.22 in the film coated group. According to the analysis of variance, it was determined that the day was very important (p<0.01), the groups were insignificant (p<0.05) and the effect of group x day interaction was significant (p<0.05).
Figure 2. The effect of edible coating with on (A); Total volatile basic nitrogen (TVB-N), (B); Thiobarbituric acid reactive substance (TBARS), (C); pH values of trout fillets during storage (12 day) at 4 ± 1°C. Mean ± standard deviation of the values (three replicates) are presented.

Microbiological load of filmed trout fillets

The results of the number of bacteria determined during storage (12 days) were given in Figure 3. The total number of aerobic mesophilic bacteria (TAMB) was 2.55±0.07 log cfu/g and 2.52±0.06 log cfu/g in the control and QSBF groups at the beginning of storage, respectively. Increases in the number of TAMB were observed in parallel with storage period and the highest value was found to 5.35±0.22 log cfu/g in the control group at the end of storage period. The results showed that the effect of group and day on the TAMB was statistically very significant (p<0.01) and that group x day interaction was significant (p <0.05). The total number of psychrotrophic bacteria (TPC) was found to 4.19±0.71 log cfu/g in both groups at the beginning of storage in Figure 3. Continuous increase in these values was observed during storage and the highest value was found to 6.84±0.11 log cfu/g in QSBF group at the end of storage period. The results showed that the effect of group and day on the number of psychrotrophic bacteria was statistically significant (p <0.01), and that the interaction of group x days was insignificant (p>0.05). The number of LAB at the first day of storage was found 2.00±0.00 log cfu / g in the control and QSBF group in Figure 3. During the storage period, no change was observed in both groups and the highest value at the end of storage period was found 3.32± 0.48 and 2.66±0.28 in the control and QSBF group, respectively. After the statistical analysis, it was determined that the effect of groups (p <0.05) and the effect of days and group x days interaction on lactic acid bacteria number (p <0.01) was significant. At the beginning of storage, the number of Pseudomonas in the control and film coating group was 2.00±0.00 log cfu/g in Figure 3. Increases were observed in both groups until the end of storage period. The highest value at the end of storage period was 5.72±0.28 log cfu/g in the control group and 5.23±0.22 log cfu/g in QSBF group. In statistical analysis, the difference between storage days was significant for both groups (p<0.05). According to the analysis of variance, the effect of group x day interaction was significant (p<0.05) and days were very significant (p <0.01). The number of Enterobacteriaceae at the beginning of storage was found to same value 2.00±0.00 log cfu/g in the control and film coating group in Figure 3. Increases were observed in both groups until the end of storage period. On the last day of storage, the highest values (4.38±0.68 log cfu/g) were observed in the control group and 4.07±0.54 log cfu/g in the QSBF group. According to the analysis of variance, the effect of group and group x day interaction on the number of Enterobacteriaceae was insignificant (p<0.05) and the difference between days was significant (p <0.01).

Figure 3. The effect of edible coating with on (A); The total number of aerobic mesophilic bacteria (TAMB) (B); Total psychrotrophic bacteria (TPB), (C); Lactic acid bacteria (LAB), (D); Pseudomonas, (E); Enterobacteriaceae values of trout fillets during storage (12 day) at 4 ± 1°C. Mean ± standard deviation of the values (five replicates) are presented.

Discussion

Structural Properties of Quinoa (Chenopodium quinoa)

Films

In our study, we measured the thickness of films as 0.195±0.010 mm. The difference in film thickness may vary depending on the bulk thickness, the difference of the main material used in the production of the film, the amount of use of the materials and the mixing ratios. Therefore, it is clear that other studies do not show similarity. In a study, which investigated the properties of gelatin films with seaweed extract, it was reported that the average thickness of films containing...
gelatin film and gelatin-seaweed extract was 0.295 mm (Rattaya et al., 2009). Valenzuela et al., (2013) reported the film thicknesses as 0.051±0.001, 0.110±0.009, 0.111±0.010 and 0.126±0.008 mm in a study in which they determined the characterization and structure of edible biofilms consisting of a mixture of quinoa protein–chitosan–sunflower oil. In another study, the characterization of edible biofilms with quinoa protein-chitosan mixture was investigated and the thicknesses of the films were reported as 0.142±0.017, 0.125±0.017 and 0.054±0.003 mm (Abugoch et al., 2011). Araujo-Farro et al., (2010) have identified the thickness of films containing quinoa starch as 0.080±0.020 mm. UV-VIS Absorption Spectroscopy has been found that % light transmittance increases parallel to the increase of light wavelength. Gomez-Guillen et al., (2007) and (Al-Hassan and Norziah 2012) reported that the transparency of the films is very important on the light transmittance of the edible films, and that this transparency may be related to the addition of any material to the main materials used. Similarly, in our study, it was found that the quinoa starch used in the film production was quite light and therefore the light transmittance values differed from other studies due to the transparent color of the films obtained.

### Chemical Changes of Filmed Trout Fillets

The TVB-N is very usually used in the assignation of spoilage of seafood (Ruiz-Capillas and Moral, 2001). It describes the sum of ammonia, methylamine, dimethylamine, trimethylamine, and other basic nitrogenous volatile compounds resulted from fish degradation (Sikorski, 1990; Ruiz-Capillas et al., 2015). As a result of endogenous enzyme and bacterial activities in fish, the amount of volatile basic nitrogenous substances increases. The acceptable limit values (25 mg/100 g) of TVB-N (Aras Hisar et al., 2004) vary according to the fish species, but the data obtained in our study are among the mentioned limit values (Nowzari et al., 2013; Volpe et al., 2015). The daily consumable limit value determined for trout was reported as 20 mg/100g by Aras Hisar et al., (2004) and Alak, (2012). At the beginning, TVB-N value was found as 13.85±0.63 mg/100g in our study. Parallel results were also published by another researchers in different works about on ice stored S. aurata (Tejada and Huidobro, 2002; Grigorakis et al., 2003; Kılınc et al., 2007). In our study, TVB-N values obtained from the control and QFW fillets remained below the daily consumable limit value during the storage period. Moreover, the lowest value was found 18.65±0.21 mg/100g in the QSBF group at the end of storage. That value suggested that coating with QSBF may cause delaying the increase of the TVB-N value. This result can shows that the TVB-N results are related to microbiological findings. Similar results were also published by different researchers (Fan et al., 2009; Gómez-Estaca et al., 2010; Ojagh et al., 2010; Alak et al., 2010; Li et al., 2013). Moreover, Ucak (2019) was reported that the TVB-N values reached 42.70, 39.90, 34.30 and 30.10 mg N/100 g in control, G0 groups (samples wrapped with gelatin film), G4 (samples wrapped with gelatin film incorporated with 4% garlic peel extract) and G8 groups (samples wrapped with gelatin film incorporated 8% with garlic peel extract), respectively at the end of the storage. When the results of our study are compared with other studies, it is seen that fish species, storage conditions, processing technologies, additives and packaging are effective in TVB-N value used as freshness criteria (Alak, 2012; Aras Hisar et al., 2004). Lipid oxidation is an important issue for quality of food. Because of TBARS is generally a significant parameter used as lipid oxidation index (Fan et al., 2009; Jeon et al., 2002; Jongjareonrak et al., 2008; Ojagh et al., 2010). Some researchers (Ojagh et al., 2010; Jouki et al., 2014; Shokri and Ehsani 2017), were published that values <3 mg MDA/kg for perfect quality material, <5 for good quality material, and 5≤ MDA/kg< 8 for suitable for human consumption. It has been reported that edible biofilms can be an important barrier in preventing light-effect lipid oxidation on foods (Gómez-Estaca et al., 2009). Furthermore, it is thought to be effective in decreasing oxidation level in the coated fillets by preventing the contact between food and oxygen due to the barrier properties of the films. In addition, fish species, preservative application, storage conditions and packaging may have an impact on TBARS values (Araujo-Farro et al., 2010). In our current study results, it was also determined that quinoa film coating applied during storage period had inhibitory effect on lipid oxidation. Because of the highest value was found 3.87±0.10 µmol MA/kg in the control group at the end of storage day. Similarly, (Sathivel et al., 2007) reported that chitosan and soy protein concentrate delays lipid oxidation in skinless pink salmon fillets. Ucak (2019) sum up lipid oxidation was also delayed in the samples wrapped with gelatin films incorporated with garlic acid extract, especially in concentration of 8%. In parallel with, (Socaciu et al., 2018), were published data reviewed in the theirs paper that TBARS values ranged from 0.2 to 0.9 mg MDA/kg for rainbow trout fillets, 1.1 to 1.8 mg MDA/kg for salmon fillets, 1.0 to 2.5 mg MDA/kg for pike-perch fillets, 0.06 to 0.12 mg MDA/kg for beluga sturgeon fillets, 3.0 to 4.0 mg MDA/kg for silver carp fillets, 0.9 to 1.2 mg MDA/kg for grass carp fillets, 0.2 to 2.0 mg MDA/kg for Japanese sea bass fillets, and 0.8 to 1.8 mg MDA/kg for red drum fillets. Owing to accumulation of alkaline compounds indicating fish spoilage, pH of trout fillets increased slowly during our study (Utami et al., 2014). Fan et al., (2009) investigated the effect of chitosan coating on the quality and shelf life of silver carp and found that the pH value of chitosan coated products was lower than the control group. In different studies with biofilm coating, it was also reported that the pH value was low (Gómez-Estaca et al., 2009; Volpe et al., 2015). The initial low level of pH in fish is due to the conversion of glycogen to lactic acid during the post mortem glycolysis phase (Sengor et al., 2000). In the next stage, the oxidation-reduction equilibrium, the change in the concentration of free hydrogen and hydroxyl ions occur due to the increase of storage time and the effect of enzyme-microorganisms and thus the pH value increases. In addition, decomposition of nitrogenous compounds in meat due to post-mortem changes leads to an increase in pH (Hernández et al., 2009). In our study, pH was determined that the day was very important (p<0.01), the groups were insignificant (p>0.05). Similarly, (Dogau and Izcı, 2015) were published that the pH values were observed as increase, although chitosan film coated fillets was lower than the control group (P< 0.05). It was observed that the data obtained in our study was consistent with the researches mentioned above.

### Microbiological Load of Filmed Trout Fillets

There are many studies on the storage of fish and other aquaculture products with antimicrobial properties of edible coatings...
in the literature. Because of microbiological analysis is very important for determining the quality of the food products. In the literature, the values of TAMB in fresh fish have been reported by different researchers as 2-7 log cfu/g (Sallam, 2007; Song et al., 2011). In our study, we found that the number of bacteria in all groups increased during storage. But the limit value (7 log cfu/g) was not exceeded in control and QFW groups at the end of on storage. Our data, which is in parallel with the results of a lot studies where the same storage process and different film techniques are applied, is thought to have a significant inhibitory effect of coating solution and coating technique on the TAMB. Some researchers also informed these coatings have antimicrobial effect (Vásconez et al., 2009; Alak et al., 2010; Zhou et al., 2011; Qiu et al., 2014; Bahram et al., 2016; Yıldız and Yanglar, 2016; Shokri and Ehsani, 2017). Ahmad et al., (2012) documented that gelatin film integrated with lemongrass essential oil may reduced microbial counts of sea bass slices. Similarly, (Lu et al., 2017) reported that 1.5% oregano (Kazemi and Rezaei 2015) and 1% lemon (Volpe et al., 2015) that were applied on rainbow trout fillet. The data obtained in our study show similarity with the results of the researchers.

**Conclusion**

Studies on the effects of edible biofilms obtained from starch extracts from Quinoa seeds on food shelf life have been found to be quite limited. In the literature review, studies related to this substance have not been found, especially in seafood processing technology. However, in recent years, there has been a growing interest in the use of quinoa, a dietary food, as an edible film. In this study, the structural properties (thickness and light transmittance) of the obtained quinoa biofilms and chemical and microbiological changes during cold storage in film coated fillets were investigated in order to determine the possibilities or suitability of use in rainbow trout fillets. In our study, it was determined that quinoa starch film coating applied to trout fillets had a significant effect especially on chemical parameters in cold storage and had inhibitory effect on bacterial growth. It is also thought that this application has a positive effect on trout fillet quality parameters in terms of shelf life and can be used as an alternative source of biofilm for the protection of aquatic products. In particular, it is considered that these products, which do not have a negative health effect on the seafood processing industry and which contribute positively to the nutritional content of the product, should be supported with further studies to be developed and enriched with the use of appropriate technical-technology.

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**Conflict of Interest**

The authors declare that there is no conflict of interest.
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