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Effect of Chitosan Coating Supplemented with Olive Leaf Extract on Oxidative Quality of Fish

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Abstract

In this study, the effect of chitosan coating supplemented with olive leaf extract on the oxidative quality of rainbow trout fillets at 4°C for 15 days of storage were determined. For this purpose, samples were divided into five groups entitled as; fillets immersed in chitosan coating (Cc), fillets immersed in chitosan coating with 0.5%, 1% and 2% olive leave extract named as O0.5, O1 and O2 and fillets without coating (C). The highest pH values were found in the C and Cc groups during storage, while the lowest pH values were found in the O2 group. Peroxide value, which is the principal oxidation products, increased in all groups until at the end of the storage and the highest values were found in C and Cc groups, respectively (9.00 meq/kg and 8.00 meq/kg). The lowest peroxide value was determined in O2 group as 5.50 meq/kg. At the beginning of storage, TBARS value of rainbow trout fillets was 0.16 mg MDA/kg showed increase in all groups at the end of the storage period. The C and Cc groups had the highest TBARS value which increased throughout the storage and reached 2.07 and 1.92 mg MDA/kg at the end of storage, respectively. Rainbow trout fillets immersed in chitosan solution enhanced with 2% OLE had TBARS values of 1.31 mg MDA/kg, which was found to be considerably low ($P<0.05$). As a result, it was determined that the supplementation of olive leaf extract raised the effectiveness of chitosan and reduced lipid oxidation in rainbow trout fillets.

Keywords: Rainbow trout, chitosan, edible coating, olive leaf extract, lipid oxidation

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INTRODUCTION

Consumers want healthy nutrient dense food due to their concern regarding food quality and their awareness about negative ecological effects of non-biodegradable food packaging which is leading to high demand of fresh fish (Angiolillo et al., 2018). Though, fish is very perishable, thus normally sold as a processed or frozen product. Unfortunately, frozen fish does not completely prevent the degradation of fish lipids. Few countries have banned the usage of chemical preservatives in foodstuff. To cope up with such issues, new approaches of preservation of food have been established, which includes active packaging like coatings or films that may control the transfer of water and gases, hence decrease microbial growth (Hassan et al., 2018).

The major purpose of food packaging is to inhibit the contact to deterioration factors that includes the effects of microbes, temperature, humidity and oxygen to reduce the nutritional loss, hence maintains quality and extends the shelf life. Though, food packaging provides additional functions like increasing communication and convenience to customers and marketing of the packed item. Various advanced packaging techniques have been established in preservation of meat like modified atmospheric packaging, vacuum packaging, edible packaging, intelligent and active packaging (Gertzou et al., 2017; Fang et al., 2017).

In recent times, edible films and coatings have fascinated much attention from scientists. In comparison with conventional packaging, edible coating or film is directly applied on food surface to maintain quality and extends shelf-life. Moreover, the edible packaging material is mostly derived organically, which have biodegradable, biocompatible, non-toxic and bioactive properties all together that might not be available in artificial packaging materials (Mihai and Popa, 2015). Growing customer demand for high quality nutritional and safe foods with long shelf lives, as well as environmental awareness of limited natural resources and the impact of packaging waste on the environment, have ignited significant interest and innovative research activity in edible packaging in the food industries (Janjarasskul and Krochta, 2010).

Enrobing with any sort of thin layer on food items for shelf-life extension that might be consumed along with food is referred as an edible coating or film. The films and coatings are applied to food with the intention to enhance the shelf life, nutritional and organoleptic features of food (Akram et al., 2019). Edible film and coating provides physical shield to save food items from mechanical loss, and also from chemical and biological events (Min et al., 2005). Edible films are made from sustainable resources, and mostly are more degradable than synthetic materials. Non-biodegradable and non-renewable packaging resources have some thoughtful environmental disadvantages. They are considered as a main cause of environmental waste and pollution by scientists (Ramos et al., 2013).

Edible coatings or films if not used along with food, may contribute to reduce environmental pollution (Embuscado and Huber, 2009). The main purpose of packaging is to save food from chemical, physical, biological factors which cause food spoilage, extend the useful effect of food processing, maintains the quality of food along with extended shelf life (Marsh and Bugusu, 2007). The purpose of this study is to determine the oxidative stability of rainbow trout fillets by using chitosan coating supplemented with different concentrations of olive leaf extract.

MATERIAL and METHOD

Materials

In this research, rainbow trout (*Oncorhynchus mykiss*) fillets, weighing 204.28 ± 8.31g with length of 24.51 ± 0.79 cm, were transported from a fish market in Niğde to the research laboratory within 1 hour in styrofoam boxes filled with ice. Commercial chitosan which produced by the deacetylation of chitin, a component of shrimp shells was obtained from Sigma-Aldrich. The olive leaves used in this study were harvested from olive trees in İzmir, Turkey in December 2021.

Methods

Extraction process of olive leaf

Olive leaves were washed two times in running tap water and dried at 45^{o}C for 48 hours. Dried olive leaf was grounded into powder with a blender. For extraction process, 10 g of olive leaf powder was dissolved in 100 mL of 70% ethanol in a flask, subjected to magnetic stirrer for 2 hours at room temperature. Afterwards, the extract was filtered by using Whatman no. 3 filter paper and evaporation was done in rotary evaporator under vacuum (IKA, HB 10 digital, Germany) at 45°C (Oomah et al., 2008).

Preparation of chitosan solution and application to rainbow trout fillets

Chitosan coating solution was prepared by the method of Ojagh et al. (2010). Chitosan solution was made by adding 1 gram of chitosan powder in 100 mL of 1 % v/v acetic acid solution and was stirred for 3 h at room temperature, followed by filtration through a Whatman no. 3 filter paper (Ojagh et al., 2010). Olive leaf extract (OLE) was added to the coating solution in three different concentrations (0.5%, 1.0% and 2.0%) (by volume per mass of chitosan). Fillets were categorized into five groups as fillets without coating (C), fillets with chitosan solution (CCh), fillets coated with chitosan solution supplemented with 0.5% OLE (O0.5), fillets coated with chitosan solution supplemented with 1.0% OLE (O1) and fillets coated with chitosan solution supplemented with 2.0% OLE (O2). Total number of rainbow trout fillets used in this study was twenty-five. For each coated group, five fillets weighing approximately 900 g were dipped in the coating solution for 30 seconds and then permitted 2-minute drain time followed by an another immersion for 30 seconds. All the samples were placed in a sterile foam plate and covered with stretch film, then stored in refrigerator at 4 °C for 15 days. Analyses were conducted every three days during the storage period.

pH measurement

In pH measurement, pH-meter probe (Thermo Scientific Orion 2-star, Germany) was immersed into the homogenized samples, mixed with distilled water in a 1: 1 ratio (Manthey et al., 1988).

Peroxide value analysis (PV)

Peroxide value analysis was carried out by following the method (AOAC, 1990). 1g of fish oil put in 30mL chloroform-glacial acetic acid solution (3chloroform and 2glacial acetic acid) and then 1mL of saturated potassium iodide solution was added. The solution after mixing was kept for 5 minutes in some dark place. Then, 30mL distilled water and few starch drops was added and titration was done with 0.1M sodium thiosulfate ($Na_2S_2O_3$) solution. Peroxide value of samples was calculated by using below formula which is expressed in meq/ kg.

PV (meq / kg) = K x (V-V0) x 12.69 x 78.8 / w
K - used on titration $Na_2S_2O_3$' starch concentration (mol / lt),
V - titration $Na_2S_2O_3$' starch amount in mL,
w - weight of the oil in grams

Thiobarbituric acid reactive substances (TBARS) analysis

The malondialdehyde in samples colored with TBA reagent, so spectrophotometric tests were performed (AOCS, 1998). The same amount of TBA reagent was combined with 0.1g of fish oil dissolved in n-butanol. It was held in 95°C water bath for 2 hours. At a wavelength of 530 nm, rapidly cooled samples were examined in a spectrophotometer, and the findings computed using the formula below, expressed as mg malondialdehyde/kg sample.

TBA = 50 x (lipid absorbance – blank absorbance) / sample weight (mg)

Statistical analyses

All analyses were carried out in duplicate. Statistical analysis was done by using SPSS (Statistical Analysis System, Cary, NC, USA) software and multiple comparison tests were performed on several applications.

RESULTS and DISCUSSION

pH

Table 1 shows the differences in pH values of rainbow trout fillets coated with 0.5%, 1% and 2% olive leaf extract and chitosan.

Table 1. Changes in pH of rainbow trout fillets immersed in chitosan coating supplemented with different concentration of olive leaf extract

	Treatments				
Storage (Day)	**C**	**Cc**	**O0.5**	**O1**	**O2**
0	6.31 ± 0.01^{Af}	6.31 ± 0.01^{Af}	6.31 ± 0.01^{Af}	6.31 ± 0.01^{Af}	6.31 ± 0.01^{Ae}
3	6.39 ± 0.01^{Ae}	6.36 ± 0.00^{Be}	6.36 ± 0.01^{Be}	6.33 ± 0.01^{Ce}	6.32 ± 0.01^{Ce}
6	6.89 ± 0.01^{Ad}	6.88 ± 0.01^{Ad}	6.49 ± 0.01^{Bd}	6.45 ± 0.01^{Cd}	6.41 ± 0.01^{Dd}
9	7.21 ± 0.01^{Ac}	7.21 ± 0.01^{Ac}	6.61 ± 0.01^{Bc}	6.56 ± 0.00^{Cc}	6.47 ± 0.01^{Dc}
12	7.40 ± 0.01^{Ab}	7.41 ± 0.01^{Ab}	6.81 ± 0.01^{Bb}	6.78 ± 0.01^{Cb}	6.68 ± 0.01^{Db}
15	7.70 ± 0.01^{Aa}	7.69 ± 0.01^{Aa}	6.94 ± 0.01^{Ba}	6.85 ± 0.01^{Ca}	6.81 ± 0.01^{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, Cc: fillets with chitosan coating, O0.5: fillets immersed in chitosan coating supplemented with 0.5% of OLE, O1: fillets immersed in chitosan coating supplemented with 1% of OLE, O2: fillets immersed in chitosan coating supplemented with 2% of OLE.

At the beginning of storage, pH of rainbow trout fillets was 6.31 and by the end of storage it became high in all groups. The rises in pH are linked to the generation of volatile amines as a result of microbes (Huss, 1995). Cobb (1977) and Finne (1982), on the other hand, cited enzymatic ammonia generation as a source of pH rises. C, Cc, and groups immersed in chitosan solution with OLE concentrations showed significant differences ($P<0.05$). The highest pH values in the C and Cc groups were 7.70 and 7.69, respectively, after 15 days of storage, while the lowest pH value in the O2 group was 6.81.

According to Ludorf and Mayer (1973) and Ozyurt et al. (2017), pH value for fresh fish should be between 6.8 and 7.0. The pH levels of OLE 0.5%, 1%, and 2% were remained within acceptable limits for fresh fish at the end of storage, but the values of C and Cc had above the limit for fresh fish after the 6th day of storage. Although the pH value is not a reliable indicator of fish deterioration, it can be used as a guideline for maintaining fish quality (Ruiz-Capillas and Moral, 2001). The accumulation of alkaline chemicals caused by the breakdown of nitrogenous compounds by spoilage bacteria activity results in an elevation in pH (Chaijan et al., 2005; Li et al., 2012). Guan et al. (2019) reported that addition of sage, oregano and grape seed extract treatment steadied the pH in hairtail fish balls during storage at 4°C. Fadıloğlu and Emir Çoban (2018) observed that pH value of chitosan + sumac effectively low pH value compared to other groups.

Peroxide value (PV)

The differences in PV of rainbow trout fillets immersed in chitosan coating incorporated with olive leaves extract concentration of 0.5%, 1% and 2% are given in Table 2.

Table 2. Peroxide value (PV) of rainbow trout fillets immersed in chitosan coating supplemented with different concentration of olive leaf extract (meq/kg)

Storage (Day)	C	Cc	O0.5	O1	O2
0	1.00 ± 0.00^{Ad}	1.00 ± 0.00^{Ac}	1.00 ± 0.00^{Ac}	1.00 ± 0.00^{Ac}	1.00 ± 0.00^{Ac}
3	1.00 ± 0.00^{Ad}	1.00 ± 0.00^{Ac}	1.50 ± 0.71^{Ac}	2.00 ± 0.00^{Ac}	1.50 ± 0.71^{Ac}
6	2.50 ± 0.71^{Abc}	3.00 ± 0.00^{Ab}	2.00 ± 0.00^{Abc}	1.50 ± 0.71^{Bc}	1.50 ± 0.71^{Bc}
9	6.50 ± 0.71^{Ab}	6.50 ± 0.71^{Aa}	6.00 ± 0.00^{Ab}	4.50 ± 0.71^{Bb}	4.00 ± 0.00^{Bb}
12	7.50 ± 0.71^{Ab}	7.50 ± 0.71^{Aa}	6.00 ± 0.00^{Bb}	5.50 ± 0.71^{Bab}	5.00 ± 0.00^{Bab}
15	9.00 ± 0.00^{Aa}	8.00 ± 1.41^{Aba}	7.50 ± 0.71^{ABCa}	6.00 ± 0.00^{BCa}	5.50 ± 0.71^{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, Cc: fillets with chitosan coating, O0.5: fillets immersed in chitosan coating supplemented with 0.5% of OLE, O1: fillets immersed in chitosan coating supplemented with 1% of OLE, O2: fillets immersed in chitosan coating supplemented with 2% of OLE.

At the beginning, PV value of rainbow trout fillets was observed as 1 meq/kg and increased in all groups during the storage period. The PV values of chitosan coated samples suplemented with 0.5%, 1% and 2% concentration of OLE were recorded as 7.50, 6.00 and 5.50 meq/kg, respectively, while 9.00 and 8.00 meq/kg were recorded in control and chitosan groups at 15th day of storage. The highest PV was observed in control group compared to chitosan coated samples, while the lowest peroxide value was observed significantly ($P<0.05$) in rainbow trout fillets immersed in chitosan solution enriched with 2% OLE because of powerful antioxidant activity. PV is a measurement of peroxides and hydroperoxides that occurs in the early stages of lipid oxidation and is extensively used for oxidative rancidity (Alsaggaf et al., 2017). Peroxides, the major product of lipid oxidation are volatile molecules that produce aldehydes, ketones, and alcohols, which cause off flavor in products (Hamilton et al., 1998).

In bovine muscle model systems Hayes et al. (2009) discovered that olive leaf extract had a positive linear dose response effect, meaning that the greater the addition level the stronger the antioxidant activity. According to Bouaziz et al. (2008), olive leaf extract at a concentration of 400 ppm demonstrated excellent antioxidant activity and was effective in preventing oil rancidity. Carpenter et al. (2006) discovered that olive leaf extract significantly reduces oxidative stress in cells. Peroxide values are classed as "verygood" if they contain less than 2 mmol O_2/kg of fish, "excellent" if they contain up to 5 mmol/kg of fish, and "acceptable" if they include 8–10 mmol/kg of fish (Varlık et al., 1993). A value of fewer than 5 meq/kg of peroxide should indicate good quality fish lipids (Hamilton et al., 1998). As a result, the peroxide value at 0 day for all samples was 1.00 meq/kg, which is considered excellent. The control and chitosan coating (Cc) groups increased to 9 and 8 meq/kg, respectively, while the O2 group was deemed good at the end of the storage period. In the current investigation, in order to prevent lipid oxidation in rainbow trout fillets during refrigerated storage usage of 2% OLE was substantially more successful.

Thiobarbituric acid reactive substances (TBARS)

TBARS values difference of rainbow trout fillets immersed in chitosan coating incorporated with olive leaves extract concentration of 0.5%, 1% and 2% are given in Table 3.

Table 3. Change in TBARS of of rainbow trout fillets immersed in chitosan coating supplemented with different concentration of olive leaf extract (mg MDA/kg)

Storage (Day)	C	Cc	O0.5	O1	O2
0	0.16 ± 0.01^{Af}	0.16 ± 0.01^{Ae}	0.16 ± 0.01^{Ad}	0.16 ± 0.01^{Ad}	0.16 ± 0.01^{Ab}
3	0.95 ± 0.01^{Ce}	1.08 ± 0.03^{Bd}	0.97 ± 0.00^{Cc}	1.25 ± 0.01^{Ab}	1.12 ± 0.02^{Ba}
6	1.31 ± 0.03^{Ad}	1.20 ± 0.05^{Ac}	1.21 ± 0.08^{Ab}	1.00 ± 0.02^{Bc}	0.97 ± 0.02^{Ba}
9	1.49 ± 0.00^{Bc}	1.67 ± 0.04^{Ab}	1.28 ± 0.04^{Cb}	1.07 ± 0.02^{Dbc}	1.02 ± 0.02^{Da}
12	1.70 ± 0.07^{Ab}	1.70 ± 0.04^{Ab}	1.30 ± 0.03^{Bb}	1.20 ± 0.13^{Bb}	1.16 ± 0.10^{Ba}
15	2.07 ± 0.01^{Aa}	1.92 ± 0.04^{ABa}	1.80 ± 0.02^{Aba}	1.55 ± 0.12^{BCa}	1.31 ± 0.37^{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, Cc: fillets with chitosan coating, O0.5: fillets immersed in chitosan coating supplemented with 0.5% of OLE, O1: fillets immersed in chitosan coating supplemented with 1% of OLE, O2: fillets immersed in chitosan coating supplemented with 2% of OLE.

The TBARS value has been widely employed as an indicator for determining the degree of lipid oxidation, which can cause off-flavor, color, and odor alterations, as well as contribute to texture deterioration in fish products (Wenjiao et al., 2013). TBARS value of rainbow trout fillets was 0.16 mg MDA/kg and increased in all samples during the storage time. TBARS values of control group and the samples immersed in chitosan solution without OLE (Cc) were higher than those of the samples incorporated with 0.5%, 1% and 2% OLE. Whereas, the value of samples immersed in chitosan solution without OLE (Cc) showed slight lower values compared with control. Chitosan coatings have been shown to prevent lipid oxidation in herring and Atlantic cod (Jeon et al., 2002).

Chitosan's antioxidant and oxygen barrier characteristics may have played a role in lipid oxidation management in pink salmon fillets. Varlık et al. (2007) offered 5 mg MDA/kg and 8 mg MDA/kg as maximum limits for "good grade" and "consumable level" qualification, respectively. The TBARS values of rainbow trout fillets were 2.07, 1.92, 1.80, 1.55, and 1.31 mg MDA/kg in the control, Cc, O0.5, O1 and O2 groups, respectively at the end of storage. The lowest TBARS value was reported in fillets immersed in chitosan coating incorporated with 2% OLE during storage period ($P<0.05$). Fadıloğlu and Emir Çoban (2018) studied that using chitosan with 2% sumac considerably reduced the lipid oxidation. According to present study, 2% olive leaves extract incorporation with chitosan coating can delay oxygen permeability due to olive leaves extract's antioxidant properties.

CONCLUSION

In this study, different concentration of OLE (0.5%, 1.0%, and 2.0%) was added to chitosan solution to prevent lipid oxidation of the rainbow trout fillets during refrigerated storage. All of the results of study demonstrated that addition of OLE (especially 2% concentration) to chitosan coating solution enhanced its efficiency and delayed lipid oxidation in rainbow trout fillets. In recent years, there has been a growing interest in alternate antioxidants agents for shelf life extension of fish. It is also suggested that chitosan coating combined with olive leaf extract might be utilized as a natural resource for the shelf life extension.

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