Microbiological Quality Alterations of Fish Fillets Coated with Chitosan Incorporated with Olive Leaf Extract

Maham ZAHID[1], İlknur UCAK[1*]

[1]Nigde Omer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Nigde, Turkiye

[*]Corresponding author: ilknurucak@ohu.edu.tr

[*]ORCID: 0000-0002-9701-0824

Abstract

The effect of chitosan coating incorporated with olive leaf extract on the microbial quality of rainbow trout fillets was determined in the present study. 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). According to the microbiological results, the O2 group had the lowest mesophilic bacteria count, psychrophilic bacteria count, yeasts and molds count and total Enterobacteriaceae count. After concluding results, it was determined that the incorporation of olive leaf extract raised the effectiveness of chitosan coating and delayed microbial deterioration in rainbow trout fillets at 4°C for 15 days of storage.

Keywords: Fish fillet, Chitosan coating, Olive leaf extract, Microbiological quality

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INTRODUCTION

Maintaining quality and preventing lipid oxidation and microbiological activity are critical in the food sector due to the rapid degradation of fresh fish. Specific spoilage microorganisms, enzymatic activity, and chemical changes are the main causes of fish spoilage, which result in nutritional quality losses. All preservation procedures tend to be energy-efficient and ecologically friendly while eradicating pathogenic and degrading microorganisms, in response to rising customer expectations for high-quality, trustworthy, and fresh-looking food items (Uçak et al., 2021). Edible films and coatings have the potential to enhance the shelf life of nearly any food system while also improving its quality. Moisture, oxygen, carbon dioxide, lipid, taste, and fragrance transfer can be controlled via edible films and coatings, either between food components or to/from the atmosphere around the meal.

The type of substance used to make biodegradable edible coatings or films can be classified. When it comes to filmmaking, each chemical class offers advantages and disadvantages. The three main ingredients employed for this function are polysaccharides, proteins, and lipids. Polysaccharides are cheap and abundantly available. Although some gums have a negative charge, the majority are neutral. Negatively charged gums like carboxymethyl cellulose, pectin and alginate exhibit varied characteristics which depend on the pH (Qiu et al., 2014).

Animals and plants produce film-forming proteins. Protein denaturation, triggered by heat, change in pH or solvents, is followed by peptide chain linkage via new intermolecular contacts (Rodriguez-Turienzo et al., 2011). Protein based films attach well to meat's hydrophilic sides and can act as barriers to O_2 and CO_2 diffusion while allowing water to pass through (Sánchez-Ortega et al., 2014). Lipids are not biopolymers and cannot form cohesive films, unlike polysaccharides and proteins. As a result of their low polarity, they can be as used coatings or mixed into biopolymers to make composite films that provide a superior water barrier (Cordeiro de Azeredo, 2012). Polysaccharides like pectin, alginate and chitosan, proteins like collagen, whey, gelatin and lipids like waxes have all been employed in the development of edible packaging (Hassan et al., 2018). Because of its unusual film-forming capabilities, little gas permeability, antioxidant action against oxidation of lipids, and most significantly, antibacterial activity against fungi and bacteria, chitosan has been widely studied and employed in the food business (Elsabee and Abdou, 2013).

Olive leaf (OL) has been utilized as food preservatives, in cosmetic industry, for human well-being and health since ancient times (Roselló-Soto et al., 2015). Olive leaf is generally ingested as a powder (capsules) or as an infusion (Ghanbari et al., 2012). During the first stage of olive oil manufacturing, large numbers of Olea europaea leaves, as well as wood, are produced. Olive leaf has the ability to weigh around 10% of the raw material that comes at the mill (Goldsmith et al., 2015). Technologies like pressured liquid extractions, supercritical fluid extractions, microwave-assisted extractions and ultrasound-assisted extractions are attracting the interest of the food industry to extract bioactive components from OL. Extracts or isolated chemicals can be obtained depending on the procedure. These processes have been used to extract high-value chemicals such as oleuropein, tyrosol, tocopherols, cinnamic acid, hydroxybenzoic acid, caffeic acid, hydroxytyrosol, elenolic acid, syringic acid, ligstroside, chlorogenic acid and verbacoside (Roselló-Soto et al., 2015). It is critical to recover in order to use natural composites as functional constituents because of their bioactivity, which includes antioxidant, hypoglycaemic, anti-inflammatory, antimicrobial, anti-hypertensive, anticholesterolemic and anticancer properties (Rahmanian et al., 2015). The most prevalent phenolic component in olive leaf is oleuropein. Oleuropein belongs to the secoiridoids, a class of coumarin-like chemicals (Fares et al., 2011). Tyrosol, verbascoside, hydroxytyrosol, rutin and caffeic acid are some of the other phenolic chemicals found in OL. Bulotta et al., 2013; Casaburi et al., 2013; García-Villalba et al., 2014 have all demonstrated beneficial biologic activity. OL also contains flavonoids such as apigenin, kaempferol, and luteolina (Rahmanian et al., 2015).

MATERIAL and METHOD

Materials

Rainbow trout (*Oncorhynchus mykiss*) fillets were used in the study. The fillets were supplied from a fish market in Niğde and transported to the laboratory within 1 hour in styrofoam boxes filled with ice. The olive leaves were harvested from olive trees in İzmir.

Methods

Olive leaf extraction

Before initiate the extraction process, olive leaves were washed in running tap water and dried at 45^{o}C for 48 hours. Dried olive leaf was grounded into powder with a blender. 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.

The extract was filtered by using Whatman no. 3 filter paper and evaporated in rotary evaporator under vacuum at 45°C (Oomah et al., 2008).

Preparation chitosan solution and application to fillets

Chitosan solution was prepared according to the method of 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 divided into five groups; fillets without coating (C), fillets with chitosan solution (CCh), fillets coated with chitosan enriched with 0.5% OLE (O0.5), fillets coated with chitosan enriched with 1.0% OLE (O1) and fillets coated with chitosan enriched with 2.0% OLE (O2). Fillets were dipped in the coating solution for 30 seconds and then permitted 2-minute drain time followed by another immersion for 30 seconds. All the samples were placed in a sterile foam plate and covered with stretch film, then stored at 4°C for 15 days. Analyses were conducted periodically as every three days during the storage.

Total mesophilic bacteria count

The spread plate method was used to calculate the total aerobic mesophilic bacteria count (ICMSF, 1982). 10 g of fish from each group was taken and each sample was homogenized for almost 1 min after adding 90 ml ringer's solution in a stomacher device. After preparing decimal dilutions, 0.1 mL of each dilution was spread on petri plate having Plate Count Agar (PCA). All plates were placed in an incubator at 37ºC for 24-48 hours. Then, Total Viable Count was determined by seeing at colonies on petri plates. Colony forming unit (CFU/ g) was calculated by using following formula.

Total psychrophilic bacteria count

Dilutions made for psychrophiles was added to PCA medium by using spread plate method and incubated at 7°C for 7 days. 10 g of fish was taken from each group and mixed with 90 mL Ringer solution, then homogenized in stomacher for almost 1 min. Total psychrophilic bacteria count was calculated according to method of ICMSF (1982).

Total yeast and mold count

Potato dextrose Agar (PDA) medium was used to calculate yeast and molds after adding citric acid for pH adjustment to 3.5. It was determined by using spread plate method (ICMSF, 1982). 10 g of fish was taken from each group and homogenized for 1 min in a stomacher after adding 90 mL Ringer solution. After making decimal dilutions, 0.1 mL of each dilution was spread on petri plate having PDA. Petri plates were put in incubator at 25ºC for 5 days.

Total Enterobacteriaceae count

Total Enterobacteriaceae count was calculated by using spread plate method (ICMSF, 1982). 10 g fish was taken from each group, homogenized for 1 min in a stomacher after adding 90 mL Ringer's solution. After preparing decimal dilutions, 0.1 mL of each dilution was spread on petri plates having Violet Red Bile Agar. Petri plates were incubated at 37ºC for 24-48 h.

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

Total mesophilic bacteria count

The differences of total mesophilic bacteria count in rainbow trout fillets coated with chitosan solution supplemented with different concentrations (0.5%, 1.0% and 2.0%) of OLE are presented in Table 1.

Table 1. Changes of total mesophilic bacteria count of rainbow trout fillets immersed in chitosan solution incorporated with different concentrations (0.5%, 1.0% and 2.0%) of OLE (log CFU/g).

Storage (Day)	C	CCh	O0.5	O1	O2
0	$1.42±0.11^{Af}$	$1.42±0.11^{Af}$	$1.42±0.11^{Af}$	$1.42±0.11^{Af}$	$1.42±0.11^{Ae}$
3	$2.45±0.01^{Ae}$	$2.39±0.04^{Ae}$	$1.91±0.09^{Be}$	$1.76±0.06^{Be}$	$1.60±0.08^{Ce}$
6	$3.37±0.10^{Ad}$	$3.35±0.01^{Ad}$	$2.43±0.02^{Bd}$	$2.13±0.07^{Cd}$	$2.02±0.03^{Cd}$
9	$5.02±0.03^{Ac}$	$4.86±0.03^{Ac}$	$4.02±0.02^{Bc}$	$3.89±0.07^{BCc}$	$3.64±0.27^{Cc}$
12	$6.31±0.02^{Ab}$	$6.10±0.03^{Bb}$	$5.21±0.04^{Cb}$	$5.13±0.05^{Cb}$	$4.95±0.05^{Db}$
15	$7.10±0.03^{Aa}$	$6.99±0.01^{Aa}$	$6.34±0.02^{Ba}$	$6.28±0.02^{Ba}$	$6.05±0.11^{Ca}$

Means specified by capital letter in same rows differ P<0.05 significantly. Means specified by lowercase letter in same columns differ P<0.05 significantly. C: control, CCh: 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 1.0% of OLE, O2: fillets immersed in chitosan coating supplemented with 2.0% of OLE.

The aerobic bacterial count is a widely used indication of microbial contamination in seafood. Total mesophilic bacteria count of rainbow trout fillets was 1.42 log CFU/g at the beginning of the storage. Following that, the total mesophilic bacteria count in all the samples increased steadily during the storage period. Control and chitosan coating fillets values were increased to 7.10 and 6.99 log CFU/g by the end of storage period. At the end of the storage, total mesophilic bacteria counts were recorded as 6.34, 6.28 and 6.05 log CFU/g in the groups O0.5, O1 and O_2, respectively. According to Shalaby et al. (2018), OLE has a positive effect on antibacterial activity in minced beef. Fan et al. (2009) reported that chitosan coating was efficient in extension of shelf life of the carp from 25 to 30 days at 4°C (6.9 log10 CFU/g). Mohan et al. (2012) determined that 2% chitosan coating on Indian oil sardine inhibit the growth of total mesophilic bacteria when compared to control. Vatavali et al. (2013) determined that the combination of chitosan and oregano essential oil reduced the total mesophilic bacteria count by 2.2 log CFU/g in Red Porgy. Jeon et al. (2002) determined that Herring and Atlantic Cod fillets coated with chitosan resulted in reduction of total aerobic mesophilic bacteria by 10^3 CFU/g. Hassanzadeh et al. (2018) determined that the combination of chitosan and grape seed extract on rainbow trout fillets inhibited the total mesophilic bacteria count till 9th day of storage. Considering the suggested limits (7 log CFU/g) for fresh fish (ICMSF, 1986; Alberle et al., 2001), the results of the current study specify that 2.0% OLE treated fillets were in good quality during the storage period when compared with the other groups.

Total psychrophilic bacteria count

The differences of total psychrophilic bacteria count in rainbow trout fillets coated with chitosan solution supplemented with different concentrations (0.5%, 1.0% and 2.0%) of OLE are presented in Table 2.

Table 2. Changes of total psychrophilic bacteria count of rainbow trout fillets immersed in chitosan solution incorporated with different concentrations (0.5%, 1.0% and 2.0%) of OLE (log CFU/g).

Storage (Day)	C	CCh	O0.5	O1	O2
0	2.08±0.10^{Af}	2.08±0.10^{Af}	2.08±0.10^{Af}	2.08±0.10^{Af}	2.08±0.10^{Ae}
3	3.33±0.05^{Ae}	3.19±0.06^{Be}	2.39±0.01^{Ce}	2.28±0.02^{De}	2.17±0.03^{Ee}
6	4.38±0.11^{Ad}	4.17±0.18^{Ad}	3.37±0.04^{Bd}	3.28±0.10^{BCd}	3.03±0.01^{Cd}
9	5.47±0.01^{Ac}	5.39±0.03^{Ac}	4.43±0.03^{Bc}	4.31±0.01^{Cc}	4.15±0.09^{Dc}
12	6.40±0.02^{Ab}	6.27±0.03^{Ab}	5.44±0.01^{Bb}	5.17±0.12^{Cb}	5.08±0.01^{Cb}
15	7.17±0.01^{Aa}	7.04±0.04^{Ba}	6.43±0.04^{Ca}	6.35±0.01^{Ca}	5.92±0.04^{Da}

Means specified by capital letter in same rows differ P<0.05 significantly. Means specified by lowercase letter in same columns differ P<0.05 significantly. C: control, CCh: 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 1.0% of OLE, O2: fillets immersed in chitosan coating supplemented with 2.0% of OLE.

The initial total psychrophilic bacteria count of rainbow trout fillets was determined as 2.08 log CFU/g. Total psychrophilic bacteria count of all groups increased until at the end of the storage. Significant differences were observed between C, CCh and the groups coated with chitosan solution supplemented with different concentrations (0.5%, 1.0% and 2.0%) of OLE ($P<0.05$). Total psychrophilic bacteria count of control and chitosan coated groups were significantly ($P<0.05$) increased during the storage period and reached to 7.17 and 7.04 log CFU/g, respectively. Total psychrophilic bacteria count of the samples supplemented with 0.5% OLE and 1.0% OLE were slightly lower than control and CCh with value of 6.43 and 6.35 log CFU/g. Compared to other treatment groups, addition of 2.0% OLE into chitosan coating solution significantly retarded the microbial growth.

Salama and Ibrahim (2011) studied the effects of different concentrations (1%, 2%, 3%) of chitosan solution on common carp fillets during 15 days of storage at 4°C. They reported that total psychrophilic bacteria count increased with the storage and 3% chitosan was the most effective treatment. According to Cosmai et al. (2017), adding OLE reduced the psychrophilic bacteria count considerably. This could be owing to OLE's direct inhibitory effect on microbial growth and the existence of anti-microbial polyphenolic chemicals in OLE. The presence of active components in olive leaf such as oleuropein, tyrosol, and hydroxytyrosol, which function as antibacterial agents, was linked to a decrease in psychrophilic bacterial populations (Cardoso et al., 2005). In the present study, chitosan solution supplemented with 2.0% OLE had an inhibitory impact on psychrophilic bacteria count. Similar results were observed by Shalaby et al. (2018). Jouki et al. (2014) reported the initial total psychrophilic bacteria count of rainbow trout fillets coated with chitosan and thyme or oregano essential oil were lower than the control. The psychrophilic bacterial count of fish fillets covered with 2% chitosan coating incorporated with 2% sumac was reached to 5.25 log CFU/g from 2.92 log CFU/g (Fadiloglu and Emir Coban 2018).

They discovered that sumac had antibacterial effects and inhibited the growth of psychrophilic bacteria. Consumable limit levels for psychrophilic bacterial count in fish have been identified as 6-7 log CFU/g in prior research (Erkan, 2007). In this study, antimicrobial action kept the total number of psychrophilic bacteria in rainbow trout fillets coated with chitosan solution supplemented with 2.0% OLE below the permitted limit value.

Total yeast and mold count

The differences of total yeast and mold count in rainbow trout fillets coated with chitosan solution supplemented with different concentrations (0.5%, 1.0% and 2.0%) of OLE are presented in Table 3.

Table 3. Changes of total yeast and mold count of rainbow trout fillets immersed in chitosan solution incorporated with different concentrations (0.5%, 1.0% and 2.0%) of OLE (log CFU/g).

Storage (Day)	C	CCh	O0.5	O1	O2
0	0.50±0.28^{Af}	0.50±0.28^{Af}	0.50±0.28^{Ae}	0.50±0.28^{Ad}	0.50±0.28^{Ad}
3	1.28±0.14^{Ae}	1.36±0.26^{Ae}	0.84±0.09^{Bd}	0.77±0.10^{Bd}	0.65±0.07^{Bd}
6	2.80±0.04^{Ad}	2.34±0.03^{Bd}	1.65±0.07^{Cc}	1.50±0.28^{Cc}	1.32±0.03^{Cc}
9	3.29±0.08^{Ac}	3.13±0.07^{Ac}	1.87±0.04^{Bc}	1.83±0.03^{Bc}	1.50±0.14^{Cc}
12	4.16±0.21^{Ab}	4.14±0.25^{Ab}	2.43±0.02^{Bb}	2.30±0.06^{Bb}	2.14±0.09^{Bb}
15	5.28±0.06^{Aa}	4.88±0.42^{Aa}	3.98±0.02^{Ba}	3.84±0.08^{Ba}	3.28±0.03^{Ca}

Means specified by capital letter in same rows differ P<0.05 significantly. Means specified by lowercase letter in same columns differ P<0.05 significantly. C: control, CCh: 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 1.0% of OLE, O2: fillets immersed in chitosan coating supplemented with 2.0% of OLE.

The initial value of total yeast and mold count was defined as 0.50 log CFU/g. At the end of the storage, the value of control (C) and chitosan coating group (CCh) increased to 5.28 log CFU/g and 4.88 log CFU/g, respectively, while this value was observed as 3.98 and 3.84 log CFU/g in the O0.5 and O1 groups, respectively. Total yeast and mold count in the O2 group was significantly (P<0.05) lower (3.28 log CFU/g) than those of the other groups. Lafka et al. (2013) found that OLE contains antibacterial chemicals that kill bacteria and fungi. OLE has also been shown to be a powerful antifungal agent by Özcan and Matthäus (2017). Feng et al. (2016) observed that golden pomfret fillets with chitosan and gelatin coating had low yeast and mold count when compared to control, proposing that the edible coating reduced the growth of yeast and molds. According to Shalaby et al. (2018), OLE had a favorable effect on reducing total yeast and mold counts in minced beef. This could be owing to the antifungal and antimicrobial effects of polyphenolic chemicals found in OLE (Verma et al., 2015). In this study, the total yeast and mold counts of rainbow trout fillets immersed in chitosan coating combined with 2.0% OLE were considerably (P<0.05) lower than the other groups, indicating that high concentration of OLE is more effective in yeast and mold inhibition.

Total Enterobacteriaceae count

The differences of total Enterobacteriaceae count in rainbow trout fillets coated with chitosan solution supplemented with different concentrations (0.5%, 1.0% and 2.0%) of OLE are presented in Table 4.

Table 4. Changes of total Enterobacteriaceae count of rainbow trout fillets immersed in chitosan coating incorporated with different concentrations (0.5%, 1.0% and 2.0%) of OLE (log CFU/g).

Storage (Day)	C	CCh	O0.5	O1	O2
0	$0.00{\pm}0.00^{Af}$	$0.00{\pm}0.00^{Af}$	$0.00{\pm}0.00^{Af}$	$0.00{\pm}0.00^{Af}$	$0.00{\pm}0.00^{Af}$
3	$1.24{\pm}0.41^{Ae}$	$0.78{\pm}0.25^{Abe}$	$0.63{\pm}0.21^{Abe}$	$0.45{\pm}0.21^{Be}$	$0.39{\pm}0.12^{Be}$
6	$2.38{\pm}0.10^{Ad}$	$2.09{\pm}0.12^{Bd}$	$1.98{\pm}0.02^{Bd}$	$1.87{\pm}0.05^{BCd}$	$1.71{\pm}0.12^{Cd}$
9	$3.43{\pm}0.05^{Ac}$	$3.31{\pm}0.01^{Bc}$	$2.45{\pm}0.02^{Cc}$	$2.36{\pm}0.03^{Cc}$	$2.18{\pm}0.06^{Dc}$
12	$5.10{\pm}0.02^{Ab}$	$5.02{\pm}0.01^{Ab}$	$4.11{\pm}0.14^{Bb}$	$3.99{\pm}0.01^{Bb}$	$3.69{\pm}0.05^{Cb}$
15	$6.37{\pm}0.01^{Aa}$	$6.06{\pm}0.08^{Ba}$	$5.23{\pm}0.03^{Ca}$	$5.18{\pm}0.03^{Ca}$	$5.02{\pm}0.03^{Da}$

Means specified by capital letter in same rows differ P<0.05 significantly. Means specified by lowercase letter in same columns differ P<0.05 significantly. C: control, CCh: 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 1.0% of OLE, O2: fillets immersed in chitosan coating supplemented with 2.0% of OLE.

The Enterobacteriaceae family has been identified as a hygiene indicator (Ahmad et al., 2012). Total Enterobacteriaceae was not detected in the rainbow trout fillets at the beginning of the storage. Total Enterobacteriaceae counts of rainbow trout fillets immersed in chitosan solution incorporated with 2.0% OLE showed significant (P<0.05) differences compared with other groups. The lowest value (5.02 log CFU/g) was observed in O2 group at the end of the storage, while this value was defined as 6.37, 6.06, 5.23, and 5.18 log CFU/g in C, CCh, O0.5 and O1 groups, respectively.

Saadony et al. (2019) found that olive leaf extracts inhibit Gram-negative bacteria, particularly those belonging to the Enterobacteriaceae family. Tsai et al. (2002) determined that using 1% chitosan coating on salmon fillets retarded microbial growth and extend shelf life up to 9 days. Vatavali et al. (2013) determined that combination of chitosan and oregano essential oil on Red Porgy caused a significant reduction in total Enterobacteriaceae count. Chitosan, in combination with sumac, functioned as an antibacterial and antioxidant agent, inhibiting the total Enterobacteriaceae count and extending the microbiological shelf life of rainbow trout fillets (Fadıloğlu and Emir Çoban, 2018). Ojagh et al. (2010) coated the rainbow trout fillets with chitosan solution combined with cinnamon oil and they observed the total Enterobacteriaceae count of treated samples was lower than control. Wenjiao et al. (2013) determined that the combination of chitosan and bamboo leaves significantly reduced the microbial growth of silver carp due to antimicrobial properties of bamboo leaf. Moosavi-Nasab et al. (2016) reported that the combination of chitosan solution incorporated with black pepper essential oil on common carp showed significant reduction in total Enterobacteriaceae count compared to other groups. Yu et al. (2017) observed that the total Enterobacteriaceae count of chitosan solution with essential oils from clove, cinnamon and lemon grass on grass carp fillets were below the acceptable limit (7 log10 CFU/g) specified by ICMSF (1986). In this study, antimicrobial properties of OLE kept the total Enterobacteriaceae count in rainbow trout fillets coated with chitosan solution supplemented with 2.0% OLE below the permitted limit.

CONCLUSION

Different concentration of OLE (0.5%, 1.0%, and 2.0%) was incorporated with chitosan solution in order to inhibit microbial growth in rainbow trout fillets during refrigerated storage. According to the results, chitosan coating enriched with OLE (especially 2% concentration) showed inhibitory effects on the bacterial growth of rainbow trout fillets during storage. It can be suggested that chitosan coating supplemented with olive leaf extract can be utilized as a natural resource in order to maintain microbial quality of fish fillets.

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