Journal of Animal and Veterinary Advances 11 (15): 2813-2816, 2012
ISSN: 1680-5593

Effects of Chitosan Prepared in Different Solvents on Quality Parameters of Mackerel Fillets

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Abstract: In this study, the use of chitosan prepared in different solvents (acetic acid, lactic acid) as a coating material was researched. For this purpose, mackerel were coated with lactic and acetic acid chitosan and the filllets refrigerated storage (4±10°C) were examined. The control and treated fillet samples were analyzed periodically for chemichal and microbiological characteristics. Both chitosans had significant effect on chemichal and microbioiogical values of the mackerel fillets during storage ($p<0.05$). Altougth, pH and Total Volatile Base-Nitrogen amount (TVB-N) values were determined higher in control group, Thiobarbituric Acid Reactive Substance (TBARS) level was low this group during the storage time ($p<0.05$). Development of bacteria was slower in fillets containing acetic acide chitosan compared with lactic acide and control group. The other hand, only lactic acide bacteria was determined high level from control and acedic acide. Altougth, the results indicated that both chitosans improved the quality characteristics of mackerel fillets and extended the shelf life during the refrigerated storage which was supported by the results of chemichal and microbial analyses, the group of acetic acide has very low value than lactic acide except TBARS and lactic acide bacteria ($p<0.05$).

Key words: Chitosan, mackerel, storage time, acetic acid, microbiological

INTRODUCTION

Particularly in recent years due to their protein quality and nutrient values, fish are a frequently preferred food. Fishery product indispensable in animal nutrition are highly perishable due to their biological composition and spoilage of fish muscle causes a short shelf life in fish. Its properties as a good substrate for decomposing microorganisms can easily spoilage (Ashie *et al*., 1996). When the recent tendency towards the consumption of these products gained importance, not only considering quality but also considering safety, the rise in the concerns on the destruction of traditional synthetic plastic material drew attention to renewable natural coating materials (Alak *et al*., 2010). Chitosan which is a natural product can be used as a coating material for the storage of fish for its effects on texture (Benjakul *et al*., 2003) as well as its antimicrobial (Cuero, 1999; Jeon *et al*., 2002) and antioxidant (Kamil *et al*., 2002) properties. The aim of the present study is to determine the chemical (pH, TVB-N and TBARS) and microbial (numbers of mesophilic, lactic acid and pseudomonas bacteria) properties of the mackerel fillets after storage at 4±1°C which were coated with chitosan film, prepared with different solvents.

MATERIALS AND METHODS

Fish and chitosan coating: The low viscosity chitosan was obtained from Sigma-Aldrich. Solutions of 1.5% chitosan were prepared by solving 7.5 g chitosan in 500 mL 1.5% acetic acid and 500 mL 1.5% lactic acid. In order to completely solubilize chitosan, the solutions were mixed at room temperature for one night using a mixer. After chitosan was completely solubilized, it was filtered using cheesecloth to remove impurities (mesh width approx. 1 mm^2). In the end, the prepared solution was poured onto Teflon-coated pans, the films were taken out of pans after being dried at room temperature for a minimum of 72 h. The resulting films were kept in the acclimatization cabin at 25°C (Alak *et al*., 2010). A total of 60 mackerel caught from the Marmara and transferred to the market on ice in 24 h were purchased at market with an average weight of 280 g and then transferred to the Erzurum. Preparation of fillets acording to Sikorski (1990). Than, fillets were divided into 3 groups and each group has 40 fillets. The 1st group was the control group (C) which not treatment. The treatment group of fillets was coated with chitosan film, 2nd group which was prepared with Acetic acid (AC) and 3rd group prepared with Lactic acide (LC). The fillets were preserved at 4±1°C for 9 days.

Chemical and microbiological analyses: The samples that were used in the chemical and microbiological analysis of the fillets were collected using lancets under aseptic conditions and an analysis sample was taken. Every analysis was made as three parallels. About 10 g samples were taken which were made smaller and 100 mL distilled water was added onto each sample. The mixture was homogenized in Ultra Turrax for 1 min and the pH values were measured using a pH meter (SCHOTT, Lab Star pH). The Total Volatile Base-Nitrogen amount (TVB-N) and Thiobarbituric Acid Reactive Substance (TBARS) value in fish was determined according to Alak *et al*. (2010). A sample of 25 g was taken for microbiological analysis and 225 mL sterile physiological saline solution (0.85% NaCl) was added to the sample. The mixture was homogenized in a Stomacher Blender (Lab Stomacher Blender 400-BA7021, Sewardmedical). The proper dilutions were prepared by taking samples from this homogenate. Plate Count Agar (PCA, Merck) was used as a medium for the total bacteria count. The dilutions were spread on petri dishes using the Spread Plate Method and the petri dishes were incubated for 2 days at 37°C for mesophilic bacteria count. The lactic acid bacteria count was performed after the incubation of the petri dishes with MRS agar for 3 days at 30°C. Pseudomonas bacteria count was taken after the incubation of petri dishes with C-F-C (Cetrimide-Fucidin-Cephloridine) added to pseudomonas agar for 2 days at 25°C. Enterobactcriaceae was determined in VioletRed- Bile-Glucose agar (VRBG-agar, Merck) plates incubated anaerobically at 30°C for 2 days. There were two parallels of each culture and the results were given in log10 CFU g^{-1} (Alak *et al*., 2010).

Statistical analyses: All of data were analyzed with variance analysis using SPSS package software and the mean values of significant variation sources were compared using the Duncan Multiple Comparison Test (Alak *et al*., 2011). However, for the statistical analysis of some bacteria counts, <2 log kob g^{-1} were taken as 2 log kob g^{-1}.

RESULTS AND DISCUSSION

Fish freshness is judged by chemichal and microbiological methods. Several chemical indices are very important with microbiological paramaters. The results of chemical (pH, TVB-N and TBARS) and microbiological parameters in mackerel fillets stored in non treatment control (C), chitosan containin Acetic Acyde (CA) and chitosan containin Lactic Acyde (LA) film for 9 days at 4°C are shown in Table 1-4. For fishery products, pH changes can be used as a spoilage indicator such as TBARS and TVB-N (Fan *et al*., 2009; Ojagh *et al*., 2010; Mohan *et al*., 2012). In this study during the storage time, the pH values increased gradually.

Similar observations were made by other researchers (Alasalvar *et al*., 2001; Manju *et al*., 2007; Fan *et al*., 2009; Song *et al*., 2011). The decrease of pH value might be attributed to the dissolution of CO_2 in the fish sample while the increase was postulated to be caused by an increase in volatile bases produced by either endogenous or microbial enzymes (Li *et al*., 2012). Control samples exhibited a significantly ($p<0.05$) higher increase reaching a TVB-N value of 18.96±6.29 and 15.94±3.99, 16.76±4.20 mg/100 g values were observed for with acetic and lactic acid chitosan treated samples, respectively on the corresponding day. This could be due to the

Table 1: Chemical properties of mackerel fillets with C, CA and LA

Treatments	pH	TBARS (μmol kg^{-1})	TVB-N (mg/100 g)
C	6.64±0.40^{a}	33.86±15.53^{c}	18.96±6.29^{a}
CA	6.24±0.32^{b}	37.00±17.72^{b}	15.94± 3.99^{c}
LA	6.01±0.17^{c}	39.44±18.79^{a}	16.76±4.20^{b}

Table 2: The influence of storage time on some chemical status of mackerel fillets

Storage time (days)	pH	TBARS (μmol kg^{-1})	TVB-N (mg/100 g)
0	6.03 ±0.06^{c}	14.01±0.17^{d}	12.42±0.36^{d}
3	6.17±0.36^{c}	29.06±2.66^{c}	13.75±0.80^{c}
6	6.34±0.44^{b}	50.68±5.18^{b}	18.96±2.23^{b}
9	6.66±0.38^{a}	53.34±2.93^{a}	23.77±2.70^{a}

Means in columns with different superscripts are significantly different at $p<0.05$

Table 3: Microorganisms in mackerel fillets with C, CA and LA (log cfu g^{-1})

Treatments	Total aerobic mesophilic bacteria	Psychrotrophic	Pseudomonas	Enterobacteriaceae	Lactic acid bacteria
C	5.50±2.13^{a}	5.96±1.31^{a}	6.66±0.16^{a}	3.09±0.55^{a}	3.34±1.31^{b}
CA	4.08±1.78^{c}	4.85±1.25^{c}	4.79±1.50^{b}	2.73±0.44^{c}	2.56±0.42^{b}
LA	4.99±2.12^{b}	5.46±1.23^{b}	4.89±1.58^{b}	2.87±0.54^{b}	3.42±0.99^{a}

Table 4: The influence of storage time on microbiological status of mackerel fillets (log cfu g^{-1})

Storage time (days)	Total aerobic mesophilic bacteria	Psychrotrophic	Pseudomonas	Enterobacteriaceae	Lactic acid bacteria
0	2.00±0.01^{d}	4.06±0.15^{d}	2.64±0.27^{d}	2.00±0.01^{d}	2.00±0.01^{d}
3	2.93±0.66^{c}	4.41±0.89^{c}	3.51±0.50^{c}	2.70±0.26^{c}	2.28±0.12^{c}
6	5.39±1.02^{b}	5.95±0.62^{b}	6.04±0.47^{b}	3.15±0.19^{b}	3.79±0.70^{b}
9	7.12±0.44^{a}	6.99±0.59^{a}	6.38±0.32^{a}	3.39±0.19^{a}	3.99±0,78^{a}

Means in columns with different superscripts are significantly different at $p<0.05$

protective chitosan coating which inhibited the bacterial growth and slowed spoilage (Mohan *et al*., 2012). In the present study, both TVB-N and pH values not exceeded the limit of acceptability on the day of storage time for untreated, chitosan treated samples, respectively indicating a good correlation. In Table 2, the TBARS values for the different treatments during storage are presented. The initial TBARS values ranged from 14.01±0.17 (μmol kg^{-1}) samples to 53.34±2.93 (μmol kg^{-1}) during the storage period. TBARS values of the controls and coated samples increased with storage time by the end of the storage period (day 9), however control samples reached significantly ($p<0.05$) lower TBARS value comparison with the chitosan treatment samples. In the current study, it was reported that TBARS values appeared to be negatively affected by the application of different solution of acids. Similar observation was made by Alak *et al*. (2010). On the other hand, Shaidi *et al*. (2002) analyzed the antioxidant effect of the addition of chitosan on processed herring, and observed that chitosan showed an antioxidant effect and the TBARS value in the samples that were treated with chitosan dropped 61% compared to the control group, after 8 storage days. Similarly, it was reported that the addition of 0.2, 0.5 and 1% chitosan with different molecular weights decreased lipid oxidation in salmon (Kyung and Thomas, 2007).

Many studies were conducted on the antimicrobial properties of chitosan (Gua-Jane *et al*., 2002; Lopez-Caballero *et al*., 2005; Fernandez-Saiz *et al*., 2010; Ojagh *et al*., 2010; Li *et al*., 2012; Mohan *et al*., 2012).

The changes in bacterial counts with the storage period for the treated and untreated fillets are shown in Table 3 and 4. For the total aerobic mesophilic bacteria, the initial quality of the fish used in the study was good as indicated by a low initial bacterial counts (5.50±2.13 log cfu g^{-1}) which further reduced to 4.08 and 4.99 log cfu g^{-1}, respectively for with acetic and lactic chitosan treated samples. With the storage period, the counts showed an increasing trend all of bacteria. The increase was significantly higher ($p<0.05$) for untreated samples compared to treated samples. A relatively long lag phase apparently indicates the effectiveness of chitosan in inhibiting the growth of microorganisms (Mohan *et al*., 2012). In this study, similar results obtained all bacteria types. Similar results recorded the antimicrobial properties of chitosan coating have been reported in the literature same as this study (Jeon *et al*., 2002; Lopez-Caballero *et al*., 2005; Gua-Jane *et al*., 2002; Arashisar *et al*., 2009; Alak *et al*., 2010; Ojagh *et al*., 2010). It was reported that the coating, a blend of chitosan dissolved in acetic acid and gelatin was observed to exert an inhibitory effect on the gram negative flora by Lopez-Caballero *et al*. (2005). The effectiveness of acetic chitosan treatment was slightly less than the samples treated with lactic acid chitosan except lactic acid bacteria.

CONCLUSION

The results of this study show that chitosan edible coating for mackerel was effective in reducing the spoilage and extending the shelf life considerably. Considering the obtained data, coating with chitosan film which is prepared in different solutions is an alternative to traditional fish preservation methods. However, coating with chitosan which was prepared using acetic acid had a positive effect on the microbiological and chemical parameters of mackerel (*Scomber scombrus*) fillets. The results indicated that the combined preservation technology have a broad potential application in the shelflife of fishery products.

ACKNOWLEDGEMENT

This research was financially supported by Ataturk University.

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