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Research Article

Effects of chitosan coating on the quality of rainbow trout fillet during storage in refrigerator

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ABSTRACT

The aim of the present study was to evaluate the effects of chitosan coating on quality and shelf life of fresh rainbow trout (*Oncorhynchus mykiss*) in refrigerator (5±1 °C). Fishes were slaughtered quickly and fresh samples were treated with a solution of 2% (w/v) medium molecular weight chitosan dissolved in glacial acetic acid randomly and stored in refrigerator for 15 days. Then all the samples were investigated for psychrotrophic bacteria count and also sensory analysis within 3 day intervals. As shown results illustrated that the initial bacterial load was 2.51±0.3 $\log_{10}$ CFU g^{-1} in chitosan coated samples followed by 2.93±0.5 $\log_{10}$ CFU g^{-1} in control samples. These values were increased to 6.51±0.3 and 8.5±0.5 $\log_{10}$ CFU g^{-1} for chitosan coated and control samples, respectively. Thus, it could be concluded that chitosan coating can retard the microbial growth for treated samples and also extend the shelf life of rainbow trout in refrigerator significantly ($p<0.05$).

Keywords: Chitosan coating; Fish; Microbial deterioration; Quality.

1. Introduction

Edible coatings made from polysaccharides, proteins and lipids due to their ability to control the influence of dissolved substances, gases and steam, can increase the shelf life of foods. Chitosan (poly-beta 1, 4-D- glucosamine) is the second most abundant natural polymer after cellulose. It occurs as a component of the cell wall of some fungi but it is generally produced by carrying out the deacetylation of chitin, an abundant polysaccharide found in the shells of crustaceans, particularly crabs and shrimps. It is a biocompatible, biodegradable and antimicrobial polymer (Alishahi, Mirvaghefi, Tehrani, Farahmand, Koshio, Dorkoosh, et al., 2011).

Chitosan is a biopolymer that has many applications in the food industry (Kanatt, Chander, & Sharma, 2008). A number of useful functional properties of chitosan in food have been reported (Shahidi, Arachchi, & Jeon, 1999) in which nontoxicity, biodegradability, biocompatibility (Kumar, 2000), anti-bacterial properties, the ability to form films and coatings (Jeon, Kamil, & Shahidi, 2002) and antioxidative properties (López-Caballero, Gómez-Guillén, Pérez-Mateos, & Montero, 2005) are important. On the other hands, chitosan has functional properties that make it useful in nutrition (Gallaher, Gallaher, Mahrt, Carr, Hollingshead, Hesslink Jr, et al., 2002). These include its antimicrobial activity and ability to form protective films (Cuero, 1998; Jeon et al., 2002), texturizing (Benjakul, Visessanguan, Phatchrat, & Tanaka, 2003), binding action (No, Lee, & Meyers, 2000); and its antioxidant activity (Kamil, Jeon, & Shahidi, 2002). Many investigators have studied chitosan as edible coating material for fishery products to enhance microbiological quality and extend the shelf life (Agustini & Sedjati, 2013; Alishahi, et al., 2011; Mohan, Ravishankar, Lalitha, & Gopal, 2012).

Fresh fish because of their biological composition, are highly perishable. Quality deterioration in fish is a result of changes made by biological reactions such as lipid oxidation, fish enzymes activities, and metabolic activities of microorganisms. This activities will cause shortening of shelf life in fish and seafood (Arashisar, Hisar, Kaya, & Yanik, 2004). In spite of numerous studies, which have indicated that improving the quality and extension the shelf life of fish through the use of chitosan, there are very little reports on the effect of

Table 1
Sensory scores for rainbow trout fillet samples during refrigerated storage[a]

parameter	treatment	Storage time (day)					
		0	3	6	9	12	15
Taste	Control	5.0±0.0[a]	4.86±0.38[b]	4.43±0.53[b]	4.14±0.38[a]	3.86±0.38[a]	2.14±0.38[a]
Smell	Control	5.0±0.0[a]	4.86±0.38[a]	4.57±0.53[a]	3.86±0.38[c]	2.29±0.49[c]	1.86±0.38[c]
Taste	Chitosan	5.0±0.0[a]	4.86±0.38[b]	4.71±0.49[b]	4.57±0.53[b]	4.00±0.00[a]	3.71±0.49[b]
Smell	Chitosan	5.0±0.0[a]	4.71±0.49[a]	4.57±0.53[a]	4.29±0.49[c]	3.57±0.53[d]	3.29±0.49[d]

[a] Values are expressed as means±SD of triplicates; Different small superscript letters indicate significant ($p<0.05$) differences between same treatments within the different storage time.

chitosan coating on quality of rainbow trout fillet during chill storage. Therefore, the aim of the present study was to evaluate chitosan as a natural biopolymer as a novel packaging for storage of rainbow trout in refrigerator.

2. Materials and Methods

2.1 Coating preparation

Chitosan powder with deacetylation degree of 75-85% (190-310 KDa) purchased (Sigma-aldrich co, Germany). A 2% chitosan solution (w/v) were prepared using glacial acetic acid 1% (v/v) and 0.75 mg g^{-1} glycerol was added as the plasticizer and the solution were mixed gently on a hotplate magnetic stirrer for 10 min. mixed solution filtered through Whatman No. 3 filter paper to remove insoluble particles. Finally, the coating solution were degassed to remove the small bubbles. It is notable that a 1% glacial acetic acid was used to treat control samples to make similar situations among treatments.

2.2. Sample preparation

Rainbow trout varying from 450 to 550 g obtained from local farm and killed by hypothermia. All fishes were transferred to laboratory in ice slurry and subjected to nobbing and evisceration. Then samples were divided to two lots randomly. Each lot dipped in its individual dipping solution for two 30 seconds with 2 min rest (Ojagh, Rezaei, Razavi, & Hosseini, 2010). After dipping, all samples were removed from the dipping solution and were put in 10 °C for 5 h and then stored in the refrigerator for15 days. Afterward, all samples were evaluated each 3 days for quality properties.

2.3 Bacteriological analysis

The total viable count of psychrotrophic bacteria were enumerated using 10 g of anterior dorsal zone of fish muscle. The tissue was obtained aseptically and following homogenization in 90 mL 0.1% peptone water, was subjected to make serial dilutions and cultured using PCA via the pour plate method (Acuamedia Manufactures, Inc., Lansing, MI, USA). The cultures were kept in 10 °C for 7 days in an incubator. All enumerations were triplicate (Arashisar et al., 2004).

2.4. Lipid oxidation

The Thiobarbituric acid (TBA) value was determined colorimetrically by the method of Porkony and Dieffenbacher as described in literature (Egan, Krik, & Sawyer, 1997). A portion (200 mg) of sample was weighted into a 25 ml volumetric flask. An aliquot (1 ml) of 1-Butanol was added to dissolve the sample. The mixture was made to volume and mixed. A portion (5.0 ml) of the mixture was pipetted into a dry stoppered test tube adding 5ml of TBA reagent (prepared by dissolving 200 mg of 2-TBA in 100 ml 1-Butanol, filtered, stored at 4 °C for not more than 7 days) were added. The test tubes were stoppered, vortexed and placed in a water bath at 95 °C for 120 min then cooled. Absorbance (As) was measured at 530 nm against water blank. A reagent blank was run and absorbance (Ab) recorded. TBA value (mg of malonaldehyde Kg^{-1} of tissue) was obtained by the formula.

$$TBA\ (mg\ MA\ Kg^{-1}) = (50.\ (As-Ab)) / 200$$

2.5. Sensory analysis

All samples were steamed at 98 °C for 60 min and then subjected to decision by 7 semi-trained panelists whom were acquaintance with sensory analysis (aged, 23-28). Sensory analysis was done based on 5-point hedonic scale for the cooked fish sample (5, excellent; 1, extremely bad) and crude fish smell (5, excellent; 1, Disgusting smell).

2.6. Statistical analysis

The analysis of variances (ANOVA) and Duncan, Kruskal-Wallis and Mann–Whitney U tests were used to investigate the statistical differences between means using SPSS (ver. 21).

3. Results and Discussion

3.1. lipid oxidation

The results of TBA are shown in Figure 1. In general, there was an incremental pattern in TBA over the time 15 days of storage but significant differences were revealed specially at the last stages ($p<0.05$). The initial TBA contents were 0.21±0.02 and 0.27±0.04 mg MA Kg^{-1} and increased to 0.72±0.02 and 0.55±0.04 mg MA Kg^{-1} for control and chitosan coated samples, respectively. As the results shown, there was no significant difference observed between coated and control samples from day 0 to 9. These results could be discussed considering barrier properties of chitosan that could affect gas transfer through the tissue which could

affect lipid oxidation. These findings are in relation with other researchers reported in the literature (Fan, Sun, Chen, Qiu, Zhang, & Chi, 2009; Mohan et al., 2012).

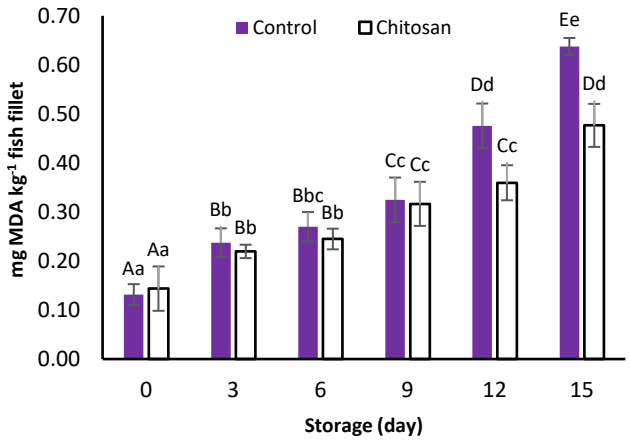

Figure 1. Changes in the TBA during refrigerated storage (Small letters a, b, c in each column indicates significant difference inside group in different times and capital letters A, B, C indicates significant difference between groups)

3.2. Microbiological analysis

Gram-negative Psychrotrophic bacteria are the main group of microorganisms causing spoilage in fish that were stored in cold temperatures and aerobic conditions (Sallam, 2007). In the present study the initial load was 2.51 and 2.93 $\log_{10}$ CFU g^{-1} in chitosan coated and control samples respectively. But, the bacterial load of control samples at day 10-11 reached to 8.5 $\log_{10}$ CFU g^{-1} that exceeded 7 $\log_{10}$ CFU g^{-1} known as maximum allowable bacterial load for crude fish

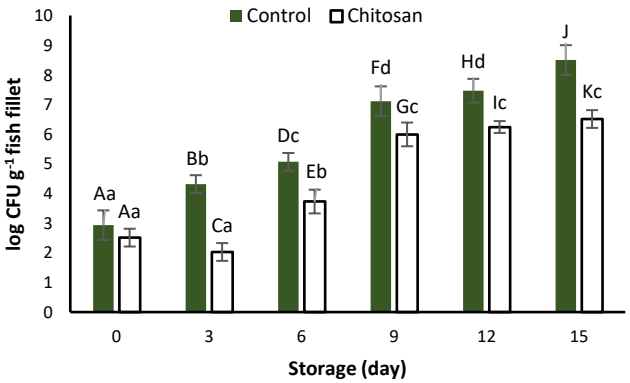

Figure 2. Changes in the Psychrotrophic bacteria count during refrigerated storage (Small letters a, b, c in each column indicates significant difference inside group in different times and capital letters A, B, C indicate significant difference between groups.

(Sallam, 2007) while, it was equal to 6.51 $\log_{10}$ CFU g^{-1} for chitosan coated samples even after 15 days of storage, that was still in the allowable range of microbial count. The differences between bacterial loads are shown in Figure 2. The results of the present study are in accordance to the literature. It was reported that pre-treatment of *Oncorhynchus nerka* with 1% chitosan for 3 hours could hinder the mesophilic, psychrophilic, Coliforms, Aeromonas and Vibrio's growth (Tsai, Su, Chen, & Pan, 2002). Also, it was reported that the mixture of chitosan and gelatin had barrier properties on Gram negative bacteria in the fish patties (López-Caballero et al., 2005).

3.2 Sensory analysis

The results of sensory analysis are shown in Table 1. The results illustrated that the coating did not cause unfavorable properties in case of sensory. In addition, the concentration of used chitosan was suitable to form the coating. The taste and smell of the samples got undesirable from day 9 onward while, it was still acceptable to day 15th for chitosan coated samples. As shown in Table 1, there were no significant differences between treatments in case of taste during time but, the differences were seen on day 6 onward for smell ($p<0.05$). This finding could be attributed to the scoring process for smell and taste that in case of taste, samples were heated that could enhance them in comparison with the smell which were subjected to evaluation in raw form.

4. Conclusions

It seems that the mechanism of action of chitosan is related to interactions between chitosan molecules with a positive electrical charge and the negative charge on the cell membrane of microbes (Barrow & Shahidi, 2007) as well as its function as a barrier against the passage of oxygen (Jeon et al., 2002). In the present study the sensory analysis was in relation to microbial growth. At the 9th day, the control samples stunk and got discolored that could be attributed to the microbial growth and lipid oxidation. The chitosan coating showed its antioxidation and antimicrobial effects and also the barrier properties against gasses to reduce lipid oxidation that leads to shelf life extension of rainbow trout in the refrigerator. It is presumption that chitosan caused the microbial growth and lipid oxidation to be retarded. As shown, the bacterial load of control samples in day 10-11 exceeded the 7 $\log_{10}$ CFU g^{-1} that is allowable limit for row fish (Sallam, 2007), While it was in the allowable range for chitosan coated samples. Considering the sensory properties of coated samples and also their microbial growth and because of well documented antioxidative and antibacterial effects of chitosan, it is concluded that chitosan made an edible coating on the samples surface which could be used safely for fish storage in refrigerator.

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