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Effect of chitosan coating on the quality characteristics of rohu (*Labeo rohita*) fillets during chilled storage

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ABSTRACT

The current work was undertaken to examine the effect of chitosan as a natural edible coating on the quality of rohu (*Labeo rohita*) fillets during 30 days of chilled storage. The fish fillets were treated with different concentrations of chitosan (0.1, 0.25, 0.5 and 1% chitosan) and fish fillet without addition of chitosan was kept as control. Sampling was done initially on the 0th day and thereafter at ten day intervals and were examined periodically for water holding capacity (WHC), water extractable protein (WEP), salt extractable protein (SEP), thiobarbituric acid reactive substances (TBARS), pH, total viable count (TVC), psychrotrophic count (PTC) and sensory characteristics. Findings indicated that chitosan coating aided in retaining the quality and extended the shelf life of rohu fillets during refrigerated storage suggesting the suitability of using chitosan as a bio-preservative to extend the shelf life of chilled stored fish.

Keywords: Chilled storage, Chitosan, Meat quality, Rohu fillet

Introduction

Fish is regarded as a worthy source of protein (15-20%), vitamins, water soluble nutrients as well as minerals and low in carbohydrates (Tayel, 2016). Fish meat also contains high concentrations of valuable polyunsaturated fatty acids (PUFAs) *viz.*, n-3 and n-6 PUFAs, which are important for human health (Calder, 2004). These PUFAs play an important role in lowering the risk of coronary and cardiovascular diseases (Ghaly *et al.,* 2010) and are also beneficial for brain and eye development in human (Gupta *et al.,* 2009).

However, fish deterioration rate is high as it contains free amino acids, autolytic enzymes having neutral pH (6-7) and high moisture level (65-80% water) (Jeyasekaran *et al*., 2006). This deterioration process is mainly catalysed by heme and non-heme containing proteins, which are present in high quantity in fish meat. Iron and other metals that enhance the spoilage process of fish are also structural part of these proteins (Alishahi and Aider, 2012). This leads to about 15% loss of fish and seafood products annually (Ghaly *et al.,* 2010).

In order to prolong the shelf life of fish and its products, different methods are employed. Freezing has been one of the most commonly practiced technique for storing fish as it keeps the fish moist, glossy and prevent it from dehydration (Jain, 2007). However, this method is not effective to address the quality issues of fish. Generally known plastic and other synthetic derivatives are extensively used for packing and storage of fish. However, these plastic products are highly dangerous causing environmental pollution issues (Aider, 2010). To overcome aforementioned problems, research has now been oriented towards adoption of new, natural biodegradable preservatives that can extend the shelf life of fish. Additionally, preservative material must have essential antimicrobial property that helps to maintain the quality of food (Dutta *et al*., 2009).

Edible coating is found effective in this regard; aiding to improve the quality of food by preventing discolouration, reducing the loss of water content and decreasing the bad aroma. They have capacity to keep proteins functional and prevent lipid from oxidation (Arancibia *et al.,* 2015) as well as act as barrier against water loss, light and oxygen. These coatings also protect fish from other hazardous environmental factors that are responsible for faster spoilage of fish products (Gennadios *et al.,* 1997).

Chitosan is a natural biopolymer, which is obtained from deacetylation of chitin (No *et al*., 2007). It is non-toxic, non-allergic, antibacterial, anti-oxidative and antifungal in nature and studies have suggested it to be less harmful towards mammalian cells (Ramezani *et al*.,

2015). The biodegradable nature and film forming property of chitosan makes it a preferable choice for use as a food preservative (Fan *et al.,* 2009). Furthermore, it decreases lipid oxidation in flesh (Gennadios *et al*., 1997). Use of chitosan is also appreciated as it appears to prevent diseases such as diabetes mellitus, high blood pressure, cancer and arthritis in humans (Lodhi *et al.,* 2014; Azuma *et al.,* 2015).

Rohu (*Labeo rohita*) is one of the most important freshwater species, belonging to the family Cyprinidae. It is basically an omnivore and a column feeder (Azim *et al*., 2001). Fast growth rate, high market value and growing consumer demand of rohu makes it a strong candidate species for farming. It is extensively cultured in China, Vietnam, Indonesia, Thailand, Philippines, India, Bangladesh and Pakistan (Dewan *et al*., 1977). Information regarding the effect of chitosan on rohu meat during refrigerated storage is lacking and hence the present study was undertaken to assess the efficiency of chitosan coating in enhancing the shelf stability and quality of rohu (*L. rohita*) during chilled storage.

Materials and methods

Sample preparation

Samples of rohu having average size of 800 g were collected from Government Fish Farm in Sargodha, Pakistan. Live fishes were transported to the laboratory immediately upon harvest under oxygenated conditions. Upon arrival, fishes were immediately dressed and filleted free of blood, viscera, scales and skin. All fillets were scrubbed under fresh running water in order to remove mud, debris and fouling organisms if any adhering to the fish fillets. Fillets were randomly divided into five groups and dip treated for two hours in different concentrations of chitosan solutions: (i) Group R_1 - untreated/control, Group R_2- treated with 0.1%, Group R_3 - 0.25%, Group R_4 - 0.5% and Group R_5 - 1% chitosan. Fillets were further drained and carefully packed in clean polyethylene bags separately and stored at 4°C for 30 days. To check the overall quality of fish fillets, sensory and chemical analyses were performed at 10 day intervals.

Preparation of chitosan based edible coatings

Chitosan powder (having 80-85% degree of deacetylation and 126.2 kDa molecular weight) was procured from VWR International, USA. Different concentrations of chitosan solution (0.1, 0.25, 0.5 and 1% w/w) were prepared in glacial acetic acid. For this,1.25, 2.5, 5 and 10 g of chitosan powder was dissolved separately in 900 ml of distilled water each, respectively; stirred for 10 min and further 10 ml of glacial acetic acid was added to the mixture; solutions were again stirred for 2 h and final solutions each of 1000 ml were made up with distilled water (Fan *et al.,* 2009).

Chemical analysis

Water holding capacity (WHC) of fish muscle, as raw and cooked (65°C, 20 min) form was determined gravimetrically by taking weight difference of samples with and without exudates. WHC was determined from percentage of the retained liquid with respect to initial water content as per the method described by Dunn *et al*. (2007). Protein extractability was measured following the methodology by Gornall *et al*. (1949) and absorbance determined at 540 nm. Thiobarbituric acid reactive substances (TBARS) were determined in fish samples at 530 nm using spectrophotometer following the methodology of Gatta *et al*. (2000). pH of filtrates were measured using a digital pH meter (Ohaus starter 3100) (Fan *et al*., 2009).

Sensory analysis

Sensory analysis of the samples was carried out by five trained panelists, using 5-point hedonic scale for parameters *viz*., colour, odour, texture and taste with scale ranging from 1 (dislike extremely) to 5 (being like extremely). The samples were served in covered plate after cooking for 20 min at 98°C in oven and cooling for 2 min. Sensory acceptance limit for rejection was kept as 4.0, below which the sample was considered to be unacceptable (Ojagh *et al*., 2010).

Bacteriological analysis

Total viable count (TVC) and psychrotrophic count (PTC) was determined following the method of Sallam (2007).

Statistical analysis

Five levels of chitosan treatments and four levels of storage time were employed for the study. Mean values±standard deviation was determined for each treatment done in triplicate and data were analysed by ANOVA. The least significant difference (LSD) was used to determine the difference between mean values ($p< 0.05$).

Results and discussion

Water holding capacity

Water holding capacity (WHC) is a qualitative parameter defined as the ability of fish muscle to retain water in raw and cooked meat systems, imperiled to external force (Risvik, 1994). Initially during storage, there was no significant difference in the WHC in all

treatments. A distinct drop in WHC was observed for all samples with increase in storage period (Table 1). This decrease in WHC value suggests denaturation of proteins during refrigeration. This reason could be explained by the fact that in muscle, myofibrillar protein network contains large amount of water and low storage temperature affects the protein structure (Duun and Rustad, 2007). The lowest value of WHC was seen in control rohu fillet samples and the highest value was recorded for 1% chitosan treated samples. Generally, decline in WHC values of chitosan coated rohu samples was significantly lower ($p<0.05$) than the control samples. These results are in agreement to Gallart-Jornet *et al.* (2007) who studied WHC values in ice stored fish muscle. The higher WHC values of chitosan coated samples could be attributed to the relative polarity of this biopolymer. Moreover, chitosan coating acted as a moisture sacrificing agent instead of moisture barrier and thus, chitosan proved very effective in maintaining the moisture content of marine fish products until evaporation of its own moisture (Mohan *et al.,* 2012).

Soluble proteins

Fish muscle proteins are important to judge the quality of fish meat (Hultmann and Rustad, 2004). In the current study, water extractable protein (WEP) and salt extractable protein (SEP) were analysed. The WEP values decreased during the period of chilled storage in all the four treatments, with 1% chitosan treatment demonstrating the highest values as compared to 0.1, 0.25 and 0.5% chitosan treatments (Table 1).These results were supported by the findings of Siddaiah *et al*. (2001) who reported decrease in the value of WEP in deboned fillets of silver carp (*Hypophthalmichthys molitrix*).

In fish, myofibrillar proteins undergo denaturation process during refrigerated storage, which results in lower extractability of proteins. Initial SEP values indicated the acceptable quality of fish muscle and non-significant differences among treatments. However, during storage, a decline in SEP values was observed with the gradual decrease in muscle quality of rohu fillets. Untreated rohu fillets presented lowest SEP values, while 1% chitosan treated samples represented highest values throughout the storage period (Table 1). In contrary to these results, a significant rise in the values of WEP and SEP was reported in Atlantic salmon (*Salmo sala*) by Hultmann and Rustad (2004). However, in a study carried out by Dunn and Rustad (2007), significantly high level of WEP than that of SEP was observed during refrigerated storage of cod (*Gadus morhua*) fillets.

The low content of extractable proteins in uncoated samples (control) might be due to degradation of protein by the activation of endogenous enzymes and spoilage bacteria which affect the muscle protein and produce volatile basic nitrogenous compounds (Ramezani *et al*., 2015). In muscle tissue, chilled storage also leads to

Table 1. Chemical parameters of chitosan coated rohu fillets during refrigerated storage for 30 days

Parameter	Storage days	0% (Control)	0.1%	0.25%	0.5%	1%
WHC (%)	0	9.74±0.03^{a}	9.75±0.04^{a}	9.74±0.01^{a}	9.71±0.01^{a}	9.74±0.01^{a}
	10	6.87±0.06^{b}	7.17±0.03^{b}	7.49±0.03^{b}	7.98±0.04^{b}	8.14±0.03^{b}
	20	4.81±0.02^{c}	5.93±0.02^{c}	6.19±0.02^{c}	6.36±0.02^{c}	6.52±0.02^{c}
	30	3.89±0.02^{d}	3.99±0.04^{d}	4.19±0.05^{d}	4.44±0.02^{d}	4.61±0.02^{d}
WEP (g 100 g^{-1})	0	5.82±0.02^{a}	5.81±0.02^{a}	5.78±0.02^{a}	5.81±0.03^{a}	5.80±0.02^{a}
	10	5.22±0.03^{b}	5.29±0.01^{b}	5.33±0.01^{b}	5.37±0.02^{b}	5.45±0.04^{b}
	20	4.56±0.02^{c}	4.65±0.02^{c}	4.71±0.01^{c}	4.80±0.01^{c}	5.00±0.01^{c}
	30	4.29±0.01^{d}	4.37±0.03^{d}	4.42±0.01^{d}	4.50±0.01^{d}	4.64±0.02^{d}
SEP (g 100 g^{-1})	0	13.39±0.02^{a}	13.39±0.02^{a}	13.41±0.04^{a}	13.41±0.04^{a}	13.44±0.02^{a}
	10	11.49±0.73^{b}	12.12±0.01^{b}	12.46±0.03^{b}	12.75±0.02^{b}	13.04±0.04^{b}
	20	10.28±0.03^{c}	10.93±0.04^{c}	11.11±0.03^{c}	11.35±0.02^{c}	11.65±0.02^{c}
	30	9.17±0.02^{d}	9.33±0.02^{d}	9.61±0.02^{d}	10.01±0.06^{d}	10.42±0.01^{d}
TBARS (mg MDA kg^{-1})	0	0.34±0.02^{d}	0.33±0.04^{d}	0.33±0.03^{d}	0.33±0.05^{d}	0.33±0.02^{d}
	10	1.08±0.04^{c}	0.81±0.02^{c}	0.71±0.02^{c}	0.60±0.01^{c}	0.43±0.02^{c}
	20	2.28±0.02^{b}	1.05±0.02^{b}	0.91±0.02^{b}	0.68±0.02^{b}	0.61±0.03^{b}
	30	3.14±0.03^{a}	1.39±0.01^{a}	1.01±0.03^{a}	0.87±0.02^{a}	0.74±0.02^{a}
pH	0	5.73±0.03^{d}	5.75±0.03^{d}	5.75±0.02^{d}	5.74±0.05^{d}	5.72±0.03^{d}
	10	5.97±0.04^{c}	5.87±0.02^{c}	5.87±0.02^{c}	5.82±0.01^{c}	5.78±0.01^{c}
	20	6.29±0.02^{b}	6.13±0.02^{b}	6.02±0.01^{b}	5.99±0.01^{b}	5.91±0.01^{b}
	30	6.72±0.03^{a}	6.52±0.04^{a}	6.43±0.02^{a}	6.34±0.02^{a}	6.06±0.03^{a}

WHC: Water holding capacity; WEP: Water extractable proteins; SEP: Salt extractable proteins; TBARS: Thiobarbituric acid reactive substances

denaturation of the protein resulting in low salt extractable protein especially myofibrillar protein. This decrease in protein values could be attributed to denaturation of proteins (Duun and Rustad, 2007).

TBARS

Basically, TBARS is used to measure the amount of malonaldehyde which is produced during the second stage auto-oxidation process of lipids in which peroxides are converted into aldehydes and ketones (Ramezani *et al*., 2015). The TBARS values increased significantly ($p<0.05$) during storage time in all lots of rohu fillets. The fillets treated with 0.5 and 1% chitosan showed a decrease in the rate of increase of TBARS values as compared to control (Table 1). According to Wenjiao *et al.* (2014) value of 1-2 mg MDA kg^{-1} is regarded as maximal permissible limit beyond which fish usually develops an unpleasant odour. After 30 days, TBARS value reached up to 3.135 mg MDA kg^{-1} for control sample, surpassing the upper acceptability limit. This rise in value could be attributed to low storage temperature, which was responsible for partial dehydration of fish and enhanced oxidation of unsaturated fatty acids (Kilincceker *et al.,* 2009). In the support of current findings, increase in TBARS values was also reported in sardine, blue whiting and silver carp fillets during refrigerated storage (Chaijan *et al*., 2006; Jezek and Buchtova, 2011). Similarly, Souza *et al*. (2010) also reported that the fish samples coated with chitosan presented a significant reduction in TBARS values after 9 days of storage, when compared to control samples.

pH

pH is one of the most widely used key to find out the alkalinity and acidity of flesh (Li *et al.*, 2016). Change in pH value can be used as a spoilage index of fish meat. In the present study, an increase in the pH value was observed for the control samples till the end of the experimental period. The subsequent rise in the pH might be related to the increase in production of volatile basic components such as ammonia and trimethylamine by the activities of either endogenous or microbial enzymes (Li *et al*., 2012). However, chitosan treated samples showed significantly lower pH values ($p<0.05$) than untreated samples. Further, 1% chitosan treatment was more effective to limit the change in pH of rohu fillets during storage. This phenomenon could be due to the acidic nature of chitosan solution. Some researchers also observed that different chitosan treatments delayed the rise in pH value of different fishes like silver carp (Ramezani *et al*., 2015), lingcod (Duan *et al.,* 2010) and white shrimp (Yuan *et al*., 2016).

Sensory evaluation

Sensory analysis is the most important parameter to assess the quality of fish meat (Mohan *et al*., 2012). The sensory evaluation results in terms of texture, colour, odour and taste using 5-point hedonic scale are represented in Table 2. Initially during the beginning of chilled storage, there were no significant differences among organoleptic attributes such as texture, colour, odour and taste, which clearly indicated that chitosan had no negative impact on organoleptic attributes. The sensory scores for all treatments gradually decreased with extended storage time. The coated rohu fillets exhibited significantly slower decrease in values as compared to control samples. Among all treatments, 1% chitosan was found more effective in delaying the spoilage rate of fish, reflective in the sensory score as well. This might be

Table 2. Sensory attributes of chitosan coated rohu fillets during refrigerated storage for 30 days

Parameter	Storage days	0 % (Control)	0.1%	0.25%	0.5%	1%
Texture	0	5.45±0.07^{a}	5.50±0.01^{a}	5.45±0.07^{a}	5.45±0.07^{a}	5.45±0.07^{a}
	10	4.25±0.07^{b}	4.30±0.02^{b}	4.65±0.03^{b}	5.00±0.14^{b}	5.15±0.02^{b}
	20	3.25±0.21^{c}	3.70±0.14^{c}	4.05±0.07^{c}	4.45±0.07	4.75±0.07
	30	2.45±0.07^{d}	3.15±0.07^{d}	3.70±0.14^{d}	3.95±0.07^{d}	4.20±0.05^{d}
Odour	0	4.65±0.21^{a}	4.75±0.04^{a}	4.65±0.07^{a}	4.75±0.02^{a}	4.65±0.07^{a}
	10	3.70±0.14^{b}	3.65±0.07^{b}	3.85±0.07^{b}	4.05±0.09^{b}	4.15±0.07^{b}
	20	2.75±0.07^{c}	3.05±0.06^{c}	3.20±0.14^{c}	3.65±0.07^{c}	3.85±0.04^{c}
	30	2.35±0.21^{d}	2.65±0.07^{d}	2.80±0.14^{d}	3.15±0.03^{d}	3.45±0.07^{d}
Colour	0	5.05±0.07^{a}	5.10±0.14^{a}	5.10±0.08^{a}	5.15±0.02^{a}	5.10±0.14^{a}
	10	4.10±0.14^{b}	4.15±0.07^{b}	4.30±0.04^{b}	4.45±0.07^{b}	4.65±0.07^{b}
	20	3.35±0.21^{c}	3.65±0.07^{c}	3.95±0.07^{c}	4.00±0.14^{c}	4.10±0.14^{c}
	30	1.95±0.07^{d}	2.85±0.21^{d}	3.40±0.14^{d}	3.65±0.07^{d}	3.85±0.07^{d}
Taste	0	5.05±0.07^{a}	5.15±0.07^{a}	5.15±0.07^{a}	5.20±0.14^{a}	5.10±0.12^{a}
	10	4.40±0.14^{b}	4.30±0.14^{b}	4.55±0.09^{b}	4.75±0.07^{b}	4.85±0.09^{b}
	20	3.60±0.14^{c}	3.80±0.15^{c}	3.90±0.02^{c}	4.05±0.03^{c}	4.50±0.14^{c}
	30	2.45±0.07^{d}	2.95±0.07^{d}	3.35±0.21^{d}	3.75±0.07^{d}	3.90±0.11^{d}

due to the antimicrobial, antioxidant and oxygen barrier properties of chitosan. Similar results were also reported for pacific oyster, silver carp and oilsardine (Cao *et al*., 2009; Fan *et al*., 2009; Mohan *et al*., 2012).

Bacteriological analysis

Changes in total viable count (TVC) and psychrotrophic count (PTC) of rohu fillets during refrigerated storage are shown in Fig. 1 and 2. The TVC and PTC (log_{10}CFU g^{-1}) of the untreated fillets were higher than the fillets treated with different concentrations of chitosan. The lower TVC and PTC of chitosan treated fillets indicated that chitosan coating reduced the microbial population. TVC and PTC were lowest in fillets treated with 1% chitosan. The antimicrobial properties of chitosan coating have been well stated in previous studies. Fernandez-Saiz *et al*. (2013) reported a significant ($p<0.05$) increase of the lag phase and a decrease in the final microbial population. Lopez-Caballero *et al*. (2005) also documented that chitosan coating along with gelatin reduced the growth of Gram negative bacteria in fish. Other researchers also reported that the complex chitosan film reduced the growth of bacteria (Gomez-Estaca *et al.*, 2010). The mechanism of antimicrobial action of chitosan could be related to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membrane. In the present study, chitosan coating led to significant ($p<0.05$) reduction in the TVC and PTC of rohu fillets during refrigerated storage.

Chemical, microbial and sensory evaluation results proved chitosan to be very effective as a natural preservative to maintain the quality of rohu fillets. Current study concluded that 1% chitosan coating on rohu fillets can lead to retention of good quality characteristics and extension of shelf life upto 30 days during chilled storage.

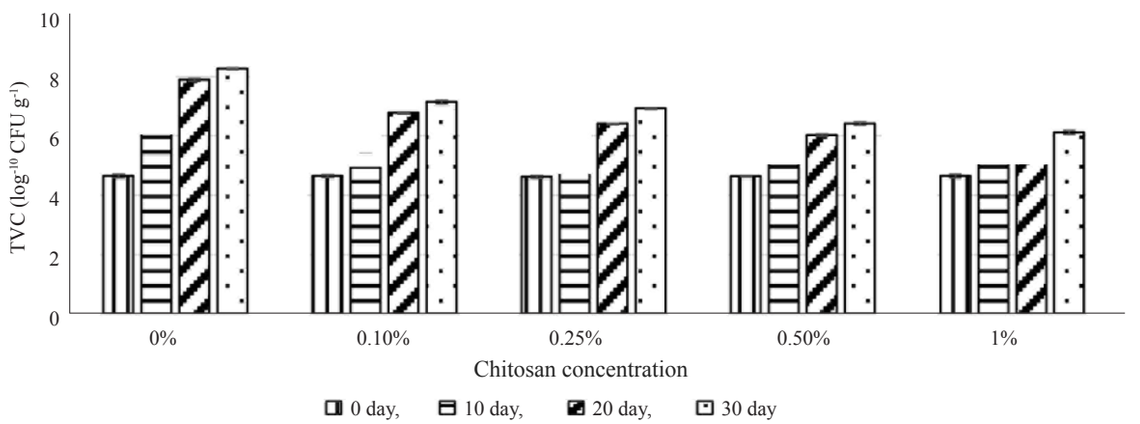

Fig. 1. Changes in total viable counts (TVC) of fish samples treated with different concentrations of chitosan during refrigerated storage

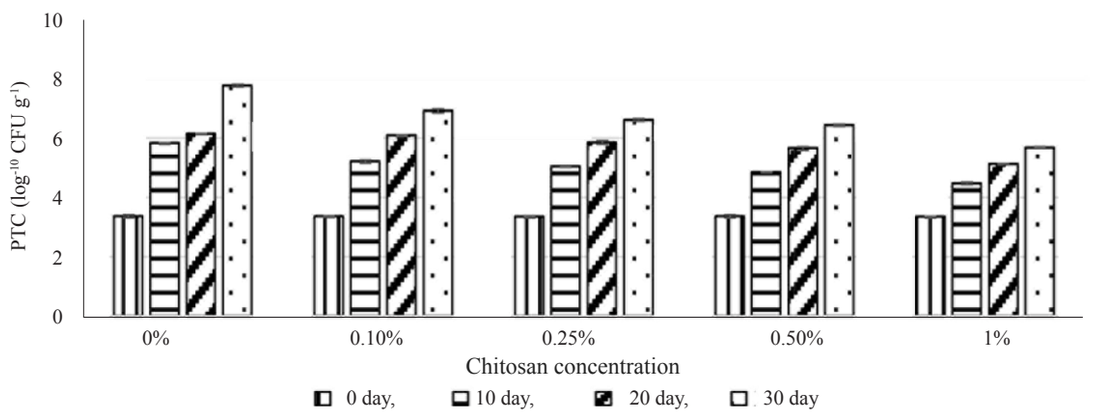

Fig. 2. Changes in psychrotrophilic counts (PTC) of fish samples treated with different concentrations of chitosan during refrigerated storage

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