Pakistan J. Zool., vol. 55(1), pp 371-377, 2023 **DOI**: https://dx.doi.org/10.17582/journal.pjz/20190519170504

Effect of Chitosan Edible Coating on the Physico-Chemical and Sensory Characteristics of Stored Mori Fillet (*Cirrhinus mrigala*)

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ABSTRACT

The fish meat is highly perishable. So, it is necessary to seek viable alternatives that help to preserve meat freshness and quality and to increase its shelf life. One of these alternatives is the chitosan which is natural preservative having antimicrobial properties. In present study, the effectiveness of chitosan coatings as natural preservative was assessed on rancidity development and quality changes in mori fillets during 28 days of storage. The control and chitosan coated samples were analyzed periodically at intervals of 7 days, for determination of pH, water holding capacity, water extractable proteins, salt extractable proteins, thiobarbituric acid reactive substances (TBARS) and sensory quality of mori fillets. The results indicated that chitosan coatings were effective in controlling pH, water loss, TBARS production, retention of water extractable proteins and salt extractable proteins in fish fillets. The sensory attributes texture, color, taste and odor were significantly improved in chitosan treated samples as compared to untreated samples. Furthermore, among chitosan treatment groups, 1% chitosan treatment showed best preservative effect on mori fillets. Therefore, it can be concluded that 1% chitosan treatment is most effective for maintaining the storage quality of mori fillets during this experiment.

Article Information
Received 19 May 2019
Revised 13 November 2020
Accepted 12 January 2021
Available online 30 March 2022 (early access)
Published 07 November 2022

Authors' Contribution
HJ designed and executed the study. MA supervised and guided in planning the research work. NM did statistical analysis. WH assisted in compiling the data. SZHS and FS helped in writing the manuscript.

Key words
Chitosan, *Cirrhinus mrigala*, Fish fillet, Fish meat, Sensory quality of fish, Mori

INTRODUCTION

Fish is a nutritious food contributing about 1/4th of the total animal protein. It is considered an excellent source of protein for human consumption due to its balanced amino acid profile and higher digestibility (Louka *et al*., 2004). It is also a best source of essential minerals, vitamins and valuable lipids with high amount of polyunsaturated fatty acids. These fatty acids are of vital importance for the improvement of human health because they perform different biological functions such as reducing the potential risk of cardiovascular disorders (Alishahi and Aider, 2012) and prevention of some types of cancers, including intestinal, prostate and breast cancer (Matsumoto *et al*., 2009).

The biological composition of fresh fish makes it highly perishable. Many internal and external factors influence the fish quality, including improper postmortem changes leading to the destruction of the meat structure thereby degrading the fish quality (Ayala *et al*., 2010). Fish preservation is a difficult task as high moisture content, neutral pH, autolytic enzymes and high non-protein nitrogen make it susceptible to chemical deterioration and microbial spoilage leading to economic and health issues (Jeyasekaran *et al*., 2006).

Food processors and consumers desire to decrease the application of synthetic chemicals in the preservation of foodstuffs due to their unhealthy side effects (Lopez-Carballo *et al*., 2012). Consequently, the demand of natural products as preservative agents has increased rapidly in the last decade (Realini and Marcos, 2014). Bacteriocins and organic acids from bacteria showed good antimicrobial activities against spoilage bacteria. Plant-derived antimicrobials could prolong fish shelf life and decrease lipid oxidation. Animal-derived antimicrobials also have good antimicrobial activities; however, their allergen risk should be paid attention. Moreover, some algae and mushroom species can also provide a potential source of new natural preservatives (Mei *et al*., 2019). However, most of natural products have narrow range of antimicrobial activity and large quantity is required for

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0030-9923/2023/0001-371 $ 9.00/0

their effectiveness (Friedman and Juneja, 2010).

Chitosan is a cationic polysaccharide obtained from the alkaline hydrolysis of N-acetyl group of chitins, the main component of the crustacean shells. Chitosan has been reported to have a number of functional properties that make it technically and physiologically useful in nutrition (Gallaher *et al*., 2002). Chitosan is an effective food preservative due to its anti-microbial and antioxidant activities, edibility and non-polluting and non-toxic nature (Falguera *et al*., 2011). Chitosan is considered best film forming biopolymer (Frenandez-Saiz *et al*., 2013) to avoid the moisture loss, aromas loss, oxygen and water penetration and transport of solutes in food products (Dutta *et al*., 2009). Chitosan is a well-known film-forming biopolymer with strong antimicrobial and antifungal activities (Duan *et al*., 2010), which has been widely applied to the preservation of seafood products (Li *et al*., 2013). A number of studies have demonstrated the antimicrobial action of chitosan as bioactive preservative for variety of fish species to control microbes and maintaining fish quality during storage (Hafdani and Sadeghinia, 2011; Li *et al*., 2013; Mitelut *et al*., 2015).

Cirrhinus mrigala is nutritionally valuable and economically important for human consumption, as it is best source of protein and long chain fatty acids which are essential for biological functioning of body (Gonzalez *et al*., 2006). It is endemic carp of subcontinent freshwater systems and is highly cultivated in Pakistan, India, Burma and Bangladesh. Carp species contribute about 87% of total freshwater aquaculture production of Indian subcontinent (ICLARM, 2001). It has high economic value and consumer demand in Asian countries (Eun *et al*., 1994). To date, literature is lacking the information regarding the effect of chitosan on mori during refrigerated storage. Current study was designed to assess/explore the effect of different chitosan percentages on frozen mori fillets. Physicochemical and sensory characteristics of the mori fillets were assessed at different interval for about a period of 28 days.

MATERIALS AND METHODS

VMR International (US) Chitosan, in powdered form was purchased from VMR International (US). The degree of deacetylation of chitosan was 90% with 10% moisture content. Different formulations of chitosan were prepared: 1%, 0.5%, 0.25% and 0.1% w/w chitosan solution in glacial acetic acid by adding 10g, 5g, 2.5g and 1.25g of chitosan powder, respectively, 900 ml of distilled water was further added and the solution was stirred for 10 min, 10 ml of glacial acetic acid was added to the mixture and stirring was done again for 2 hr. Total volume of 1000 ml was obtained with distilled water (Fan *et al*., 2009).

Live farmed mori fish weighing approximately 750-1000 g were obtained from local fish farm of Sargodha. The live specimens were transported to laboratory in plastic oxygenated nylon sacks within one hour. Fish were slaughtered, skinned and gutted in laboratory. Fish samples were divided into five groups (each group contains two fish) and subjected into immersion treatment for 2 h in different concentrations of chitosan solutions i.e. 1%, 0.5%, 0.25 % and 0.1% while the control group was kept without chitosan treatment. All samples of each treatment group were well drained and individually packed in polyethylene bags and stored at -18°C for 28 days. During this period, physico-chemical and sensory analyses of fish samples were performed at 7-days interval with 3 replicates from each group and averages were used for quality assessment.

Physico-chemical analysis

Fish muscles (10 g) were homogenized in distilled water and the mixture was filtered. pH of filtrate was measured using a digital pH meter (Ohaus starter 3100) as described by Fan *et al*. (2009). Water holding capacity of fish muscles as raw and cooked form was determined gravimetrically by taking weight difference of samples with and without exudates. Water holding capacity was determined from percentage of the retained liquid with respect to initial water content according to method devised by Dunn and Rustad (2007).

Extractable protein was measured by following Gornall *et al*. (1949). The absorbance of samples was checked at 540 nm. Thiobarbituric acid reactive substances (TBARS) were determined in fish samples at 530 nm on spectrophotometer following Gatta *et al*. (2000).

Sensory evaluation

Sensory evaluation of frozen mori fillets was carried out by five trained panelists of Department of Zoology, University of Sargodha. Samples were served in covered plate after cooking for 20 min in oven and cooling for 2 min. To restore the taste sensitivity, a glass of water was provided with samples. The panelists were asked to evaluate fish sample using 5-point hedonic scale for all four parameters considering texture, color, odor and taste. Texture of raw fish fillets were measured by “finger method” (pressing fillets by finger) to check the firmness of muscles. Overall acceptability was calculated by sum of the 4 parameters score (wherein 1= extremely undesirable; 5= extremely desirable) (Ojagh *et al*., 2010; Cheng *et al*., 2014).

Statistical analysis

All data were subjected to analysis of variance

(ANOVA). The least significant difference (LSD) procedure was used to test the differences among means (significance was defined at $p < 0.05$). Statistical analysis was performed through SPSS software.

RESULTS

In present study, the pH value of fish samples increased gradually with the increase in storage time. Untreated samples showed faster increase in pH value with storage period and reached unacceptable limit at the end of storage (7.35±0.07). In chitosan treated samples the values of pH were recorded as 6.37±0.03, 6.44±0.04, 6.51±0.06 and 6.56±0.01 for 1 %, 0.5 %, 0.25 % and 0.10 %, respectively while it was maximum in control fish. The highest concentration of chitosan treatment (1%) showed maximum control on pH increase. In the present study it was observed that water holding capacity (WHC) in mori fillets steadily increased during whole storage by applying chitosan coating. The decrease in WHC was significantly higher ($P<0.05$) in untreated samples and lower in chitosan treated samples. Mori fillets treated with 1 % chitosan level showed maximum WHC throughout the study period.

Table I. Physico-chemical analysis of chitosan coated mori fillets during storage at -18 °C for 28 days.

Attributes	Chitosan levels	Storage days					Overall Means
		0 day	7 day	14 day	21 day	28 day	
pH	0%	6.25±0.04^{a}	6.35±0.02^{a}	6.45±0.08^{a}	6.86±0.04^{a}	7.35±0.07^{a}	6.65±0.04^{A}
	0.10%	6.25±0.02^{a}	6.34±0.01^{a}	6.44±0.07^{a}	6.80±0.02^{a}	7.00±0.03^{b}	6.56±0.01^{B}
	0.25%	6.25±0.03^{a}	6.30.±0.05^{ab}	6.40±0.03^{ab}	6.73±0.03^{a}	6.86±0.01^{bc}	6.51±0.06^{B}
	0.50%	6.25±0.04^{a}	6.28±0.03^{bc}	6.30±0.10^{b}	6.62±0.01^{b}	6.77±0.02^{c}	6.44±0.04^{C}
	1%	6.25±0.03^{a}	6.26±0.02^{c}	6.28±0.02^{b}	6.48±0.02^{c}	6.60±0.02^{d}	6.37±0.03^{D}
	Overall means	6.25±0.00^{D}	6.31±0.04^{C}	6.37±0.02^{C}	6.70±0.01^{B}	6.92±0.03^{A}	
WHC (%)	0%	9.08±0.04^{a}	7.38±0.04^{e}	6.07±0.04^{d}	5.42±0.04^{d}	3.43±0.03^{e}	6.27±0.06^{E}
	0.10%	9.08±0.02^{a}	7.58±0.02^{d}	6.60±0.02^{c}	5.54±0.03^{d}	3.79±0.03^{d}	6.52±0.02^{D}
	0.25%	9.07±0.06^{a}	7.92±0.02^{c}	6.90±0.02^{b}	5.71±0.02^{c}	4.35±0.04^{c}	6.79±0.05^{C}
	0.50%	9.09±0.07^{a}	8.31±0.01^{b}	7.01±0.07^{b}	6.00±0.03^{b}	4.72±0.08^{b}	7.02±0.04^{B}
	1%	9.07±0.02^{a}	8.74±0.02^{a}	7.49±0.02^{a}	6.19±0.03^{a}	5.09±0.02^{a}	7.31±0.02^{A}
	Overall means	9.08±0.01^{A}	7.98±0.03^{B}	6.81±0.03^{C}	5.77±0.05^{D}	4.27±0.02^{E}	
WEP (g/100 g)	0%	5.76±0.01^{a}	5.50±0.02^{c}	4.98±0.03^{c}	4.59±0.06^{d}	4.39±0.02^{d}	5.04±0.03^{E}
	0.10%	5.74±0.02^{a}	5.51±0.01^{c}	5.05±0.02^{bc}	4.70±0.02^{c}	4.50±0.02^{cd}	5.10±0.05^{D}
	0.25%	5.74±0.01^{a}	5.56±0.01^{bc}	5.10±0.01^{b}	4.71±0.02^{c}	4.59±0.03^{bc}	5.14±0.01^{C}
	0.50%	5.75±0.01^{a}	5.59±0.02^{ab}	5.22±0.02^{a}	4.85±0.07^{b}	4.70±0.02^{ab}	5.22±0.04^{B}
	1%	5.74±0.07^{a}	5.66±0.02^{a}	5.31±0.01^{a}	4.99±0.02^{a}	4.74±0.02^{a}	5.29±0.01^{A}
	Overall means	5.74±0.02^{A}	5.56±0.04^{B}	5.13±0.05^{C}	4.77±0.02^{D}	4.58±0.06^{E}	
SEP (g/100 g)	0%	13.39±0.02^{a}	12.75±0.03^{d}	11.93±0.04^{e}	10.46±0.03^{e}	09.34±0.03^{e}	11.58±0.03^{E}
	0.10%	13.41±0.01^{a}	12.86±0.02^{cd}	12.31±0.02^{d}	11.58±0.03^{d}	10.38±0.02^{d}	12.11±0.01^{D}
	0.25%	13.40±0.07^{a}	12.96±0.02^{bc}	12.52±0.02^{c}	11.90±0.04^{c}	11.21±0.02^{c}	12.40±0.04^{C}
	0.50%	13.40±0.01^{a}	13.02±0.03^{ab}	12.68±0.02^{b}	12.21±0.02^{b}	11.86±0.03^{b}	12.64±0.05^{B}
	1%	13.38±0.02^{a}	13.13±0.02^{a}	12.99±0.02^{a}	12.45±0.02^{a}	12.10±0.04^{a}	12.81±0.02^{A}
	Overall means	13.39±0.03^{A}	12.94±0.04^{B}	12.48±0.02^{C}	11.72±0.01^{D}	10.97±0.04^{E}	

TBARS (mg-MDA/Kg)	0%	0.66±0.06^{a}	1.82±0.02^{a}	2.64±0.03^{a}	3.78±0.03^{a}	4.04±0.03^{a}	2.59±0.01^{A}
	0.10%	0.68±0.02^{a}	1.29±0.02^{b}	1.57±0.01^{b}	1.78±0.02^{b}	2.34±0.02^{b}	1.53±0.03^{B}
	0.25%	0.66±0.03^{a}	1.05±0.02^{c}	1.32±0.02^{c}	1.64±0.01^{c}	2.09±0.05^{c}	1.35±0.02^{C}
	0.50%	0.66±0.01^{a}	0.97±0.01^{c}	1.16±0.02^{d}	1.49±0.02^{d}	1.86±0.03^{d}	1.23±0.01^{D}
	1%	0.68±0.02^{a}	0.85±0.02^{d}	1.01±0.02^{e}	1.33±0.04^{e}	1.68±0.02^{e}	1.11±0.01^{E}
	Overall means	0.66±0.01^{E}	1.20±0.03^{D}	1.54±0.02^{C}	2.01±0.04^{B}	2.40±0.05^{A}	

Mean±S.E; n=3; Values in the same column for each attribute followed by a different letter are significantly different ($p<0.05$).
WPC, water holding capacity; WEP, water extractable proteins; SEP, salt extractable proteins; TBARS, thiobarbituric acid reactive substances

Table II. Changes in sensory attributes of chitosan coated mori fillets during storage at -18 °C for 28 days.

Attributes	Chitosan levels	Storage days					Overall means
		0 day	7 day	14 day	21 day	28 day	
Texture	0%	5.30±0.10^{a}	4.20±0.14^{b}	3.65±0.05^{c}	3.20±0.11^{c}	2.60±0.05^{c}	3.79±0.02^{D}
	0.10%	5.25±0.07^{a}	4.25±0.07^{b}	3.75±0.07^{c}	3.30±0.01^{c}	2.90±0.09^{bc}	3.89±0.03^{D}
	0.25%	5.25±0.04^{a}	4.40±0.01^{b}	3.90±0.14^{bc}	3.55±0.20^{bc}	3.20±0.14^{ab}	4.06±0.02^{C}
	0.50%	5.35±0.07^{a}	4.75±0.07^{a}	4.25±0.07^{ab}	3.80±0.11^{ab}	3.55±0.07^{a}	4.34±0.01^{B}
	1%	5.25±0.07^{a}	4.95±0.07^{a}	4.55±0.06^{a}	4.15±0.07^{a}	3.70±0.14^{a}	4.52±0.04^{A}
	Overall means	5.28±0.03^{A}	4.51±0.04^{B}	4.02±0.04^{C}	3.60±0.03^{D}	3.19±0.06^{E}	
Taste	0%	5.05±0.07^{a}	3.85±0.14^{c}	3.45±0.09^{c}	2.70±0.14^{c}	2.20±0.14^{d}	3.52±0.02^{D}
	0.10%	5.10±0.05^{a}	4.05±0.07^{bc}	3.45±0.05^{c}	2.95±0.15^{bc}	2.45±0.07^{cd}	3.56±0.01^{D}
	0.25%	5.05±0.07^{a}	4.20±0.04^{bc}	3.75±0.08^{bc}	3.15±0.03^{b}	2.85±0.04^{bc}	3.77±0.04^{C}
	0.50%	5.05±0.05^{a}	4.35±0.02^{b}	4.05±0.07^{ab}	3.55±0.09^{a}	3.15±0.08^{ab}	4.03±0.03^{B}
	1%	5.10±0.03^{a}	4.75±0.07^{a}	4.30±0.04^{a}	3.85±0.07^{a}	3.40±0.04^{a}	4.28±0.03^{A}
	Overall means	5.07±0.02^{A}	4.24±0.04^{B}	3.80±0.02^{C}	3.24±0.03^{D}	2.81±0.02^{E}	
Color	0%	5.25±0.07^{a}	4.10±0.14^{c}	3.65±0.03^{c}	2.70±0.14^{b}	1.90±0.14^{e}	3.52±0.03^{E}
	0.10%	5.20±0.08^{a}	4.25±0.07^{bc}	3.75±0.07^{cd}	2.95±0.07^{b}	2.55±0.10^{d}	3.74±0.02^{D}
	0.25%	5.25±0.07^{a}	4.35±0.08^{bc}	3.95±0.09^{c}	3.15±0.07^{b}	2.95±0.13^{c}	3.93±0.01^{C}
	0.50%	5.25±0.09^{a}	4.55±0.07^{ab}	4.25±0.12^{b}	3.70±0.14^{a}	3.35±0.07^{b}	4.22±0.02^{B}
	1%	5.30±0.01^{a}	4.85±0.03^{a}	4.65±0.01^{a}	4.10±0.13^{a}	3.75±0.04^{a}	4.53±0.04^{A}
	Overall means	5.25±0.02^{A}	4.42±0.03^{B}	4.05±0.03^{C}	3.32±0.01^{D}	2.90±0.02^{E}	
Smell	0%	4.80±0.14^{a}	3.95±0.07^{c}	3.45±0.06^{c}	2.90±0.08^{c}	2.30±0.14^{d}	3.48±0.03^{E}
	0.10%	4.85±0.07^{a}	4.05±0.07^{c}	3.65±0.08^{bc}	3.10±0.14^{c}	2.65±0.02^{cd}	3.66±0.02^{D}
	0.25%	4.85±0.06^{a}	4.05±0.03^{bc}	3.85±0.012^{b}	3.55±0.07^{b}	2.95±0.06^{bc}	3.87±0.02^{C}
	0.50%	4.75±0.07^{a}	4.35±0.02^{b}	4.15±0.06^{a}	3.85±0.07^{ab}	3.25±0.07^{b}	4.07±0.01^{B}
	1%	4.85±0.03^{a}	4.75±0.09^{a}	4.35±0.07^{a}	4.00±0.01^{a}	3.65±0.03^{a}	4.32±0.02^{A}
	Overall means	4.82±0.03^{A}	4.25±0.02^{B}	3.89±0.04^{C}	3.48±0.03^{D}	2.96±0.05^{E}	

Mean±S.E; n=3; Values in the same column for each attribute followed by a different letter are significantly different ($p<0.05$).

Results of present study, indicated that water extractable proteins (WEP) and salt extractable proteins (SEP) in mori fillets significantly decreased during storage while the same parameters were gradually increased with the increase in level of chitosan treatments. The maximum WEP and SEP was recorded in mori fillets treated with 1% chitosan. The results of present study indicated, TBARS values of all samples rose with the increase in storage time. This increase was significantly higher ($p<0.05$) in untreated samples, compared with chitosan treated samples (Table I). Results also indicated that the higher concentration of chitosan treatment showed higher antioxidant property in fish muscles. Among chitosan treated groups, 1% chitosan treatment was most effective for maintaining good quality of fish fillets. It was noted that the score of all sensory attributes gradually declined with time in all samples of

mori irrespective of chitosan treatment. At initial day of storage all samples had good taste, pleasant odor and characteristic texture and coloration of fresh mori fish. At end of storage, untreated samples showed a strong off-odor and flavor associated with spoiled fishery products and development of yellow to reddish coloration (Table II).

DISCUSSION

pH value is best indicator to check the spoilage of fishery products during storage (Mohan *et al*., 2012). The subsequent increase in pH value was presumably because of high level of volatile basic nitrogenous compounds which are produced by either microbial metabolism or endogenous enzymes of fish, during storage (Zhou *et al*., 2011). This might be due to acidic nature of chitosan (Mohan *et al*., 2012). Chitosan treatment inhibits the microbial propagation and their metabolites, it is also effective in controlling activities of proteases (Fan *et al*., 2009), which contributes to extending shelf life of fish during chill storage. Similarly, significant decrease ($p<0.05$) in pH value was examined in Sword fish (Tsiligianni *et al*., 2012), Atlantic cod and herring (Jeon *et al*., 2002) by application of chitosan coating.

Results also showed that as concentration of chitosan increased the retention of water contents in postmortem muscles. Similar to the findings of current study, chitosan treatment was reported to be effective in controlling the WHC in fillets of Indian oil sardine (Mohan *et al*., 2012) and cod (Jeon *et al*., 2002). Water holding capacity (WHC) is the retention of water in postmortem muscles (meat) after the application of external pressures including gravity and temperature. Decrease in WHC in untreated samples could be due to degradation of myosin fibrils which results in the increase of loss of less tightly bound water (Mohan *et al*., 2012). The higher WHC in chitosan treated samples might be due to relative polarity of this polysaccharide (Jeon *et al*., 2002). Moreover, chitosan coating acted as a moisture sacrificing agent instead of moisture barrier. Thus, chitosan was proved very effective in maintaining the moisture contents of marine products until evaporation of its own moisture (Mohan *et al*., 2012).

The process of protein extraction involved two steps resulting in salt soluble and water-soluble protein fractions (Hultmann and Rustad, 2002). Lower contents of extractable proteins in uncoated samples might be due to denaturation of the protein in muscle (Duun and Rustad, 2007) or 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 denatures the protein resulting in low salt extractable proteins especially myofibrillar protein (Dunn and Rustad, 2007).

The thiobarbituric acid reactive substances (TBARS) assay is widely used to measure lipid oxidation and antioxidant activity in food and physiological systems (Ghani *et al*., 2017). TBARS value is the best indicator to assess the degree of lipid oxidation in fish tissue. It is widely used to account the second stage auto-oxidation of muscle tissues, during which peroxidase are oxidized to aldehyde and ketone (Ramezani *et al*., 2015). Due to lipid oxidation many substances are produced, some of which gives unpleasant flavor and odor to meat (Fernandez *et al*., 2013). Chitosan treatment showed higher inhibitory effect on microbial growth which in turn affected oxidation of lipid (Zhao *et al*., 2011). Ramezani *et al*. (2015) reported the quality enhancement of silver carp by application of 2% chitosan coating which controlled the TBARS production and maintained the odor and flavor during 12 days of refrigerated storage. Sensory evaluation is most reliable and satisfactory method to check the organoleptic properties of food products (Hassan and Ali, 2011).

The chitosan treatment notably enhanced the organoleptic properties of fish fillets during storage period. Among chitosan treated groups, 1% chitosan treatment was most effective for maintaining good quality of fish fillets. Similar to current findings, chitosan treatments had positive effect on maintaining good sensory attributes and extending the shelf life of sword fish (Tsiligianni *et al*., 2012), Atlantic cod and herring (Jeon *et al*., 2002). Mohan *et al*. (2012) reported that 1 and 2% chitosan treatment had extended the shelf life of Indian oil sardine.

From these results, it can be concluded that chitosan coating is effective in maintaining the good sensory attributes and extending the shelf life of mori fillets. Present study indicated that chitosan treatments helped to control the pH, WHC, protein extractions. These coatings also showed antioxidant effect as TBARS values were lower in treated samples than control. Therefore, chitosan coating is effective for preservation of mori fillets during storage. To the best of our awareness, this is the first study reporting the chitosan as safe preservative for mori fish in refrigerated storage. The efficacy of further chitosan levels on mori fillets can be checked.

Statement of conflict of interest

The authors have declared no conflict of interest.

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