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Effect of Chitosan Edible Coating on the Microbial Quality of Ribbonfish, *Lepturacanthus savala* (Cuvier, 1929) Steaks

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Abstract

The demand for healthy food products without any added chemical preservative is increasing globally. Chitosan can find application in food products due to its bio-preservation effects. The present study was aimed to assess the effectiveness of chitosan edible coating on the microbial quality of ribbon fish, *Lepturacanthus savala*. Ribbonfish steaks treated with (1%) and without chitosan were packed in multi-layered films and stored under refrigerated conditions (3±1°C). The fish used in the study is medium fatty fish with good quantity of crude protein. The initial total plate count of 4.5 log cfu g^{-1} indicates the good quality of fish used in the study which exceeded limit of 7 log cfu g^{-1} on 10th days in control samples indicating the shelf life of ~8-9 days. In chitosan treated samples, total plate counts were observed to be 6.81 log cfu g^{-1}on 15th day and the limit of 7 log cfu $g^{-1}$was exceeded only on 20th day indicating a shelf life of ~16-17 days. This clearly indicates the antimicrobial effect of chitosan. Further, significantly ($p<0.01$) lower counts for *Pseudomonas* spp., H_2S forming bacteria and enterobacteriaceae were observed for chitosan treated samples. The study reveals that chitosan can be used effectively to enhance the microbial quality as well as shelf life of the fishery products.

Keywords : Chitosan, Ribbon fish, microbial quality, refrigerated storage

Introduction

Ribbon fish belonging to the family Trachipteridae is one of the common fish consumed in Gujarat. Contribution of ribbon fish to total fish landings of Gujarat was to the tune of 14.26% in 2014 (CMFRI, 2015). Apart from its domestic utilization, it finds major export markets like China, Japan and other Southeast Asian countries mainly in frozen form. Nearly 64% of ribbon fish landed is exported in frozen form. Belly bursting is one of the major problem faced by the ribbon fish processing industries and this is mainly due to the autolysis and microbial activity, which leads to loss of quality and nutritive value of ribbon fish. Chitosan which is a derivative of chitin is a natural biodegradable polysaccharide which find varied application in food industry. Chitin is a copolymer of N-acetylglucosamine and glucosamine residues linked by β-1, 4-glycosidic bonds and insoluble in dilute acids. Chitosan [β-(1,4)-2-amino-2-deoxy-D-glucopyranose] is the deacetylated form ofchitin and in most cases, will be soluble in dilute acid. It is the second most abundant natural polymer in nature after cellulose (Shahidi et al., 1999). Fungi synthesize chitin and chitosan in their cell walls, while the shells of crabs and shrimps and the bone plates of squids and cuttlefish are composed of chitin only (Ng et al., 2007; Nwe et al., 2002). Pure chitosan is non-toxic, free of antigenic effects, biocompatible, biodegradable and polar in nature (VandeVord et al., 2002). Chitosan has been reported to have a number of functional properties that make it useful in nutrition (Gallaher et al., 2002; Shahidi et al., 1999). These include its antimicrobial activity and its ability to form protective films (Cuero, 1999; Jeon et al., 2002), its texturizing (Benjakul, et al., 2003), and binding action (No et al., 2000); and its antioxidant activity (Kamil et al., 2002). Many workers have studied chitosan as edible coating material for fishery products to enhance quality (Jeon et al. 2002; Augustini & Sedjati, 2007; Mohan et al., 2012). Although there are reports on the

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application of chitosan in food, very limited report is available on the edible coating on the microbial quality of ribbonfish. Hence the present study aimed to assess the effect of chitosan edible coating on the microbial quality and shelf life of ribbonfish in refrigerated storage condition.

Materials and Methods

Chitosan with 85% degree of deacetylation with moisture content of less than 10% was procured from Mahatani Chitosan Pvt. Ltd. (Veraval, Gujarat, India). From this, 1% (w/v) chitosan solution was made by dissolving in 1% acetic acid and filtered through cheese cloth in order to eliminate the insoluble material. Fresh ribbonfish (*Lepturacanthus savala)* was procured from local fish market of Veraval, Gujarat, India and brought to the laboratory within 30 min in iced condition. The fish samples were immediately beheaded, gutted, cleaned and made in to steaks (ca. 190g±10g) and subsequently kept under refrigeration (3±0.5°C) until further use. The fishes were divided into two batches and one batch was dipped in 1% chitosan for 10 minand labelled as chitosan treated ribbon fish (CT). The steaks were then allowed to drain for 10 min on a pre-sterilized metal net. Another batch,the chitosan treatment was not given and labelled as control (C). Fish steaks were packed in synthetic multilayer film (nylon, EVOH and polyethylene) pack (Sealed Air (India) Pvt. Ltd., Bangalore, India) and stored at 3±1°C for further analysis.

The proximate compositionof ribbon fish was determined as per AOAC method (AOAC, 2000). The microbial assessment was done by the standard method of USFDA Bacteriological Analytical Manual (2001). For microbial assessment, ribbon fish steak of 25g was transferred into a stomacher bag and homogenized for 2 min (Laboratory Blender 400, Seward, London, U.K.) with peptone saline solution. From this appropriate decimal dilutions were prepared, and used to enumerate the total plate count (TPC), *Pseudomonas* spp., H_2S-producing bacteria, Enterobacteriaceae and coagulase positive Staphylococci. Results were presented as log colony forming units (cfug^{-1}). The microbial quality assessment was carried out immediately after the preparation of steaks from whole fish (day 0) followed by every 5 days interval during the study period.

Statistical Analysis

Triplicate samples were used for the analysis and the results are expressed as mean ± standard deviation. Significant differences for different samples were evaluated at $P < 0.01$ for microbiological quality by analysis of variance (ANOVA).

Results and discussion

Proximate composition of the fish is affected by various factors like season, size, age, sex, diet etc. The proximate composition of ribbonfish studied is shown in Table 1. The results revealed that major contributor is moisture followed by crude protein and crude fat. The fish used in the study had good quantity of protein (20.05%) and is medium fatty fish (3.18%). This indicates that ribbonfish is very ideal for providing nutritional security effectively as it is considerably low cost variety of fish which contains good quantity of protein. The nutritional security can be achieved by supplying to distant markets using appropriate preservation technologies of which chitosan edible treatment can be one option. Prasad et al. (1990) have also reported similar protein content for ribbonfish.

Changes in TPC and *Enterobacteriaceae* counts of control and chitosan treated ribbon fish steaksare shown in Fig. 1. On the initial day TPC of ribbonfish steaks was 4.5 logcfug^{-1}, which indicate the sample had a relatively higher microbial load (Fig.1). This indicates good quality of fish used in the study. Contrary to this, very high TPC counts ca. 5.5 log cfu^{-1}are reported for ribbon fish by Imtiaz (2013). This variation could be due to the season of study as well as prevailing hygienic practices in the region. Total plate count increased with the storage period in both the samples, however, the increase was faster

Table 1. Proximate composition of Ribbonfish

Fish	Proximate composition (% in wet wt basis)			
Ribbonfish	Moisture	Crude Protein	Crude Fat	Ash
(*Lepturacanthus savala*)	76.17±1.02%	20.05±0.91%	3.18±0.08%	0.08±.01%

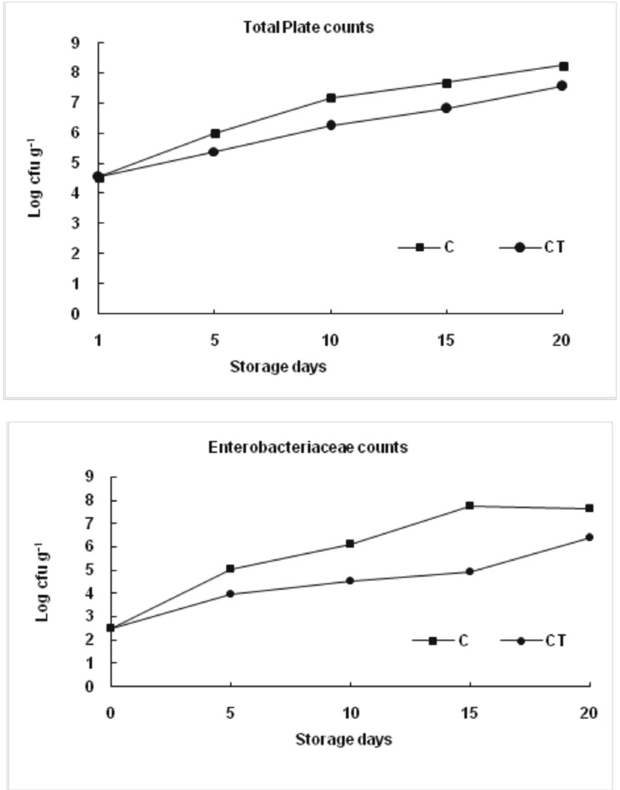

Fig. 1. Changes in total palte count and *Enterobacteriaceae* of Ribbonfish during refrigerated storage

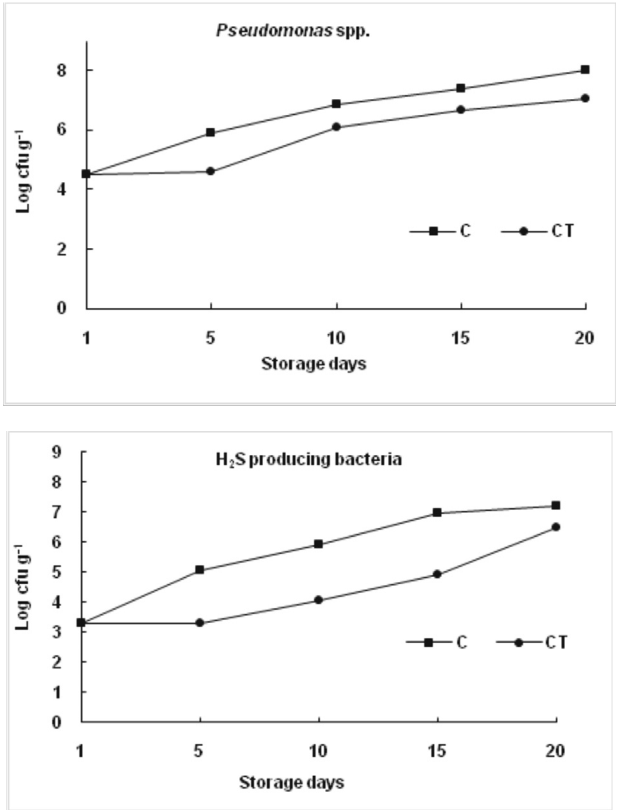

Fig. 2. Changes in *Pseudomonas* spp. and H2S-producing bacteria of Ribbonfish during refrigerated storage

in control samples compared to chitosan treated samples. TPC levels exceeded 7 log cfug^{-1}, which was higher than the maximum recommended level for raw fishery products (ICMSF, 1998) on day 10 for control samples. Up to 15 days, the TPC of chitosan treated samples were observed to be 2 log less than control samples. Total plate counts in chitosan treated sample was less than 7 log cfug^{-1} until the day 15. This indicates the effectiveness of chitosan treatment in inhibiting microbial growth. The total enterobacteriaceae counts of the initial sample was 2.47 log cfu g^{-1} which reached a level of 6.13 log cfu g^{-1}on 10th day for control samples. In chitosan treated samples, the counts were below 5 log cfu g^{-1}up to day 15 indicating its antimicrobial effect.

In control, the initial counts of *Pseudomonas* spp and H_2S producing bacteria were 4.5 and 3.2 logcfug^{-1} respectively and the counts reached 6.8 and 5.9 logcfug^{-1} on day 10 (Fig 2). In chitosan treated samples, the counts of *Pseudomonas* spp and H_2S producing bacteria were 6.0 and 4.0 logcfug^{-1}, which is lower than the control samples on day 10. Tsiligianni et al. (2012) reported that 1% chitosan coating resulted in 2-3 log reductions in aerobic, 3-4 log reduction in anaerobic *Pseudomonas* spp and H_2S producing bacteria of sword fish steaks after 8 days of refrigerated storage. Present result showed that the chitosan coating retard the microbial proliferation in 1-2 log reduction on ribbon fish in the refrigerated storage. Fan et al. (2009) demonstrated that 2% chitosan coating on silver carp (*H. molitrix*) stored at -3^{o}C extended the shelf life upto 30 days. Li et al. (2013) studied the effect of chitosan coating combined with natural preservatives (grape seed extract and tea polyphenols) on red drum (*Sciaenops ocellatus*) fillets extended the shelf life of 6-8 days during refrigerated storage. In another study conducted by Vásconez et al. (2009) reported that chitosan coating with tapioca starch reduced the mesophilic and psychrophilic count and extend the shelf life of salmon to 6 days.

Coagulase positive Staphylococci were not detected in both control as well as chitosan treated ribbonfish steaks during storage period. Fresh fish does not contain any coagulase positive Staphylococci. Its incidence in fish is indicative of poor personnel hygiene. The above results reveals there is no cross contamination of coagulase positive Staphylococci in ribbon fish steaks. The effect of chitosan alone or combined with hydrocolloids on shelf life of fish has been studied by several authors (Soares et al., 2015; Mohan et al., 2012; Li et al., 2013; Tsiligianni et al., 2012; Ojagh et al., 2010; Fan et al., 2009; Vasconezet al., 2009). Several hypotheses have been published on mechanism of antimicrobial activity of chitosan. Chitosan is a cationic polysaccharide, can easily interact with anionic charged cell membrane, which induces the damage of cell (Duanet al., 2010; Devlieghere et al., 2004; Fang et al., 1994). Moreover the chitosan also act as physical barrier create an anaerobic condition so that it inhibit the growth of aerobic bacteria and also inhibit the microbial growth by chelation of protein in the microbes (Cuero et al., 1991; Sudarshan et al., 1992).

The edible chitosan treatment had beneficial effects by inhibiting the microbial counts significantly compared to control samples. This study reveals that the chitosan coating on ribbon fish steaks retard the growth of gram positive as well as gram negative bacteria in effective manner and it can be used as a coated material to extend the shelf life of the fish and fishery products. Chitosan edible coating extended the shelf life by 7-9 days compared to control samples during refrigerated storage indicating its application as natural preservative.

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