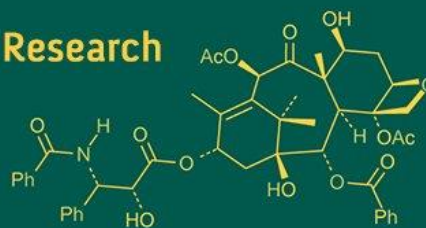
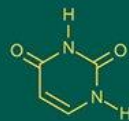
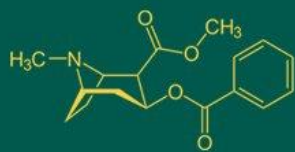


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## Effect of marine biopolymers as edible coatings incorporated with cinnamon essential oil on shelf-life extension of black pomfret steak stored under refrigeration storage

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### Abstract

The present study investigated the effectiveness of marine biopolymer-based edible coatings enriched with cinnamon essential oil for extending the shelf life of Black Pomfret (*Parastromateus niger*) steaks during refrigerated storage at 4±1°C. Fish steaks were treated with different coating formulations: untreated control (T<sub>0</sub>), 1% chitosan with 1% cinnamon essential oil (T<sub>1</sub>), 1% gelatin with 1% cinnamon essential oil (T<sub>2</sub>), and a blended coating of 1% chitosan + 1% gelatin with 1% cinnamon essential oil (T<sub>3</sub>). Samples were packed in LDPE bags and analyzed periodically for proximate composition, biochemical quality, and microbial load. Fresh Black Pomfret showed good nutritional quality with moisture, protein, lipid, and ash contents of 77.64%, 19.19%, 1.41%, and 1.52%, respectively. During storage, Total Plate Count (TPC) increased gradually in all treatments; however, coated samples exhibited significantly lower microbial growth than the control. Among the treatments, T<sub>3</sub> demonstrated the highest antimicrobial effectiveness and maintained the lowest microbial count throughout storage. The synergistic effect of chitosan, gelatin, and cinnamon essential oil effectively delayed spoilage and preserved fish quality. The findings indicate that marine biopolymer-based edible coatings can be used as a natural, sustainable, and effective preservation method for refrigerated seafood products.

**Keywords:** Black Pomfret, edible coating, chitosan, gelatin, cinnamon essential oil, refrigerated storage, shelf life, microbial quality

### Introduction

Seafood is a crucial component of a balanced diet due to its high protein content, low-fat levels, and rich supply of long-chain polyunsaturated fatty acids like EPA and DHA, offering various health benefits (Hassoun *et al.*, 2022; Petricorena, 2015; Carlucci *et al.*, 2015). However, post-harvest deterioration occurs rapidly due to biochemical, physicochemical, and microbial processes, leading to protein degradation, oxidation, and spoilage (Liu *et al.*, 2013; Zhan *et al.*, 2018; Wang, 2019). Traditional preservation methods like drying, curing, and fermentation help slow deterioration (Hassoun *et al.*, 2020a; Hassoun *et al.*, 2020b; Tavares *et al.*, 2021), but incorporating natural preservatives in low concentrations provides additional antimicrobial and antioxidant benefits (Baptista *et al.*, 2020; Hassoun & Çoban, 2017; Batiha *et al.*, 2021). These can be applied directly to seafood or integrated into edible coatings and packaging materials (Hassoun *et al.*, 2022). Edible coatings, made from biopolymers like polysaccharides, proteins, and lipids, form a thin protective layer on food surfaces (Ju *et al.*, 2019; Santhosh *et al.*, 2020). Polysaccharide-based coatings, such as chitosan and seaweed extracts, offer limited moisture barriers but can be enhanced with essential oils (EOs) like oregano, cinnamon, and thyme, which improve antibacterial action and prolong shelf life (Dehghani *et al.*, 2018; Aider, 2010; Ju *et al.*, 2019). Combining polysaccharide coatings with functional EOs provides strong antimicrobial and antioxidant properties, effectively preserving seafood quality (Ju *et al.*, 2019) [1, 3, 4, 6, 7, 13-16, 21, 25, 32, 33, 37, 39, 40].

Edible coatings are thin layers of edible biopolymers, such as polysaccharides, proteins, and lipids, applied to food surfaces via immersion or spraying to enhance preservation (Ju *et al.*, 2019; Santhosh *et al.*, 2020). Polysaccharide-based coatings like chitosan and seaweed extracts

offer limited moisture barriers due to their hydrophilic nature but can be improved with essential oils (EOs) like oregano, cinnamon, and thyme, which enhance antibacterial activity and prolong shelf life (Dehghani *et al.*, 2018; Aider, 2010; Ju *et al.*, 2019). Cinnamon (*Cinnamomum zeylanicum* L.), rich in cinnamaldehyde and terpenes, exhibits strong antibacterial and antioxidant properties by disrupting bacterial cell walls and enzyme formation (Tzortzakis, 2009; Helander *et al.*, 1995). Chitosan, a biodegradable and biocompatible polysaccharide, is widely used in food coatings and packaging due to its potent antimicrobial and antioxidant effects (Li *et al.*, 2013; Kulawik *et al.*, 2019). Similarly, gelatin-based coatings offer strong mechanical properties and act as effective barriers against oxygen and moisture, reducing oxidation and extending food shelf life (Hanani *et al.*, 2013; Molinaro *et al.*, 2015; Krochta & Mulder-Johnston, 1997) [1, 7, 12, 17, 21, 22, 23, 24, 28, 38].

Mainly fishery products develop objectionable odours and flavours due to bacterial activity during refrigerated storage. So, natural bio-preservatives are used to prevent or destroy the growth of these spoilage bacteria and improve the quality of the fish product (Gilliland & Ewell, 1983). Therefore, this work aimed to evaluate the effect of marine biopolymers such as chitosan and gelatin-based coatings incorporated with the essential oil such as cinnamon essential oil on the shelflife extension of Black Pomfret, *Parastromateus niger*, steak during refrigerated storage [10].

## 2. material and methods

### 2.1 Preparation and treatment of fish samples

**2.1.1 Fish preparation:** Fresh black pomfret (*Parastromateus niger*) were purchased from Veraval fish landing center, (Gujarat) and kept in an insulated box in iced condition with a temperature range of 0-2 °C and transported to the Fish Processing Laboratory of the Department of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Veraval. After that, the fish was washed and dressed. Then dressed fish was cut into the steak and again washed with chilled potable water.

#### 2.1.2 Preparation of coating solutions and treated steaks

Chitosan and Gelatin powder purchased from Myochem Pvt. Ltd. For preparing chitosan coating solution, first of all, Chitosan dissolved in 1% glacial acetic acid overnight and then filtered with Whatman filter paper no. 3 and then make up a solution with distilled water and add 0.75ml/gm glycerol as plasticizer and stir for 10 minutes. For preparing a gelatin coating solution, gelatin powder is dissolved in distilled water on a magnetic stirrer at 35 °C for 15 minutes. Then, cool down the solution at 7 °C for 15 minutes and allow the solution to swell. Again, warm the solution at 55 °C for 30 minutes in a hot air oven. 0.2 ml of Tween 80 was added in 1 ml of cinnamon essential oil as an emulsifier for complete dispersion of oil into the above biopolymers coating solutions.

After dressing fish were divided into 4 groups, such as T0(C), T1, T2, and T3. In T0, it was considered a control treatment where no treatment was given. In the T1 treatment, the fish steaks were treated with 1% chitosan with 1% cinnamon essential oil coating solution. In the T2 treatment, the fish steaks were treated with 1% gelatin with 1% cinnamon essential oil coating solution. In the T3 treatment, the fish steaks were treated with 1% chitosan and 1% gelatin coating blended with 1% cinnamon essential oil coating solution.

All treatment solutions were mixed with the help of a centrifuge for 5 minutes at 9,000 rpm. Then after, the fish steaks were dipped for 30 seconds in a solution. Then allow standing for 2 minutes. After that, again immersed for 30 seconds in a solution. Then remove from the solution and allow to drain for 2 minutes. After that, put it in a hot air oven for 15 minutes at 35 °C for proper coating. After that, treated fish steaks were packed in air packaging of an LDPE bag and stored at 4±1 °C refrigerated storage. The biochemical and microbial analysis was conducted at every 5 days of interval to determine the overall quality of fish steaks.

### 2.2 Proximate Analysis

The moisture content and crude ash were determined in an oven at 105 and 550 °C, respectively, until the weight became constant. The total crude protein was determined by the Kjeldahl method. The crude lipid was determined by the Soxhlet extraction method (AOAC, 1984) [2].

### 2.3 Microbial Analysis

Total Plate Counts (TPC) and Psychrotrophic Bacterial Counts (PBC) were determined by preparing serial decimal dilutions of homogenized fish steaks and inoculating them onto plate count agar using the pour plate method. The plates were incubated at 37°C for TPC (2 days) and at 7°C for PBC (5 days). The results were expressed in log CFU/g. For *E.coli* determination, a 0.1 ml aliquot of aseptically prepared serial dilutions was spread onto Tergitol-7 agar plates using a sterile glass rod, followed by incubation at 37±1°C for 24 hours. Yellow colonies with a pinkish tinge were counted, and *E. coli* counts were reported as CFU/g. *Staphylococcus aureus* in fish samples was determined using the AOAC (2006) method by plating 1 ml of food homogenate on Baird Parker Agar, followed by incubation at 35-37°C for 1 hour upright and 45-48 hours inverted. Results were expressed as *S. aureus* CFU/g [2].

### 2.3 Biochemical Analysis

Total volatile base nitrogen (TVB-N) was determined using the micro-diffusion method of Beatty & Gibbons (1937). The unreacted acid in the inner chamber of the diffusion unit, after overnight reaction with TCA extract and potassium carbonate, was titrated against 0.01N NaOH using Tashiro's indicator. Results were expressed as mg% TVB-N.

Trimethylamine (TMA) was determined using the micro-diffusion method of Beatty & Gibbons (1937). After reacting the TCA extract with formaldehyde and potassium carbonate in a sealed diffusion unit overnight, the unreacted acid was titrated with 0.01N NaOH using Tashiro's indicator. Results were expressed as mg% TMA [5].

**2.3 Statistical Analysis:** Data were statistically analyzed using a completely randomized design, with analysis of variance (ANOVA) conducted as per Snedecor and Cochran (1989). Experimental data were processed using IBM BASIC Windows release 1.13 to identify significant differences among treatment combinations and determine the optimal treatment [35].

## 3.0 Results and discussion

**3.1 Proximate analysis:** The average proximate composition of the fresh black pomfret as raw material was demonstrated

in Figure 3.1. The black pomfret was caught in December 2022. The proximate composition includes moisture content was about  $77.64 \pm 0.26\%$ , crude protein at  $19.19 \pm 0.12\%$ , crude lipid at  $1.41 \pm 0.08\%$ , and ash content was  $1.52 \pm 0.12\%$  on a wet weight basis. The nutritional values of the raw material align with Jamilah *et al.* (2009), reporting  $78.77 \pm 0.13\%$  moisture but lower fat content ( $1.54 \pm 0.07\%$ ) in Black Pomfret. Similar findings were observed by Manju *et al.* (2007) with  $75.92\%$  moisture,  $2.48\%$  crude fat,  $20.04\%$  crude protein, and  $1.56\%$  ash, as well as by Taheri (2015) with  $75.5 \pm 0.3\%$  moisture,  $2.4 \pm 0.1\%$  crude fat,  $19.9 \pm 0.4\%$  crude protein, and  $1.7 \pm 0.04\%$  ash. Jai Ganesh *et al.* (2011) also reported comparable values except for a lower crude protein content of  $14.4 \pm 0.5\%$ . These results confirm that all proximate characteristics are within the prescribed limits, indicating the freshness of the fish used in this study [19, 20, 27, 36].

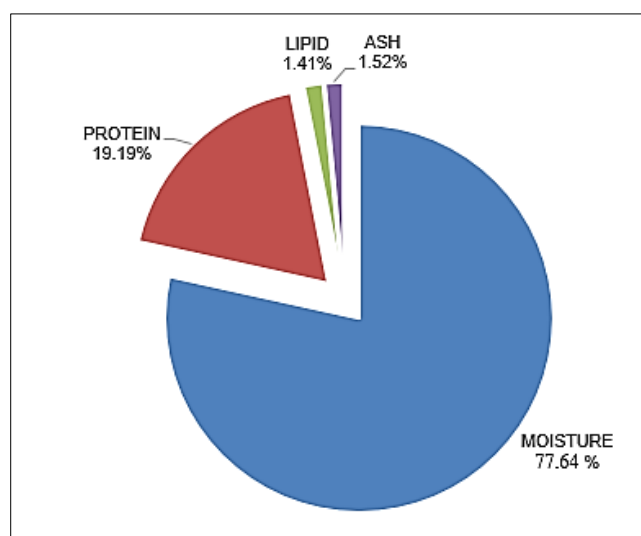


**Fig 3.1:** Proximate Composition of Fresh Black Pomfret (*Parastromateous niger*)

## 3.2 Microbial analysis

### 3.2.1 Total Plate Count (TPC)

The changes in the TPC content of Black Pomfret are shown in Fig. 3.2. Initially, the TPC values for T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> were  $3.32 \pm 0.26$ ,  $3.18 \pm 0.19$ ,  $2.99 \pm 0.17$ , and  $3.01 \pm 0.10$  log cfu/gm, respectively, which increased to  $9.31 \pm 0.97$ ,  $7.88 \pm 0.29$ ,  $7.93 \pm 0.31$ , and  $6.88 \pm 0.34$  log cfu/gm at the end of storage, showing significant differences ( $CV\% = 7.003$ ,  $p < 0.05$ ). Coated samples had lower initial bacterial loads due to the antimicrobial properties of the coating ingredients. The maximum acceptable TPC limit for fresh fish is 7 log cfu/g (ICMSF & Thatcher, 1978). Studies by Devlieghere *et al.* (2004) and Pereda *et al.* (2011) explained that chitosan and gelatin have antimicrobial effects, while Shan *et al.* (2007) and Gupta *et al.* (2008) highlighted the role of trans-cinnamaldehyde and other compounds in cinnamon oil in inhibiting microbial growth. Similar findings were reported by Ojagh *et al.* (2010), Nowzari *et al.* (2013), and Farajzadeh *et al.* (2016), confirming that coatings like chitosan-gelatin and chitosan-cinnamon oil effectively reduce TPC during storage. Manju & Srinivasa (2005) reported a higher initial TPC ( $5.52$  log cfu/g) at  $20^\circ\text{C}$  compared to the present study [8, 9, 11, 26, 29, 30, 31, 34].

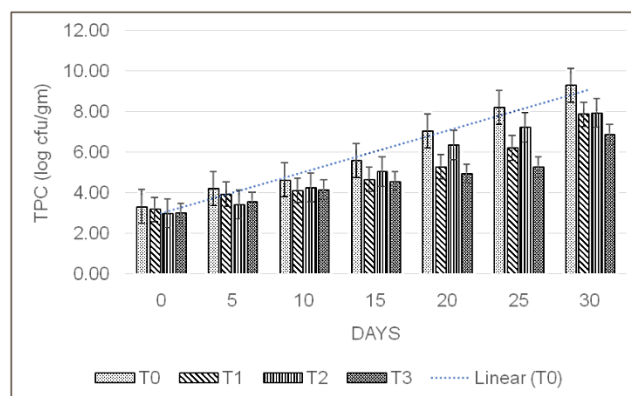


**Fig 3.2:** Changes in Total Plate Count (log cfu/g) in Black Pomfret steak during storage

### 3.2.2 Total Psychrotrophic Bacterial Count (log cfu/g):

The changes in the Total Psychrotrophic Bacterial Count (PBC) of Black Pomfret steak are presented in Table 4.12 and Fig. 4.11. Initially, PBC was not detected in any sample; however, the count gradually increased during refrigerated storage. The final PBC values of T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> were  $9.25 \pm 0.18$ ,  $8.08 \pm 0.13$ ,  $9.38 \pm 0.18$ , and  $6.45 \pm 0.21$  log cfu/g, respectively. Significant differences ( $p < 0.05$ ) were observed among treatments, with a  $CV\%$  of 0.633. Psychrotrophic Gram-negative bacteria are the major spoilage microorganisms in refrigerated fish products (Nirmal & Benjakul, 2011). Coated samples exhibited lower PBC values compared to the control, indicating the antimicrobial effectiveness of chitosan and cinnamon essential oil. Chitosan acts as an oxygen barrier and disrupts microbial cell membranes through interactions between its protonated amino groups and negatively charged bacterial surfaces, thereby inhibiting microbial growth (Jeon *et al.*, 2002; Feng & Xia, 2011). Similar reductions in psychrotrophic bacterial growth in coated seafood products were also reported by Vieira *et al.* (2019), Ojagh *et al.* (2010), and Farajzadeh *et al.* (2016). These findings suggest that chitosan and cinnamon essential oil effectively delayed microbial spoilage and improved the microbiological quality of fish steaks during refrigerated storage.

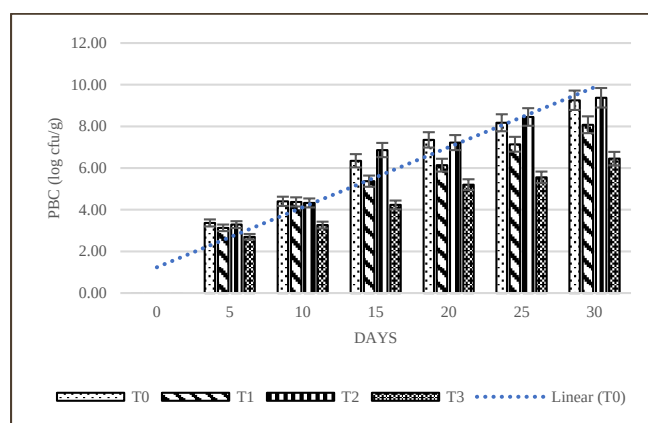


**Fig.3.3:** Changes in Total Psychrotrophic Bacterial Count (log cfu/g) in Black Pomfret steak during storage

## 3.3 Biochemical Analysis

### 3.3.1 TVB-N (mg%)

The changes in Total Volatile Base Nitrogen (TVB-N) content of Black Pomfret steak are presented in Table 4.8 and Fig. 4.7. The initial TVB-N values of all treatments ranged

from  $4.58 \pm 0.24$  to  $4.92 \pm 0.26$  mg%, which gradually increased during refrigerated storage. At the end of storage, TVB-N values of T0, T1, T2, T3, and T4 were  $35.91 \pm 0.28$ ,  $35.19 \pm 0.19$ ,  $35.39 \pm 0.19$ , and  $32.56 \pm 0.54$  mg%, respectively, showing significant differences ( $p < 0.05$ ) among treatments with a CV% of 3.27. TVB-N is an important indicator of fish spoilage and reflects the formation of volatile nitrogenous compounds such as ammonia, trimethylamine, and dimethylamine produced by microbial and enzymatic degradation (Nowzari et al., 2013). The acceptable limit for marine fish products is generally considered to be 30–35 mg/100 g (Connell, 1995). In the present study, coated samples showed lower TVB-N values compared to the control, indicating reduced spoilage activity. Similar findings were reported by Farajzadeh et al. (2016), Ojagh et al. (2010), and Haghighi & Yazdanpanah (2020), who observed that chitosan- and gelatin-based coatings incorporated with cinnamon essential oil effectively delayed TVB-N formation by inhibiting microbial growth and reducing protein degradation. The lowest TVB-N value was observed in chitosan-gelatin coated samples blended with cinnamon essential oil, confirming the antimicrobial and preservative effects of the coating materials during refrigerated storage.

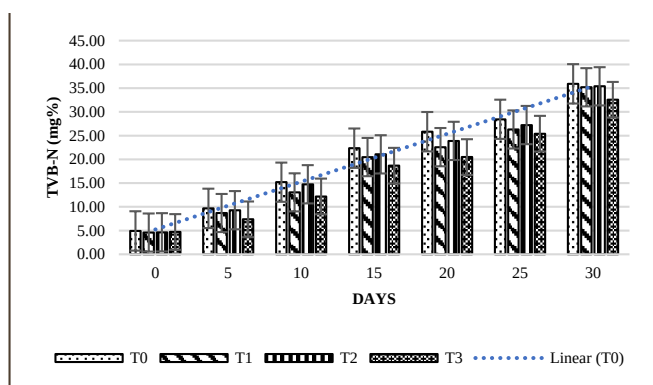


**Fig 3.4:** Changes in TVB-N (mg%) in Black Pomfret steak during storage

### 3.3.2 TMA-N (mg%)

The changes in Trimethylamine Nitrogen (TMA-N) content of Black Pomfret steak are presented in Table 4.7 and Fig. 4.6. The initial TMA-N values ranged from  $2.61 \pm 0.21$  to  $2.74 \pm 0.18$  mg%, which gradually increased during refrigerated storage. At the end of storage, the TMA-N values of T0, T1, T2, T3, and T4 were  $15.15 \pm 0.32$ ,  $14.54 \pm 0.20$ ,  $14.20 \pm 0.06$ , and  $13.40 \pm 0.28$  mg%, respectively, with significant differences ( $p < 0.05$ ) among treatments and a CV% of 5.58. TMA-N is an important spoilage indicator formed due to the microbial reduction of trimethylamine oxide (TMAO), resulting in the development of characteristic fishy odours during storage. The acceptable limit for TMA-N in fish products is reported to be 15 mg N/100 g (Manju et al., 2007). In the present study, coated samples showed lower TMA-N values compared to the control, indicating reduced spoilage activity and delayed microbial decomposition. Similar increasing trends during refrigerated storage of Black Pomfret were reported by Manju and Srinivasa (2005). Studies by Haghighi and Yazdanpanah (2020) and Aşık and Candoğan (2014) also demonstrated that chitosan-based coatings incorporated with cinnamon or garlic oil effectively inhibited TMA-N formation due to their antimicrobial properties. The lower TMA-N values observed in coated samples suggest that chitosan, gelatin, and cinnamon essential oil effectively retarded bacterial activity and

maintained the quality of fish steaks during refrigerated storage.

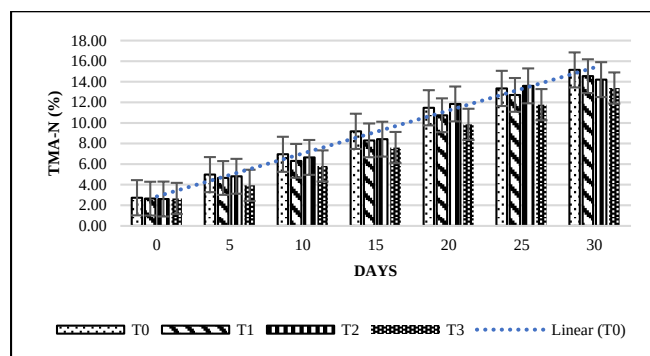


**Fig 3.5:** Changes in TMA-N (mg%) in Black Pomfret steak during storage

## 4. Conclusion

The findings of the present investigation revealed that edible coatings prepared from marine biopolymers and incorporated with cinnamon essential oil were effective in preserving the quality of Black Pomfret (*Parastromateus niger*) steaks during refrigerated storage. The treated samples exhibited slower microbial proliferation compared to the untreated control, indicating the antimicrobial potential of the coating materials. Among the different treatments, the combination of chitosan, gelatin, and cinnamon essential oil showed the best preservation effect by maintaining lower microbial counts throughout the storage period.

The coated fish steaks also showed delayed spoilage and better retention of freshness during storage, which may be attributed to the barrier properties of chitosan and gelatin along with the antioxidant and antimicrobial activities of cinnamon essential oil. The synergistic interaction of these natural components helped in minimizing quality deterioration under chilled conditions.

Overall, the study suggests that marine biopolymer-based edible coatings enriched with cinnamon essential oil can serve as an effective and sustainable preservation method for refrigerated seafood products. The use of such natural bioactive coatings may contribute to extending shelf life, improving product quality, and reducing post-harvest losses in fish and fishery products.

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