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Effect of chitosan coating and drying on quality of Bombay duck (*Harpodon nehereus*)

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Abstract

Bombay duck (*Harpadon nehereus*) is one of the most abundant and preferable marine species in the Maharashtra and Gujrat regions. While Bombay duck is mostly consumed in dried form, the major problems associated with traditionally sun-dried Bombay duck are contamination by insects or pests; uneven drying which leads to spoilage; and poor quality of the end product. Chitosan and its derivatives are used as an edible coating in food applications due to its antioxidant and antimicrobial actions. In the present work, the effect of chitosan coating and drying on the quality of Bombay duck was studied. Five different samples were prepared as follows: (i) control (without any treatment); (ii) dip-treated in 1% acetic acid (AA); (iii) dip-treated in 0.5% chitosan dissolved in 1% acetic acid (CAA); (iv) dip-treated in 1% malic acid (MA); and (v) dip-treated in 0.5% chitosan dissolved in 1% malic acid (CMA). All samples were dried at 50°C. When biochemical, microbiological, and sensory analyses were assessed for four months, results indicated the sample coated with chitosan pre-dissolved in either acetic acid or malic acid had lower TVB-N (86.5 - 115.25 mg/100 g) and TPC (5.3 - 5.5 $\log_{10}$ CFU/g) than the control (TVB = 163 mg/100 g; TPC = 7.4 $\log_{10}$ CFU/g); a similar trend was also observed for TBA values. Even though the microbial analysis revealed that TPC crossed the limit of acceptability (5.2 $\log_{10}$ CFU/g) by the second month in the control, AA, CAA, MA, and CMA levels were acceptable up to three months. Sensory analysis showed that the overall acceptability score was higher for sample coated with chitosan. These results suggested that chitosan coating and drying could improve the quality of dried Bombay duck.

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Introduction

Fish and fishery products are important sources of proteins, fats, essential amino acids, minerals, vitamins, and other nutrients. Approximately 20% of the artisanal catch of fishery products is dried by traditional sun drying methods, and consumed in the domestic market (Mukharjee *et al*., 1990). Bombay duck (*Harpodon nehereus*) is one of the most abundant and preferable marine species in the Maharashtra and Gujrat regions (Chakrabarti, 2010). Traditionally, the drying of Bombay duck is done by interlocking their jaws on bamboo scaffolds, which are then fixed in the sand by bars tied with thick ropes. Some significant problems associated with traditionally sun-dried Bombay duck is contamination by insects or pests, uneven drying, and longer drying times, which leads to spoilage and poor quality of the end product. For this reason, a drying process under controlled temperature conditions has become very important in fish drying to achieve better quality dried fish products. Although the drying process reduces the moisture content and prevents bacterial spoilage, the addition of antioxidants or antimicrobial agents will further enhance the shelf life of dried fish products. Consequently, there is growing interest in antioxidants or antimicrobial agents from natural sources for food application.

Chitosan (β-(1,4)-2-amino-2-deoxy-D-glucopyranose) is a versatile biopolymer obtained by the deacetylation of chitin, which is found in the shell of crustaceans. The application of chitosan in seafood has been studied by various researchers for shelf-life extension and textural modification due to its intrinsic antioxidant and antimicrobial properties, and good film-forming ability (Mohan *et al*., 2012; Remya *et*

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al., 2015; Renuka *et al.*, 2016; Jeyakumari *et al.*, 2016a, 2016b; Morachis-Valdez *et al.*, 2017; da Silva Santos *et al.*, 2017). There is currently a need for novel fish-drying methods to control the enzymatic and microbial spoilage of fish. Organic acids such as acetic, citric, and lactic acids are most commonly used for various food preservation to inhibit metabolic reactions, microbial growth, and are generally recognised as safe (Dibner and Buttin, 2002; Rey *et al.*, 2012; Smyth *et al.*, 2018). Malic acid and its salts are also approved food additives by the European Union. It has been reported that malic acid could reduce microbial growth in meat products (Mohan and Pohlman, 2016; Olaimat *et al.*, 2018; Fernández *et al.*, 2021).

Very few studies have established the chitosan film wrapping for dried fish to improve the quality (Agustini and Sedjati, 2007; Vimala *et al.*, 2015). Viji *et al.* (2021) studied the edible coating of carboxymethyl chitosan for shelf-life extension of dried anchovies. To the best of our knowledge, there is no report on chitosan dip treatment using acetic and malic acids for improved production quality of dried Bombay duck. Based on these information, the present work was focused to study the effect of chitosan coating and drying on the quality of dried Bombay duck in comparison with fish treated with only organic acids (*i.e.*, acetic and malic acids).

Materials and Methods

Sample preparation

Bombay ducks (200 - 250 g size) were procured from a fish market in Vashi, Navi, Mumbai, and brought to the laboratory in iced conditions. The fresh Bombay duck was cleaned, sliced, and washed with potable water. It was then subjected to a dip treatment of a 5% salt solution for 10 min to achieve an ideal salt content in the finished product. The five different samples were then prepared as follows: (i) control (without any treatment); (ii) dip-treated in 1% acetic acid (AA); (iii) dip-treated in 0.5% chitosan dissolved in 1% acetic acid (CAA); (iv) dip-treated in 1% malic acid (MA); and (v) dip-treated in 0.5% chitosan dissolved in 1% malic acid (CMA).

All samples were given a 10-min dip treatment. The control sample was dip-treated in a 5% salt solution after being drained, and was subjected to drying. In the case of the chitosan-organic acid treatment, the samples were first dip-treated in a 5% salt solution after being drained, then it was subjected to a dip treatment in chitosan/1% organic acid solution and dried. All samples were dried at 50°C for 14 h. The dried samples were then packed treatment-wise (200 g/pack) in high-density polyethylene pouches, which are impervious to microbial and insect attacks. Biochemical, microbiological, and sensorial analyses were carried out at monthly intervals for four months.

Biochemical analysis

Proximate composition and salt were analysed in accordance with the AOAC (2015) methods. Total volatile base nitrogen (TVB-N) was evaluated using the Conway micro-diffusion method (Conway, 1950). Peroxide value (PV) was estimated using the titrimetric method (AOAC, 2015). Thiobarbituric acid (TBA) was analysed according to Tarladgis *et al.* (1960).

Sensorial evaluation

A sensory analysis of the dried fish samples was conducted after they were fried at 160 - 180°C for 2 min. The panellists were asked to evaluate the appearance, colour, flavour, texture, taste, and overall acceptability using 9-point hedonic scale; scores of 7 - 9 indicated "extremely liked," scores of 5 - 7 indicated "liked," and scores below 5 were the limit of acceptability (Tahra *et al.*, 2018).

Microbiological analysis

Total plate count (TPC) and *Staphylococcus aureus* were determined following the FAO (1992) method. *Escherichia coli* was determined following the FDA (2002) method.

Statistical analysis

The data obtained were analysed by a One-way analysis of variance (ANOVA) using IBM® SPSS® Version 16.0 software (SPSS Inc., Chicago, Illinois, USA). All mean separations were tested at a significance level of 5%.

Results and discussion

Proximate composition

Proximate composition is one of the major factors that determine the nutritional value of food. The proximate composition of fish generally varies between species, and also from species to species; this variation is influenced by various factors, such as size, sex, age, geographical location, and season. The proximate composition and salt content of dried Bombay duck are presented in Table 1. The moisture content was 11.25 - 11.58%. The protein and lipid contents were 69.35 - 71.02 and 3.8 - 4.8%, respectively. These results were in accordance with previous reports for sun-dried Bombay duck (Bhattacharya *et al.*, 2016; Jamil *et al.*, 2017).

The protein content observed in the present work confirmed that Bombay duck could be good source of protein. The highest ash content was found in the control, AA, and CAA. These results were in accordance with previous reports on dried Bombay duck (Hossain *et al.*, 2015; Jamil *et al.*, 2017). Except for the control (10.25%), all samples had a salt content of 6.10 - 6.5% (Table 1), which was within the acceptable limit (*i.e.*, 7.5% salt) per Indian standards for fish products (IS 14950; Indian Standard, 2001). The variation of salt content between samples might have been due to different treatment processes followed during the sample preparation.

Table 1. Proximate composition of dried Bombay duck.

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Salt (%)
Control	11.58 ± 0.15	69.35 ± 0.20	3.80 ± 0.01	14.04 ± 0.40	10.25 ± 0.20
AA	11.33 ± 0.20	69.94 ± 0.25	4.20 ± 0.02	14.15 ± 0.55	6.20 ± 0.25
CAA	11.25 ± 0.05	71.02 ± 0.01	4.8 ± 0.01	13.05 ± 0.60	6.35 ± 0.30
MA	11.30 ± 1.20	70.65 ± 0.55	3.95 ± 0.05	13.50 ± 0.85	6.10 ± 0.35
CMA	11.45 ± 0.25	71.41 ± 0.25	4.2 ± 0.12	13.80 ± 0.35	6.50 ± 0.40

Results are mean ± SD (n = 3). Control: without acid or chitosan treatment; AA: 1% acetic acid treated; CAA: 0.5% chitosan dissolved in 1% acetic acid treated; MA: 1% malic acid treated; and CMA: 0.5% chitosan dissolved in 1% malic acid treated.

Changes in biochemical quality

Moisture and pH

The moisture content of the dried samples showed an increasing trend during storage. Hoque *et al.* (2018) also observed a gradual increase in the moisture content of dried fish during storage. While the control was observed to have a higher moisture content (14.5%) at the end of the fourth month, and the others followed in the order of MA > AA > CMA > CAA, the moisture content in all the samples was within the acceptable limit of 15% (IS 14950; Indian Standard, 2001) throughout the storage period. The measurement of the pH value of fish muscle indicates the quality of the fish; the initial pH of dried Bombay duck varied between 5.8 - 6.54. Farid *et al.* (2014) observed an increasing trend in the pH value of dried fish stored at room temperature due to an increase in basic compounds. In the present work, there was no significant difference in the pH of dried fish during storage. There was, however, a significant difference ($p < 0.05$) in the pH of dried Bombay duck between treatments (Table 2), which might have been due to differences in the composition of the treatment used in the present work.

TVB-N

TVB-N measures biochemical quality indices of fish and fishery products. The initial TVB-N content of dried Bombay duck was 35.5 - 60.5 mg/100 g; this variation might have been due to different treatments applied in the present work. Several studies observed higher TVB-N values (71 - 115 mg/100 g) for dried Bombay duck collected from local fish markets (Vijayan and Surendran, 2012; Hossain *et al.*, 2015; Jamil *et al.*, 2017). The lower TVB-N obtained in the present work indicated that the fish were dried under controlled conditions. In the present work, TVB-N showed an increasing trend during storage. This was also observed by previous researchers (Bhattacharya *et al.*, 2016; Das *et al.*, 2018). In the present work, CAA had lower TVB-N values (86.5 mg/100 g) than the other treatments and the control; this might have been due to the fact that chitosan coating could reduce the capacity of bacteria for oxidative deamination of non-protein nitrogen substances. A reduction in TVB-N content in chitosan-coated smoked tilapia fillets and fish steaks was also observed by previous researchers (Remya *et al.*, 2015; da Silva Santos *et al.*, 2017). There was a

Table 2. Changes in moisture, pH, and TVB-N contents in Bombay duck during storage.

Storage days	Samples	Moisture (%)	pH	TVB-N (mg%)
0	Control	11.58 ± 0.50^{aA}	6.54 ± 0.1^{cA}	60.5 ± 1.5^{cA}
	AA	11.33 ± 0.25^{aA}	5.8 ± 0.06^{Aa}	45.2 ± 3.5^{bA}
	CAA	11.25 ± 0.40^{aA}	6.1 ± 0.15^{aB}	36.5 ± 1.2^{aA}
	MA	11.25 ± 0.45^{aA}	5.9 ± 0.18^{abA}	48.6 ± 3.5^{bA}
	CMA	10.8 ± 0.50^{aA}	6.1 ± 0.08^{abA}	35.5 ± 2.5^{aA}
1st Month	Control	11.9 ± 0.60^{aA}	6.56 ± 0.05^{cA}	75.5 ± 2.8^{dB}
	AA	11.65 ± 0.30^{abA}	5.85 ± 0.08^{aA}	58.6 ± 4^{bcBA}
	CAA	11.58 ± 0.60^{aA}	6.05 ± 0.1^{abA}	48.6 ± 2^{aB}
	MA	11.45 ± 0.55^{abA}	5.9 ± 0.15^{aA}	65.8 ± 4^{cB}
	CMA	11.5 ± 0.55^{abA}	6.2 ± 0.15^{bA}	52.4 ± 1.8^{abB}
2ndMonth	Control	12.45 ± 1.2^{aA}	6.58 ± 0.1^{cA}	97.5 ± 4^{cC}
	AA	12.35 ± 0.55^{abcA}	5.85 ± 0.09^{aA}	63.2 ± 5.5^{aB}
	CAA	11.9 ± 0.55^{aA}	6.1 ± 0.09^{abA}	62.6 ± 1.8^{aC}
	MA	11.9 ± 0.35^{abA}	5.9 ± 0.07^{aA}	84.2 ± 5.5^{bC}
	CMA	11.9 ± 0.45^{bcA}	6.25 ± 0.06^{bA}	67.25 ± 3^{aC}
3rdMonth	Control	13.85 ± 1.2^{bAC}	6.62 ± 0.06^{cA}	145 ± 3.5^{dD}
	AA	12.65 ± 0.55^{bcAB}	5.9 ± 0.15^{aA}	82.4 ± 4.2^{abC}
	CAA	12.04 ± 0.55^{aA}	6.1 ± 0.15^{abA}	75.5 ± 2.5^{aD}
	MA	12.8 ± 0.35^{bcAB}	5.85 ± 0.05^{aA}	92.55 ± 4.2^{cC}
	CMA	12.25 ± 0.45^{bcA}	6.3 ± 0.04^{bA}	84.25 ± 1.2^{bD}
4thMonth	Control	14.5 ± 0.4^{bC}	6.62 ± 0.15^{cA}	163 ± 5.2^{aE}
	AA	13.1 ± 1.2c^{AB}	5.95 ± 0.12^{aA}	110.65 ± 3.8^{aD}
	CAA	12.25 ± 0.8^{aA}	6.15 ± 0.12^{abA}	86.5 ± 2^{aE}
	MA	13.65 ± 0.45^{cBC}	5.9 ± 0.1^{aA}	125.2 ± 3.8^{aD}
	CMA	12.8 ± 0.55^{cAB}	6.3 ± 0.08^{bA}	115.25 ± 1.8^{aE}

Results are mean ± SD (n = 3). Means with different lower- and uppercase superscripts are significantly (p < 0.05) different between treatment and storage days, respectively. Control remained without acid or chitosan treatment; AA was treated with 1% acetic acid; CAA was treated with 0.5% chitosan dissolved in 1% acetic acid; MA was treated with 1% malic acid; and CMA was treated with 0.5% chitosan dissolved in 1% malic acid.

significant difference (p < 0.05) in the TVB-N content of dried Bombay duck during storage (Table 2). Connell (1995) suggested a limit of 200 mg/100 g TVB-N for salted and dried fish; accordingly, TVB-N values were within the limit of acceptability in all samples during the storage period.

Peroxide values and thiobarbituric acid-reactive substances

PV and TBARS are most commonly used to assess lipid oxidation in food, and regarded as useful index to measure the degree of oxidative rancidity. Initial PV ranged between 0.98 - 1.74 meq. O_2/kg of fish. The PV then demonstrated an increasing trend during storage (Figure 1). The highest PV value was observed in the control (10.5 meq. O_2/kg of fish), and the lowest PV value was found in CAA (6.2 meq O_2/kg of fish), followed by CMA (6.8 meq. O_2/kg of fish) at the end of the storage period. A PV of more than 20 meq. O_2/kg of oil for fish usually results in a rancid smell and taste (Reza, 2006). Accordingly, all the samples in the present work showed PV values within the limit of acceptability. Das *et al.* (2018) observed higher PV for dried Bombay duck collected from a local market in different seasons; they also reported that temperature plays a major role in the fat oxidation of dried fish. In the present work, lower PV indicated that fish were under a controlled environment. Like PV, TBA values also demonstrated an increasing trend during storage (Figure 2). While the result showed that the control had TBA values of 1.7 mg malonaldehyde/kg at the end of the storage period, CAA and CMA had lower TBA values of 0.82 - 0.85 mg malonaldehyde/kg. Vimaladevi *et al.* (2015) observed lower PV and TBA values for dried anchovies wrapped in chitosan-based film. TBA values lower than 3.0 mg malonaldehyde/kg in cured fish are generally considered an acceptable limit. Accordingly, all the samples had a TBA value within the acceptable limit throughout the storage period.

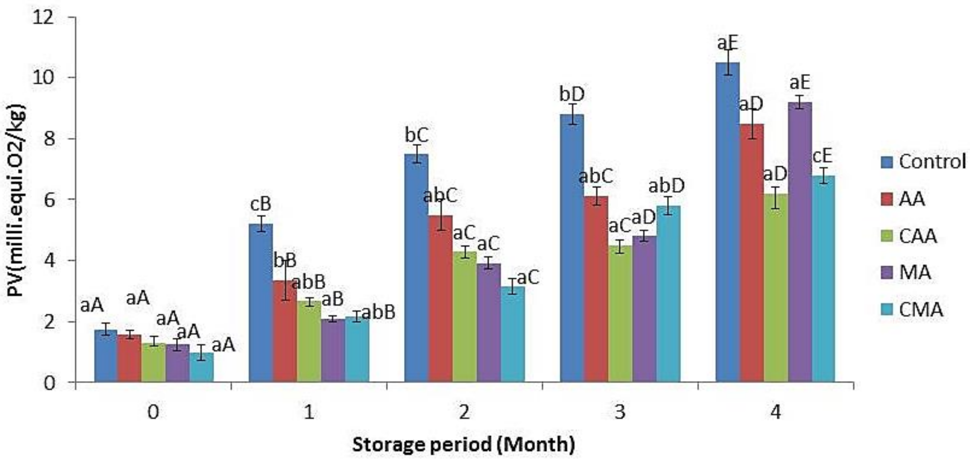

Figure 1. Changes in peroxide value (PV) of dried Bombay duck during storage.

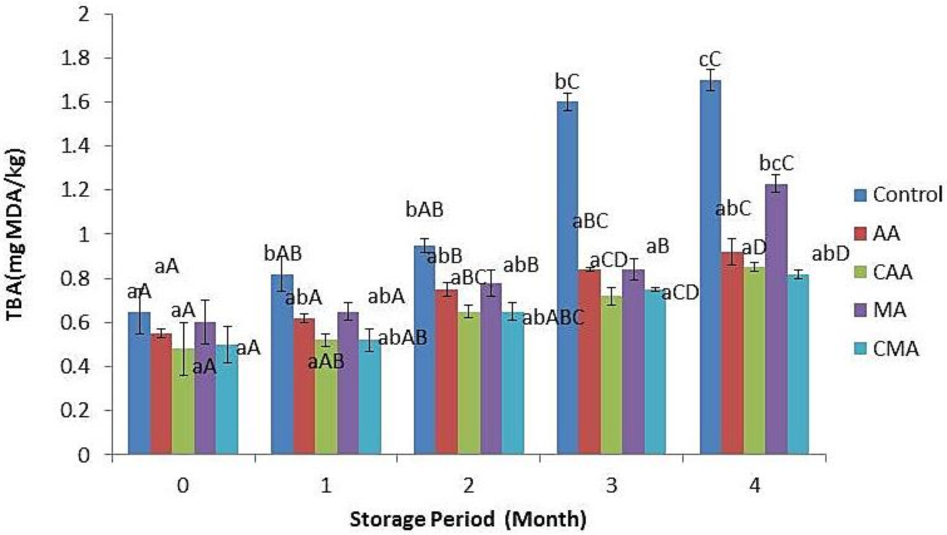

Figure 2. Changes in thiobarbituric acid value (TBA) of dried Bombay duck during storage.

Overall acceptability

A sensory analysis showed that the scores for all dried fishes ranged from 6.8 - 9 using the 9-point hedonic scale. No insect infestation or broken pieces were observed in any of the dried samples throughout the storage period. These results indicated that the control had characteristic colour with a slight rancid odour during storage. Moreover, the chitosan treated samples (*i.e.*, CAA and CMA) had no rancid odour, and achieved a higher score for appearance and overall acceptability during storage. Sulieman and Allaahmed (2012) reported that TVB-N values were found to have an inverse relationship with the sensory scores of dried fish products. In the present work, overall acceptability coincided with biochemical quality as indicated by lower TVB-N, PV, and

TBARS values; these results were in agreement with previous reports for chitosan- and thyme-oil-coated dried fish (Tahra *et al.*, 2018; Gore *et al.*, 2019) All dried samples in the present work were rated between “liked” and “extremely liked”, and had TVB-N values between 35.5 ± 1.2 to 163 ± 5.2 mg/100 g.

Changes in microbial quality

The initial TPC in the dried Bombay duck ranged between 3.6 - 3.9 log_{10} CFU/g, and showed an increasing trend during storage (Figure 3); the recommended TPC for dried fish products for human consumption is 5 log_{10} (IS 14950; Indian Standard, 2001). A higher TPC value (5.1 log_{10} CFU/g) was observed for open sun-dried Bombay duck and shark meat by previous researchers (Guizani *et al.*, 2008; Das *et al.*, 2018). In the present work, while TPC values surpassed the acceptable limit in the control during the second month of storage; AA, CAA, MA, and CMA levels were acceptable for up to three months. These results indicated that the sample coated with chitosan pre-dissolved in either acetic acid or malic acid had lower TPC levels than the samples treated by acetic acid or malic acid treated alone and the control.

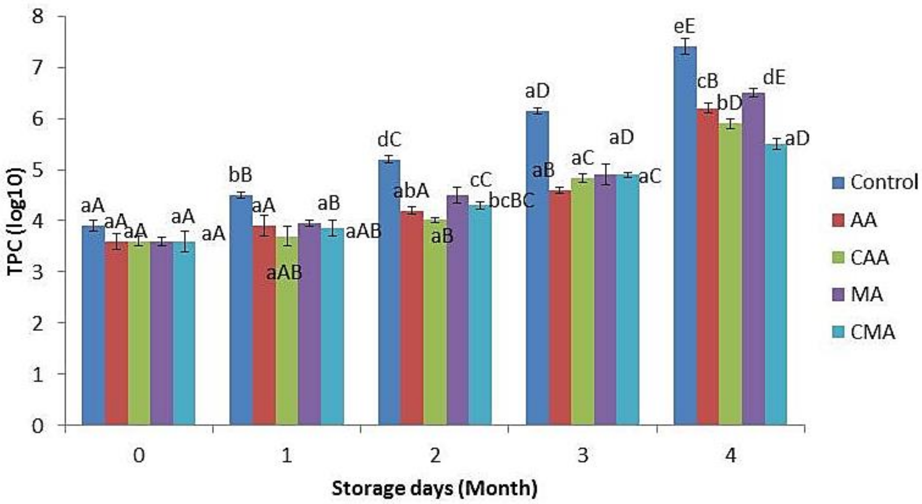

Figure 3. Changes in total plate count (TPC) of dried Bombay duck during storage.

The bioactivity of chitosan is mainly due to the presence of the amine group in the C-2 position (Muthu *et al.*, 2021). While chitosan is not soluble in water, it is soluble in an acid-aqueous solution. When the chitosan is dissolved, the amino group ($-NH_2$) of glucosamine units is converted to soluble protonated form ($-NH^+3$) which provides the antimicrobial activity due to electrostatic interaction between polycationic chitosan and microbial cell surface (Jamilah *et al.*, 2008; Ghaly *et al.*, 2010; Masson, 2021). The electrostatic interaction results in changes in the permeability of the membrane wall, which provokes internal osmotic imbalance, thereby inhibiting microorganism growth.

Malic acid is generally used in food and beverage products as an acidulant, antioxidant, pH control agent, flavouring agent, and flavour enhancer (Fernández *et al.*, 2021). Several studies have observed microbial reduction in meat products treated with malic acid at a 0.5 - 3% concentration (Mohan and Pohlman, 2016; Olaimat *et al.*, 2018; Fernández *et al.*, 2021); they also reported that the antimicrobial activity of organic acids depends on the pH and buffering capacity of the food matrix. Vimaladevi *et al.* (2015) observed a lower TPC for dried anchovies wrapped in chitosan-based film; Agustini and Sedjati (2007) observed a significant decrease in TPC for 0.5% chitosan-coated and dried anchovies, and reported that apart from salting and drying, the application of chitosan as a coating material could reduce the bacterial load in dried fish than control; and Viji *et al.* (2021) observed that an edible carboxymethyl chitosan coating effectively controlled the spoilage in dried anchovies, which might have been due to the antibacterial activity of chitosan. Notably, *Staphylococcus aureus* and *Escherichia coli* were absent throughout the storage period, which indicated that the fish were cleaned and dried in hygienic conditions.

Conclusion

It can be concluded that the combined effect of chitosan coating and drying of Bombay duck in controlled conditions reported in the present work could produce superior quality dried fish products with an extended shelf life. Furthermore, the chitosan-coated sample showed lower TVB-N, PV, TBA, and TPC values than the samples treated with acetic or malic acid, and the control. Sensory analysis concluded that the overall acceptability score was higher for fish coated with chitosan; these results suggested that chitosan coating and drying could reduce oxidation and the total bacterial count, and extend the shelf life of dried Bombay duck.

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