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Preservation of Indian Mackerel (*Rastrelliger kanagurta*) Using Fish Protein Hydrolysate Based Bioactive Edible Coating Incorporated with Chitosan and Clove Oil

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Abstract

The study was conducted to develop ready to disperse bioactive edible coating (BEC) powder using fish protein hydrolysate added with chitosan and clove oil and evaluate their bio-preservation effect in mackerel fillets during refrigerated storage. Mackerel fillet's quality was measured by monitoring the changes in the microbial growth (mesophilic and psychrotrophic bacterial counts), biochemical quality parameters (pH, TBARS, TVB-N, TMA and PV), and sensory quality during 18 days of storage. The initial mesophilic and psychrotrophic bacterial count of mackerel fillets were 4.51 ± 0.37 and 3.68 ± 0.25 log CFU/g respectively and a significant increase was observed in control samples compared to 10 % and 20 % BEC solution coated samples during storage. Control samples exceeded the acceptable limit on day 9, whereas the samples coated with 10 % and 20 % BEC solution exceeded the limit on 15th and 18th day of storage, respectively. In biochemical quality analysis, the pH value increased steadily in control sample than the BEC solution coated samples. Lowest TBARS value was measured in 20 % BEC treated sample (1.72 ± 0.05 mg MDA/kg) followed by 10 % BEC solution treated sample (1.94 ± 0.09 mg MDA/kg) at the end of storage, while control sample exceeded the acceptable limit (2.22 ± 0.12 mg MDA/kg) on 12th day, which revealed that BEC solutions effectively prolonged the shelf life of mackerel fillets. The sensory values gradually decreased in all the samples corresponding to the microbial and biochemical quality changes. The study revealed that the FPH based BEC powder incorporated with chitosan and clove oil showed an excellent bio-preservation effect in mackerel fillets and could have broad potential applications in food products as a natural bio-preservative.

Keywords: Bioactive edible coating, refrigerated storage, psychrotrophic bacteria, sensory quality, mackerel fillet

Introduction

Indian mackerel (*Rastrelliger kanagurta*) belongs to Scombridae family and is abundantly found in the Indian coast. It is a commercially important species, because of the nutritional significance, availability, low cost and industrial use (Kumuda et al., 2018). However, it is a highly perishable commodity and generally stored in ice or under refrigerated/chilled conditions during retail distribution. In this situation, shelf life is very limited, due to microbial deterioration and lipid oxidation (Bahram et al., 2016; Srinivasan et al., 2020). This leads to huge economic loss to fish processing industries and traders. To retard the quality loss, chemical preservatives are being used to minimize lipid oxidation and microbial deterioration (Boyd et al., 1993). Unfortunately, the usage of chemical or synthetic preservatives causes several health related problems to public. Therefore, the industries and researchers are focusing on developing innovative and new technologies from natural sources to extended the shelf life during marketing and storage.

Recently, bioactive edible coating (BEC) has gained much attention due to their edibility, biodegradability, biocompatibility, functional and barrier proper-

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ties (Rennie & Sunjka, 2018; Sánchez-González et al., 2011). In precise, it acts as a natural preservative and carrier of food additives, active antimicrobial and antioxidant agent to prolong the product's shelf life (Fernández-Pan et al., 2014; Merino et al., 2019; Liu et al., 2020). Furthermore, edible coatings enrich the nutritional and sensory quality of the products. Generally, edible coatings are developed using polysaccharide, protein and lipid sources or their combinations. Among that, protein based edible coatings have better advantage due to superior mechanical properties, which will reduce the oxygen and CO_2 transfer during storage (Sánchez-Ortega et al., 2014). During seafood processing, nearly 30-50 % of seafood waste consisting of head, skin, viscera and bone etc. is generated that contain high amount of protein, fat and minerals (Jayathilakan et al., 2012). Extraction of protein from seafood waste could be an option for industries to solve the waste disposal and environmental pollution problems.

Fish protein hydrolysate (FPH) is one of the high value fishery products and can be produced from edible portion or processing discards from seafood. Fish Protein hydrolysates can be used as coating material, which will improve the nutritional value with better antioxidant, functional and bioactive properties (Henriques et al., 2021; Yathisha et al., 2022). Adding essential oil to edible coatings is one of the innovative methods to improve the antimicrobial properties and sensory quality of the product (Fernández-Pan et al., 2012). However, the poor solubility, high volatility and thermal decomposition limit its applications in food packaging (Weiss et al., 2009). So, it needs to be encapsulated with other carrier materials to retain their functional properties and also to minimize their impact on the sensory characteristics of the food (Singh et al., 2021). Chitosan is another biopolymer used to develop edible coatings (Kanatt et al., 2008; Mohamed et al., 2013), due to its coating or film forming ability (Jeon et al., 2002), antioxidant and antibacterial properties (López-Caballero et al., 2005), barrier properties against gas and aroma, which makes it more suitable for BEC development (Caner, 2005). Several studies reported that chitosan-based coatings effectively prolong the shelf life of vegetables, fruits, seafood, and meat (Sagoo et al., 2002; Devlieghere et al., 2004; Fan et al., 2009; Kanatt et al., 2013). Encapsulating the clove oil using FPH and chitosan could be an effective technique for controllable release of active materials such as antimicrobial and antioxidant compounds with good stability and dispersibility. Moreover, the development of ready-to-use BEC powder will be much useful to processors, retailers and consumers to extend the food products' shelf life. With this background, the present work aimed to develop the FPH based ready to use or disperse BEC powder incorporated with clove oil and chitosan using encapsulation method (spray drying) and to determine their bio-preservation effect on mackerel fillets during storage at refrigerated condition (4 ± 1 ^{o}C).

Materials and Methods

Indian mackerel (*Rastrelliger kanagurta*) was purchased from local fish market, (Cochin, India) and transported to the research laboratory in iced condition. The weight and length of mackerel fishes were measured (188.66 ± 6.11 g and 16.16 ± 0.76 cm) before filleting for storage study. Clove essential oil was purchased from Hi-Media, Mumbai, India. Chitosan with deacetylation degree of 90 % and viscosity of 150-500 CP was bought from SRL, Mumbai, India. AR grade chemicals and reagents were used for this study.

Fish protein hydrolysate was prepared from pink perch head waste based on the method of Elavarasan & Shamasundar (2016). Initially, pink perch head waste was minced, mixed with distilled water (1:2, w/v) and then subjected to enzymatic hydrolysis by adding papain enzyme (0.5 %) on wet weight basis of pink perch head waste (w/w). During hydrolysis process, conditions were as follows: Enzyme to substrate ratio (E/S) of 0.63 %, pH of 6, incubation time of 90 min and temperature at 55 ^{o}C. Thereafter, the hydrolysis reaction was terminated by keeping the mixture in water bath at 85-90 ^{o}C for 15-20 min. Finally, the FPH solution was collected by filtering the mixture using Whatman filter paper.

To prepare the microencapsulated BEC powder, 1 % acetic acid (v/v) followed by 1 % chitosan (w/v) was added into FPH solution and stirred for 30 min to achieve complete dispersion. Thereafter, clove oil (1 %) (v/v), was incorporated into the solution and continued the stirring process for another 30 mins. Then clear BEC solution was spray-dried into powder form and stored for preservative study (Fig. 1).

For the storage study, mackerel fillets were dipped in the BEC solutions (10 % (w/v) and 20 % (w/v)) for 5 min and allowed to drain the excess solution

for 1 min. Then the BEC treated mackerel fillets were packed in sterile polyethylene bags and kept in refrigerated condition (4 ± 1 °C) to study the preservative effect. Samples without BEC solution treatment were kept as control. The samples from each group were randomly selected for microbial and biochemical analysis at predetermined time intervals (3 days).

Approximately, ten gram of mackerel fillet was homogenized aseptically with 90 mL of sterile normal saline (NS) (0.85 %). The resulting suspension was serially diluted in a tube containing sterile NS (1:10) and mixed thoroughly using vortex mixer. Further, spread plate technique was employed in plate count agar (Oxoid, UK). The mesophilic and psychrotrophic bacterial counts were determined after the incubation period of 24 hrs at 37 °C and 96 hrs at 4 °C, respectively. Finally, the bacterial colonies were counted and results were expressed in logarithmic average count values was (Log CFU/g).

pH values of samples were measured with a digital pH meter (Cyberscan 510, Singapore) by homogenizing 5 g of fish muscle in 25 mL of distilled water (1:5, w/v). The thiobarbituric acid (TBA) value was estimated according to Tarladgis et al. (1960) method. Total volatile base nitrogen (TVB-N) and trimethylamine (TMA) were analysed using micro diffusion method (Conway, 1962). Peroxide value (PV) was calculated according to AOAC method (2019).

Sensory evaluation of mackerel was carried out based on the 9-point hedonic scale method (Meilgaard et al., 1999). using the parameters such as colour, appearance, odour and overall acceptability of the product. Samples from each group were taken out randomly and evaluated by panellists throughout the storage. An overall acceptance score of 6 and above was considered acceptable. The overall acceptability for each groups were calculated and expressed as Mean ± Standard Deviation (n=3).

Results and Discussion

Variations in mesophilic bacterial counts of mackerel fillets coated with BEC solutions during storage are given in Fig. 2A. The initial mesophilic count of sample was 4.51 ± 0.37 log CFU/g, which indicates the freshness of mackerel fish. During the refrigerated storage, the mesophilic counts steadily increased in all the treatments and it was observed that bacterial growth was significantly lowered in samples treated with BEC solution than in the control samples (p<0.05). On 3rd day of chilled storage, mesophilic count got reduced due to the application BEC along with chilling effect. After 3 days of storage, the bacterial count started increasing and significant difference was observed between the control and the samples treated with BEC solutions. The control sample crossed the acceptable limit of 7 log CFU/g on 9th day of storage (7.32 ± 0.07 log CFU/g), whereas samples coated with 10 % and 20 % BEC solution exceeded the limit on 15th (7.02 ± 0.16 log CFU/g) and 18th (7.06 ± 0.15 log CFU/g) day of storage, respectively. This shows the effectiveness of BEC solution against bacterial growth during the refrigerated storage.

The psychrotrophic bacterial count is an important indicator for fish spoilage in refrigerated condition and their changes are shown in Fig. 2B. The initial bacterial count was 3.68 ± 0.25 log CFU/g and showed an increasing trend in all the samples during refrigerated storage. The psychrotrophic bacterial count in the control sample surpassed the upper limit of 7 log CFU/g on 9th day (7.03 ± 0.52 log CFU/g), while sample coated with 10 % and 20 % BEC solution reached to 7.23 ± 0.15 and 7.56 ± 0.16 log CFU/g on 15th and 18th day, respectively. The samples coated with BEC solutions have shown significant differences from the control sample from the third day onwards (p<0.05). Among the two BEC solutions, 20 % BEC solution showed better antibacterial effect against both mesophilic and psychrotrophic bacteria, which demonstrated the potential of BEC as a natural antimicrobial substance. A similar study reported that whey protein isolate edible coating added with oregano essential oil effectively reduced the mesophilic and psychrotrophic bacteria in chicken (Fernández-Pan et al., 2014). Other studies also revealed the effectiveness of bioactive edible coatings, when combined with chitosan and/or essential oils on mackerel (Sathish et al., 2015; Kumuda, et al., 2018; Li et al., 2019).

pH value indicates the acidic and alkaline condition of sample and also displays the freshness of fish samples after death. Fig. 3A showed the variations in the pH of mackerel fillets during storage. The initial pH of mackerel sample was 6.38 ± 0.01, which was similar to the previous reports (Li et al., 2019) and the pH of BEC was 5.23. The pH of BEC treated samples showed an initial decrease until day 3,

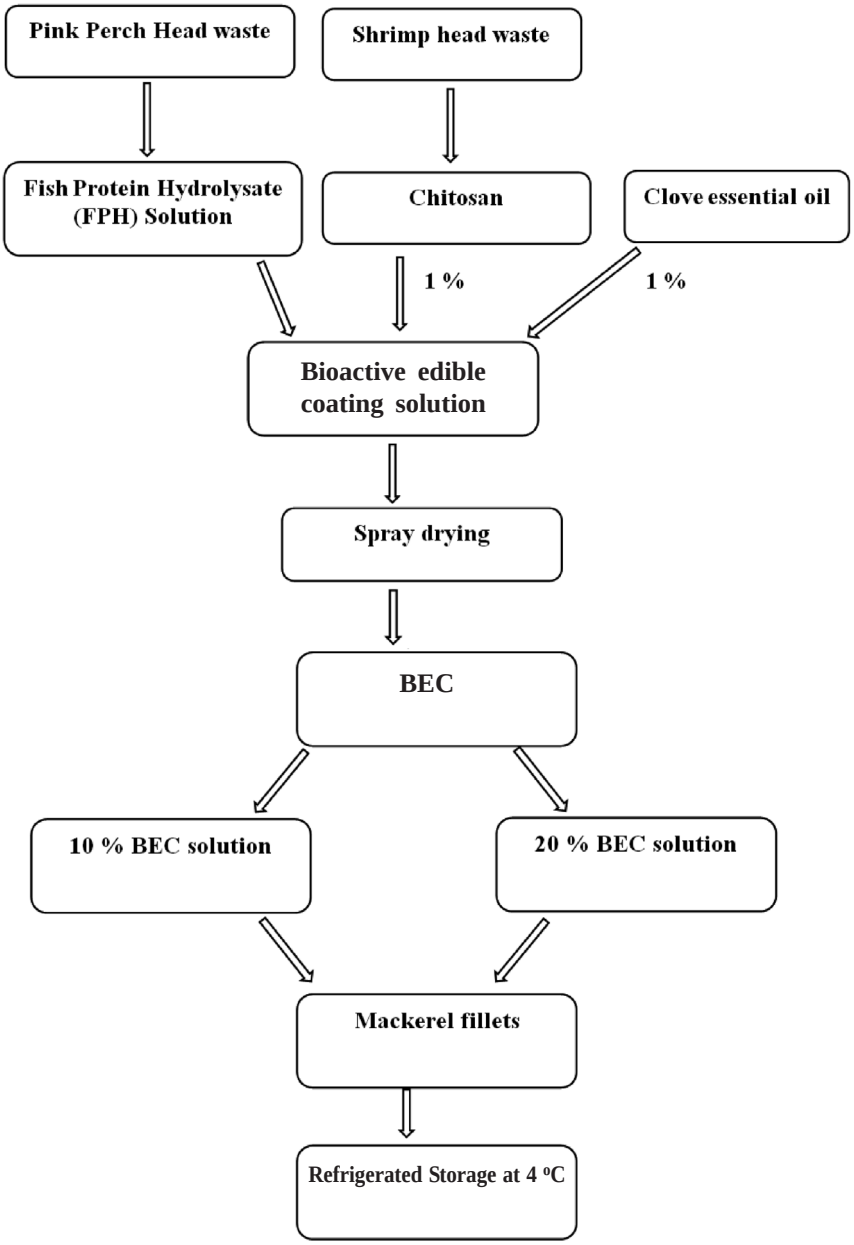

Fig. 1. Schematic diagram of BEC powder preparation and application for the storage study

which may be due the presence of acetic acid used for dissolving chitosan in the coating solutions and also the dissolution of CO_2 or lactic acid accumulation (Yu et al., 2017), while control sample showed an increasing trend. pH values were significantly different between the control and BEC treated samples from day 3 onwards (p<0.05). After the initial drop in the BEC coated samples, pH value steadily increased during chilled storage period, which may be due to the action of microorganisms and endogenous enzymes present in the fish (Ramezani et al., 2015; Li et al., 2019). Interestingly, the pH value of control sample increased dynamically to 8.26 ± 0.005 on 9th day of storage while 10 % and 20 % BEC treated samples crossed the pH above 8 on 15th and 18th day of storage, respectively. These results suggested that, BEC coating effectively minimized the spoilage bacterial growth and preserved the mackerel quality by minimizing the decomposition rate.

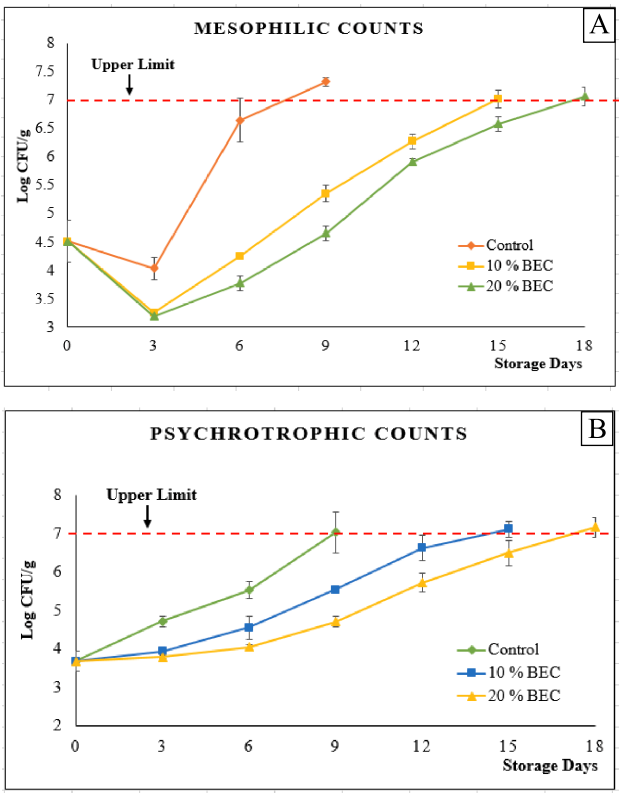

Fig. 2. Changes in the mesophilic (A) and psychrotrophic (B) bacterial counts in mackerel fillets treated with different concentrations of BEC coatings during chilled storage

TBARS value measures the oxidative rancidity in fishery products and the changes in mackerel fillets during storage are plotted in Fig. 3B. The initial TBARS value of mackerel fillet was 0.034 ± 0.005 mg MDA eq/kg and the value steadily increased, as the storage period progressed. TBARS value of control sample was significantly (p<0.05) higher than the sample coated with BEC solutions, which exceeded the spoilage limit of 1–2 mg MDA/kg on 12th day of storage (2.22 ± 0.12 mg MDA/kg). The TBARS values of 10 % and 20 % BEC solution treated samples gradually increased and did not cross the limit during the 18-day storage. The results revealed that, the BEC solutions delayed lipid oxidation commendably during storage. In particular, chitosan present in the BEC solution reduced the oxygen permeability and therefore controlled the rate of lipid oxidation.

TVB-N is another method to measure the index of spoilage in seafood (Liu et al., 2016) and the changes in TVB-N during the storage are displayed in Fig. 3C. The initial TVB-N value was 3.65 mg-N/100g, which showed the freshness condition of the fish. Later, value of control sample increased significantly (p<0.05) and exceeded the recommended level of 30–35 mg TVB-N/100g of muscle, on 12th day of storage (40.02 ± 1.35 mg-N/100g, while samples coated with 10 % and 20 % BEC solutions almost touched the limit on 15th (35.38 ± 0.83 mg-N/100g) and 18th (34.01 ± 1.45 mg-N/100g) day of storage, respectively. The study demonstrated the overall tendency of BEC solutions to slow down bacterial growth during chilled storage, which confirms the direct relationship between the TVB-N content and microbial growth of mackerel fillets.

TMA values of control and BEC solution coated samples during chilled storage are shown in Fig. 3D. The Initial TMA values of all the sample were 1.58 ± 0.09 mg-N/100g and showed an increasing tend during the storage period. A significant difference was noted between the control and BEC solution coated samples (p<0.05). The TMA value of control sample steadily increased and crossed the recommended level of acceptance (10 - 15 mg-N/100g) on 12th day of storage (19.14 ± 1.60 mg-N/100g) whereas the samples treated with 10 % and 20 % BEC solutions exceeded the limit on day 15 (18.38 ± 0.53 mg-N/100g and day 18 (16.69 ± 0.51 mg-N/100g) respectively. It can be concluded that BEC solutions effectively minimized the accumulation of TMA in fish muscle during storage as compared with control samples.

Peroxide value of BEC coated fillets increased significantly during the course of refrigerated storage (p<0.05), as represented in Fig. 3E. The initial PV of mackerel fillet was 1.84 ± 0.08 meq O_2/kg and increased gradually as the storage days progressed, which was also stated by Sofi (2015). The value of control sample increased rapidly and exceed the acceptable limit of 5–8 meq of O_2/kg (Ludorff & Meyer, 1973) on day 12 (8.14 ± 0.05 meq O_2/kg). While the mackerel fillets coated with 10 % and 20 % BEC solutions increased from 0.78 ± 0.03 to 7.71 ± 0.48 and 7.42 ± 0.16 meq O_2/kg lipids respectively on 15th and 18th day of storage. The reason might be the presence of chitosan, which act as an oxygen barrier material and helps in reducing hydroperoxide production (Coban & Pelin Can, 2013). Furthermore, the clove oil present in the BEC solution might have scavenged the free radicals and hindered the production of secondary lipid oxidation products during the storage. Therefore, the

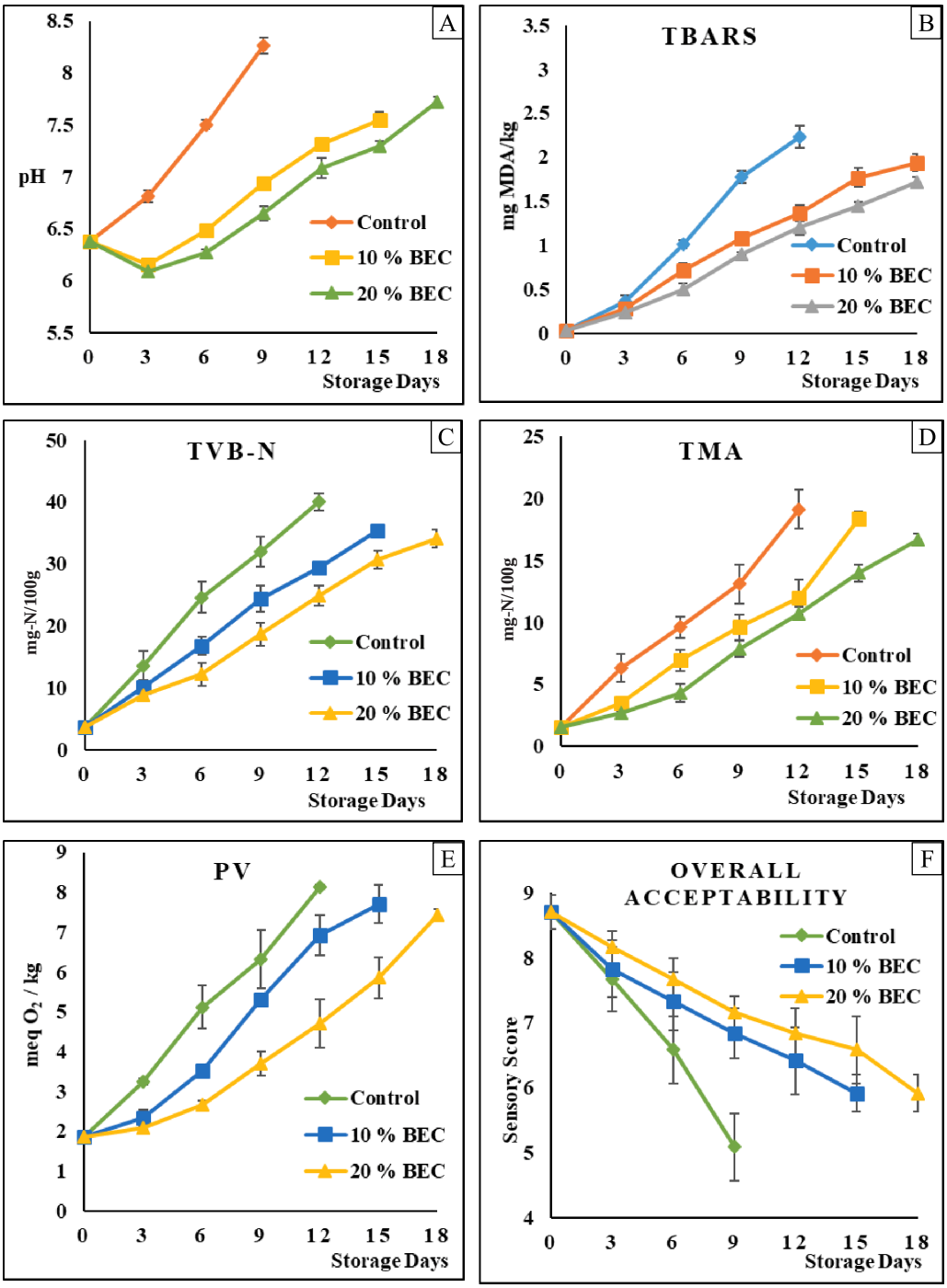

Fig. 3. Changes in the biochemical quality parameters such as (A) pH, (B) TBARS (C) TVB-N (D) TMA (E) PV and (F) Overall acceptability of mackerel fillets treated with different concentrations of BEC coatings during refrigerated storage

incorporation of clove essential oil and chitosan in protein hydrolysate based BEC powder played a major role in minimizing the peroxide generation during storage.

The overall acceptability score of mackerel fillets dipped in BEC solutions and stored in refrigerated conditions is depicted in Fig. 3F. There was a significant gradual decrease in sensory score of all

samples during chilled storage (p<0.05) and similar results was observed by Kumuda et al. (2018). The initial sensory score of mackerel fillets was 8.70 ± 0.25 and significant differences between the samples were observed from 3rd day onwards. Control samples exceeded the acceptable limit on 9th day of storage (<6.00) due to odour development and colour changes. While the sample treated with 10 % and 20 % BEC solutions crossed the acceptable limit on 15th (5.91 ± 0.28) and 18th (5.83 ± 0.38) day of chilled storage, respectively. These results were coinciding with changes in microbial and biochemical quality and hence it can be suggested that the BEC solutions effectively extended the shelf life of mackerel fillets as compared with control samples.

Development of BEC with the combination of two or more materials will provide better protection or extend the shelf life of food products than a single material. In this study, it was found that fish protein hydrolysate based BEC powder incorporated with chitosan effectively prolonged the shelf life of mackerel fillets by controlling bacterial growth during storage. Similarly, adding clove oil had more significant effect in maintaining biochemical and sensory qualities of mackerel fillets by minimizing the lipid oxidation. Thus, our study demonstrated the effectiveness of BEC powder, which holds great potential to be used as a natural food-grade bio-preservative in food industries and offered a promising way for preserving the mackerel fillets.

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