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# IMPLEMENTATION OF CHITOSAN COATING FOR PRESERVATION OF DRIED SPINY EEL (*MASTACEMBELIDAE*)

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## Abstract

Lipid oxidation and proteolytic activities accounts for major changes in spiny eel (*Mastacembelidae*). Edible coatings are a promising preservation technology for dried fish because they provide good barrier against spoilage and pathogenic microorganisms, limit the lipid oxidation. The films gas barrier properties contribute to extended shelf life because physicochemical changes, such as color, texture, and moisture, may be significantly minimized. Objective of the current research studied the feasibility of chitosan coating in preservation of dried spiny eel (*Mastacembelidae*). The dried spiny eels were treated by different concentration of chitosan (1.0%, 1.5%, 2.0%, 2.5%, 3.0%). The effectiveness of chitosan coating was based on quality changes of dried spiny eels such as lipid oxidation: Peroxide value (mEqO2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg); proteolytic changes: total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g). All treated samples were monitored during 12 months of storage at ambient temperature in 3 months of interval sampling. Results revealed that the incorporation of 2.5% w/w chitosan could control microbial spoilage and lipid oxidation. By this approach, the dried spiny eels (*Mastacembelidae*) are stable at normal environment for 12 months. The study indicated that the edible coating could be commercially utilized to prolong the shelf-life of the dried oil fishes. The improvement in the quality of the seafood products is achieved through inhibition of microbial growth, reduction of lipid oxidation reduction and enhancement of sensorial attributes.

***Keywords:*** *Mastacembelidae, chitosan, microbial spoilage, lipid oxidation, shelf-life*

## Introduction

Spiny eel (*Mastacembelidae*) is a tropical fish founding in the rivers of Southeast Asia. Coming from the natural resource, it flesh has valuable nutrients. Spiny eel (*Mastacembelidae*) has been recorded to reach a maximum length of 30 centimetres (12 in). Spiny eel (*Mastacembelidae*) is a freshwater species, generally found at the bottoms of bodies of water. It is found in the rivers of Southeast Asia, including the Mekong, Chao Phraya, and Mae Klong. During the day, Spiny eel (*Mastacembelidae*) buries itself in the river bottom, coming out at night to feed on insects, crustaceans, and worms. Spiny eel (*Mastacembelidae*) is a distinct group of percomorph fishes restricted to fresh waters (Tyson R Roberts, 1986). Spiny eel (*Mastacembelidae*) is distinguished from all other congeners by the following combination of characters: lack of rostral tooth plates, dorsal-fin spines 15-19, dark spots like imperfect ocelli along the base of dorsal soft branched fin rays 7-11, dark blotches at the mid-lateral sides of body 22-27, dorsal fin rays with two rows of parallel greyish streaks, caudal fin rays with 4-6 striated greyish streaks and body width 59.0- 67.4% of its depth (Arunkumar L, 2016). The genetic variation of spiny eel (*Mastacembelidae*) was influenced by immigration and isolation events as well as by environmental factors (AP Takagi *et al*., 2011). A research focused on the processing of spot fin spiny eel into the curcumin-dry-salted product by salting and drying method. With addition of 0.75% of curcumin, drying at 50ºC in 4 hours, the curcumin-dry-salted spotfin spiny eel products had the good physicochemical, microbiological and sensory characteristics. By preserving under vacuum at 4ºC, the curcumin-dry-salted spotfin spiny eel could be maintained shelf-life for 12 months without any deterioration (Nguyen Phuoc Minh *et al*., 2018)

Chitosan is a polysaccharide obtained from the alkaline hydrolysis of N-acetyl group of chitin, 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; Shahidi *et al*., 1999). The use of edible coatings could have a beneficial effect on the preservation of seafood products, since they function as a barrier against moisture and oxygen penetration (Pereira de Abreu *et al*., 2012). 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 (Duan *et al*., 2010; Fan *et al*., 2009; Li *et al*., 2013; Ojagh *et al*., 2010). Chitosan offers the possibility of obtaining coatings to cover fresh or processed foods to extend their shelf-life, being an excellent film-forming material showing antifungal and antimicrobial activity due to its polycationic nature (Aider, 2010). The mechanism of the antimicrobial activity. s based on: (i) interactions by electrostatic forces between chitosan amine groups and microbial cell membranes; (ii) the action of chitosan as a chelating agent; (iii) the penetration of low molecular weight chitosan molecules through the cell membrane; or (iv) modifications on cell surfaces that may affect the integrity of the microbial cell membrane interfering with energy metabolism and nutrient transport in bacteria cells (Elsabee and Abdou, 2013).

Rancidity is a problem in oily fish associated primarily with frozen and dried storage. Indeed, the shelf-life of frozen oily fish usually ends with the onset of rancid flavors. In canned fish the total elimination of $O_2$ during processing is sufficient to give these products a shelf-life of many years. In the living animal the ingestion and regeneration of antioxidants prevents excessive oxidative deterioration of important biological components. Post-mortem, the protective systems become depleted and are unable to regenerate themselves. Thus, the edible muscle tissues of fish are liable to react with $O_2$ in the presence of air. Generally the preliminary products of fatty acid oxidation (lipid hydroperoxides) do not have a flavor impact and are measured as peroxide value. Volatile secondary oxidation products derived from the breakdown of these lipid

hydroperoxides are believed to lead to rancid flavors and aromas. At the same time, an increase in free fatty acid (FFA) lipolysis resulting from the enzymatic hydrolysis of esterified lipids also occurs in fish tissue post-mortem (Bremner, 2002). The rate of hydroperoxide formation correlates with lipid oxidation in its early stages. Aldehydes, ketones and similar compounds are the secondary products which form as the hydroperoxides react. The reactions lead to aldehydes and other products that can be measured using the thiobarbituric acid (TBARS) test (Rodriguez-Turienzo *et al*., 2011).

Fish processing, especially freezing, has a significant impact on final product quality attributes which are mostly associated with changes in chemical composition and the degradation of muscle proteins. Functional properties of fish proteins and sensory quality, such as loss of protein solubility, emulsifying capacity, waterbinding capacity, thaw drip, and texture scores are mostly affected by postharvest handling and the method of preservation. Due to the action of enzymes present in fish products or microbial activities nitrogen compounds such as trimethylamine-N-oxide (TMAO) are degraded to ammonia, formaldehyde and trimethylamine (measured as TMA-N). These may cause protein aggregation, thus reducing the proteins' ability to bind water (Barraza *et al*., 2015). At death, the pH value begins to decrease due to formation of lactic acid from glycogen by a series of enzymatic reaction in the tissues. Certain critical enzymes, particularly phosphofructokinase, are inhibited and pH drops as pyruvate is shunted to lactic acid. This triggers the release of proteolytic enzymes like cathepsins. Enzymes from spoilage microorganisms produce a wide variety of volatile compounds causing off-flavors. The combined total amounts of ammonia ($NH_3$) and TMA in fish is measured as the total volatile base (TVB-N) nitrogen content of the fish and is commonly used as an estimate of spoilage. With the increase of spoilage bacteria after death in fish, a subsequent increase in TMAO reduction to TMA take places. On the other hand, the increase in the TVB-N is mainly caused by the formation of TMA, which is prevalent in spoiled fish that have TMAO (mainly in marine pelagic fish) and is the most common cause of fishy odor. Aeromonas spp., psychrotolerant Enterobacteriaceae, Photobacterium phosphoreum, Shewanella putrefaciens-like organisms and Vibrio spp. are the bacteria that are able to reduce TMAO to TMA (Heising *et al*., 2014).

There were several notable researches mentioned to application of chitosan coating in fishes to control oxidation and proteolytic change. The effect of chitosan–gelatin coating and film on the rancidity development in rainbow trout fillets during refrigerated storage (4 ± 1 C) was examined over a period of 16 days. The results indicated that chitosan–gelatin coating and film retained their good quality characteristics and extended the shelf life of fish samples during refrigerated storage .The coating was better than the film in reducing lipid oxidation of fillets (Nowzari et al., 2013). Vacuum packaging with chitosan-based edible films significantly reduced trimethylamine and total volatile basic nitrogen and growth of total mesophilic and total psychrophilic aerobic bacterial counts (P ⌋ 0.05) during cold storage at 4 C. Prolonging in shelf life by about 20 days was observed (Günlü & Koyun, 2013). Chitosan coating significantly (P ⌋ 0.05) reduced lipid oxidation as displayed in peroxide value, conjugated dienes, 2-thiobarbituric acid reactive substances and headspace volatiles, chemical spoilage as reflected in total volatile basic nitrogen, trimethylamine, and hypoxanthine, and growth of microorganisms as reflected in total plate count in both fish model systems compared to uncoated samples (Jeon *et al*., 2002). Chitosan coating was effective in inhibiting bacterial growth and reduced the formation of volatile bases and oxidation products significantly in Indian oil sardine. The chitosan coating improved the water holding capacity, drip loss and textural properties significantly (Mohan *et al*., 2012). Qiu *et al*. (2014) also showed that citric acid can enhance the preserving function of chitosan significantly by slowing lipid oxidation and limiting microbial growth using thiobarbituric acid reactive substances and total plate count, respectively (Qiu *et al*., 2014). However there was not any research mentioned to the investigation of chitosan coating to the dried spiny eels (*Mastacembelidae)*. The aim of this current work was to study the application of chitosan coating in maintaining product quality of dried spiny eels (*Mastacembelidae)* stored at ambient temperature.

## Material and Method

### Material

Spiny eel (*Mastacembelidae)* fishes were naturally collected from My Xuyen district, Soc Trang province, Vietnam. After collecting, they must be kept in ice chest below 4$^o$C and quickly transferred to laboratory for experiments. They were washed and sanitized under washing tank having 30 ppm chlorine with a support of air bubble blowing to remove foreign matters. Besides black tiger shrimps we also used other material during the research such as chlorine, salt, whey protein isolate, cacboxymethyl cellulose, corn protein, gelatin. Lab utensils and equipments included digital weight balance, Rotronic, stomacher, incubator, colony counter, and dry oven.

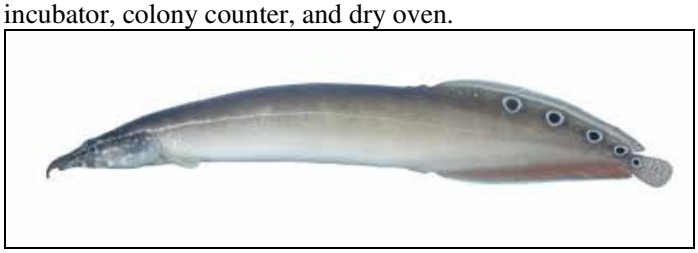

**Fig. 1 :** Spiny eels (*Mastacembelidae*)

### Researching Method

**(i) Lipid oxidation of dried spiny eels (Mastacembelidae) by chitosan coating during preservation**

Raw spiny eels (*Mastacembelidae)* were treated with 3.0% salt, 1.5% sugar, 0.1% monosodium glutamate, 0.3% garlic extract, 0.3% turmeric powder in 20 minutes. After that they were dried at 50$^o$C to 11% moisture content. Different concentrations of chitosan were examined (1.0%, 1.5%, 2.0%, 2.5%, 3.0%) were examined on the dried spiny eels. Lipid oxidation was estimated by Peroxide value ($mEqO_2$/ kg), Thiobarbituric acid (mg maloaldehyde/ kg). All treated samples were monitored during 12 months of storage at ambient temperature in 3 months of interval sampling.

**(ii) Proteolytic changes of dried spiny eels (Mastacembelidae) by chitosan coating during preservation**

Raw spiny eels (*Mastacembelidae)* were treated with 3.0% salt, 1.5% sugar, 0.1% monosodium glutamate, 0.3% garlic extract, 0.3% turmeric powder in 20 minutes. After that they were dried at 50$^o$C to 11% moisture content. Different concentrations of chitosan were examined (1.0%, 1.5%, 2.0%, 2.5%, 3.0%) were examined on the dried spiny

eels. Proteolytic change was estimated by the total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g). All treated samples were monitored during 12 months of storage at ambient temperature in 3 months of interval sampling.

**Physico-chemical, microbial and sensory evaluation of dried shrimp during storage**

Peroxide value ($mEqO_2$/ kg) was determined using the CDR FoodLab® instrument. Thiobarbituric acid (mg maloaldehyde/ kg) was measured by 1,1,3,3-tetraethoxypropane (Torres-Arreola *et al*., 2007). Standard methods recommended by Food and Agriculture Organization of the United Nations (FAO, 1986) were used for the determination of total volatile base (TVB-N, mg N/100 g) and trimethylamine (TMA, mg N/100 g).

**Statistical Analysis**

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT). Statistical analysis was performed by the Statgraphics Centurion XVI.

## Result and Discussion

**Lipid oxidation of dried spiny eels (*Mastacembelidae*) by chitosan coating during preservation**

Raw spiny eels (*Mastacembelidae)* were treated with 3.0% salt, 1.5% sugar, 0.1% monosodium glutamate, 0.3% garlic extract, 0.3% turmeric powder in 20 minutes. After that they were dried at 50°C to 11% moisture content. Different concentrations of chitosan were examined (1.0%, 1.5%, 2.0%, 2.5%, 3.0%) were examined on the dried spiny eels. Lipid oxidation was estimated by Peroxide value ($mEqO_2$/ kg), Thiobarbituric acid (mg maloaldehyde/ kg). All treated samples were monitored during 12 months of storage at ambient temperature in 3 months of interval sampling. Results from table 1 and 2 revealed that 2.5% chitosan coating could control rancidity in dried spiny eels during 12 month storage

**Table 1** : Peroxide value ($mEqO_2$/ kg), Thiobarbituric acid (mg maloaldehyde/ kg) in dried spiny eels (*Mastacembelidae*) by different concentration of chitosan coating (1.0%, 1.5%, 2.0%, 2.5%, 3.0%) after 3 months of storage

| Chitosan concentration | Peroxide value ($mEqO_2$/ kg) | Thiobarbituric acid (mg maloaldehyde/ kg) |
|---|---|---|
| **Control** | $1.12\pm0.01^{a}$ | $0.75\pm0.02^{a}$ |
| **1.0%** | $0.74\pm0.03^{b}$ | $0.46\pm0.01^{b}$ |
| **1.5%** | $0.54\pm0.00^{bc}$ | $0.33\pm0.00^{bc}$ |
| **2.0%** | $0.35\pm0.02^{c}$ | $0.21\pm0.02^{c}$ |
| **2.5%** | $0.24\pm0.01^{cd}$ | $0.13\pm0.01^{cd}$ |
| **3.0%** | $0.22\pm0.03^{d}$ | $0.12\pm0.00^{d}$ |

*Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).*

**Table 2 :** Peroxide value ($mEqO_2$/ kg), Thiobarbituric acid (mg maloaldehyde/ kg) in dried spiny eels (*Mastacembelidae*) by 0.25% chitosan coating during preservation

| Storage (months) | Peroxide value ($mEqO_2$/ kg) | Thiobarbituric acid (mg maloaldehyde/ kg) |
|---|---|---|
| **0** | $0.17\pm0.00^{c}$ | $0.05\pm0.02^{c}$ |
| **3** | $0.24\pm0.01^{bc}$ | $0.13\pm0.01^{bc}$ |
| **6** | $0.29\pm0.02^{b}$ | $0.18\pm0.00^{b}$ |
| **9** | $0.33\pm0.03^{ab}$ | $0.22\pm0.03^{ab}$ |
| **12** | $0.38\pm0.00^{a}$ | $0.25\pm0.02^{a}$ |

*Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).*

Rodriguez-Turienzo *et al*. (2011) indicated that whey proteinbased coatings delayed lipid oxidation of salmon fillets measured using both peroxide values and TBARS. They were helpful with frozen fish with low or high fat including retaining the fish sensory properties sensitive to lipid oxidation (Rodriguez-Turienzo *et al*., 2011). Oxidation rates have been reduced by using different antioxidant (e.g., vitamin C and tea polyphenols) in coatings (Song *et al*., 2011). The previously discussed work of Li *et al*. (2013) and Duan *et al*. (2010) are examples of this. The TBARS values after the addition of cinnamon leaf essential oil, vitamin E and other natural antioxidants (i.e., grape seed extracts and tea polyphenols) in chitosan coatings did not provide significant additional antioxidant effects (P − 0.05). The chitosan coatings and packaging might have been sufficient to retard lipid oxidation in lingcod (Ophiodon elongates) fillets (Duan, Jiang *et al*., 2010). The work of Kim *et al*. (2012) with DMM showed that it had sufficient antioxidant activity to lower TBARS.

**Proteolytic changes of dried spiny eels (*Mastacembelidae*) by chitosan coating during preservation**

Raw spiny eels (*Mastacembelidae)* were treated with 3.0% salt, 1.5% sugar, 0.1% monosodium glutamate, 0.3% garlic extract, 0.3% turmeric powder in 20 minutes. After that they were dried at 50°C to 11% moisture content. Different concentrations of chitosan were examined (1.0%, 1.5%, 2.0%, 2.5%, 3.0%) were examined on the dried spiny eels. Proteolytic change was estimated by the total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g). All treated samples were monitored during 12 months of storage at ambient temperature in 3 months of interval sampling. Results from table 3 and 4 revealed that 2.5% chitosan coating could control proteolytic change in dried spiny eels during 12 month storage.

**Table 3 :** Total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g) in dried spiny eels (*Mastacembelidae*) by different concentration of chitosan coating (1.0%, 1.5%, 2.0%, 2.5%, 3.0%) after 3 months of storage

| Chitosan concentration | TVB-N (mg N/100 g) | TMA (mg N/100 g) |
|---|---|---|
| **Control** | 47.49±0.23$^{a}$ | 35.21±0.15$^{a}$ |
| **1.0%** | 36.12±0.07$^{b}$ | 20.37±0.07$^{b}$ |
| **1.5%** | 31.20±0.03$^{bc}$ | 17.23±0.04$^{bc}$ |
| **2.0%** | 28.40±0.02$^{c}$ | 13.57±0.02$^{c}$ |
| **2.5%** | 25.16±0.03$^{cd}$ | 10.21±0.01$^{cd}$ |
| **3.0%** | 23.85±0.01$^{d}$ | 9.59±0.02$^{d}$ |

*Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).*

**Table 4 :** Total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g) in dried spiny eels (*Mastacembelidae*) by 0.25% chitosan coating during preservation

| Storage (months) | TVB-N (mg N/100 g) | TMA (mg N/100 g) |
|---|---|---|
| **0** | 12.38±0.00$^{c}$ | 6.71±0.04$^{c}$ |
| **3** | 25.16±0.03$^{b}$ | 10.21±0.01$^{b}$ |
| **6** | 26.34±0.01$^{ab}$ | 11.39±0.00$^{ab}$ |
| **9** | 27.15±0.02$^{ab}$ | 11.86±0.03$^{ab}$ |
| **12** | 27.52±0.04$^{a}$ | 12.03±0.02$^{a}$ |

*Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).*

Qiu *et al*. (2014) studied the effects of chitosan and different organic acid on fresh Japanese sea bass fillets. They showed that the TVB-N of the fish fillet samples increased with microorganism growth. Their growth decreased with the treatments and the TVBN also decreased (Qiu *et al*., 2014). Cai *et al*. (2015) evaluated the effects of e-polylysine (PL), sodium alginate (SA) and e-polylysine/sodium alginate (PLSA) treatments on the quality characteristics of Japanese sea bass (*Lateolabrax japonicus*) during refrigerated storage. They showed that the TVB-N of PL, SA and PLSA treatments were lower than that of the control during storage and the differences became more pronounced in the latter periods of storage ($P < 0.05$) (Cai *et al*., 2015). The effectiveness of edible chitosan coating on the quality changes of Indian oil sardines (*Sardinella longiceps*) was studied by Mohan *et al*. (2102). They used the volatile bases TMA-N and TVB-N as indices of spoilage. Untreated samples showed a significantly ($P < 0.05$) higher increase in TMA-N and TVB-N on day 5 than those samples treated with chitosan (Mohan *et al*., 2012).

## Conclusion

Consumption of spiny eels has increased during recent years as consumers have become more aware of its nutritional benefits and of the health concerns associated with other meat products. Edible coating provide a replacement and fortification of the natural layers at the product surfaces to prevent moisture losses, gas aromas and solute movements out of the food, while selectively allowing for controlled exchange of important gases. Biodegradability, barrier properties, biocompatibility, and edibility as well as being nontoxic and non-polluting are a few advantages of edible films and coatings over plastic packages. Chitosan films have been successfully applied as edible material in films and coatings for the quality preservation of different seafoods. The current research showed that 2.5% w/w chitosan was appropriated for coating the dried spiny eels for 12 months of storage at ambient temperature.

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