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Feasibility of Edible Coating, Storage Temperature and Packaging for Rancidity and Proteolytic Activity of Dry-Salted Snakeskin Gourami, *Trichopodus pectoralis*

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ABSTRACT

Snakeskin gourami (*Trichopodus pectoralis*) has been considered as a valuable and suitable species for breeding in fresh and brackish water regions in Vietnam. It has a high meat yield and favouritely consumed as dry-salted fish. However the dry-salted snakeskin gourami quality has gone down very fast owing to rancidity and proteolytic activity because it has high fat content and protease in its abdomen. It's necessary to have an appropriate processing and preserving approach to accelerate its commercial value in local and international markets. Edible coating supports a natural cover on the product surface to control weight loss, oil rancidity and solute movements. Biodegradability, edibility and barrier attributes are some benefits of edible coating superior to plastic bag. Storage temperature and packaging procedure affected the rancidity and proteolytic activity in the dry-salted snakeskin gourami (*Trichopodus pectoralis*). Purpose of this research demonstrated the efficacy of carrageenan coating on storage of dry-salted snakeskin gourami (*Trichopodus pectoralis*). The dried snakeskin gouramis were coated by various concentrations of carrageenan (0.5%, 0.75%, 1.0%, 1.25%, 1.5%). The efficacy of carrageenan coating was defined via quality indicators of dry-salted snakeskin gouramis such as fat rancidity: Peroxide value (mEq02/ 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). In 3 month-interval, all coated samples were periodically analyzed during 12 months of preservation at ambient condition. Results showed that the carrageenan coating of 1.25% w/w, packed in vacuum bag and stored at 4oC could avoid microbial decomposition and fat oxidation of the dry-salted snakeskin gouramis. From this investigation, the dry-salted snakeskin gouramis had shelf-life at ambient storage for 12 months without deterioration. The research implied that the edible coating would be ideal to extend the stability of this valuable oil fish by inhibition of proteolytic reaction, reduction of fat rancidity and enhancement of overall acceptance.

KEY WORDS: *TRICHOPODUS PECTORALIS*, CARRAGEENAN, PROTEOLYTIC REACTION, RANCIDITY, STABILITY.

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INTRODUCTION

Snakeskin gourami (Trichogaster pectoralis) is one of the most common fish in paddy fields and rivers of Vietnam. It lives in waters at low dissolved oxygen and high organic accumulation and feeds on zooplankton, crustaceans and insect larvae (Jafaryan et al., 2014). It achieves good maturity stages after 3 months of rearing. It has a high meat yield and is consumed as a dried fish in Vietnam. Farming area of snakeskin gourami has been opened dramatically in recent years offering attractive income for local farmers (Minh et al., 2019). The aquaculture sector contributes an important source of nutritious food for human consumption. Fish and fishery products are among the most important agricultural commodities (Bharda et al., 2017). This species is considered as an alternative species to shrimp farming in rainy season. They are good sources of proteins, macro-nutrients, minerals and some vitamins. The mineral elemental levels and vitamins of this species is a function of the availability preferential accumulation. Aquaculture of P. elongatus in the Ca Mau province (Vietnam) has developed rapidly during the past decade due to relatively high demand and high marketable value (Minh et al., 2019).

At death, the pH value in fish muscle begins to decrease due to formation of lactic acid from glycogen via a series of enzymatic reactions in the muscles. Enzymes from spoilage microorganisms produce different volatile compounds causing bad smell. The combination of ammonia (NH3) and TMA in fish is accounted for the total volatile base (TVB-N) nitrogen content of the fish and is normally considered as an indicator of quality decomposition. With the proliferation of spoilage bacteria after death in fish, a subsequent increase in TMAO reduction to TMA happens. On the other hand, the increase in the TVB-N is mainly created 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).

Rancidity is a matter in fatty fish related to the dried preservation. Indeed, the shelf-life of dried fatty fish usually ends with the onset of rancid flavors. The speed of hydroperoxide formation strongly relates to fat 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 evaluated using the thiobarbituric acid (TBARS) test (Turienzo et al., 2011). There were several notable researches mentioned to application of edible coating in fishes to control oxidation and proteolytic change. Whey proteinbased coatings delayed lipid oxidation of salmon fillets (Turienzo et al., 2011). The chitosan coatings and packaging might have been sufficient to retard lipid oxidation in lingcod (Ophiodon elongates) fillets (Duan et al., 2010). The effects of chitosan and different organic acid on fresh Japanese sea bass fillets were studied (Qiu et al., 2014). Quality characteristics of Japanese sea bass (Lateolabrax japonicus) during refrigerated storage were affected by e-polylysine, sodium alginate and e-polylysine/sodium alginate (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).

Several studies mentioned to the processing and preservation of snakeskin gourami. The effect of various salt concentrations and other soluble elements on the moisture content and water activity (aw) of dried snakeskin fish was studied (Muoi et al., 2008). The influence of sorbitol and ethanol on the water activity and quality changes of dried snakeskin fish was examined (Truc et al., 2009). Ethanol treatment to eliminate fishy ordor; addition of salt, sorbitol, as well as dry temperature that affected to to water activity (aw), microbial load (coliform, cfu/g), sensory score of dried snakeskin gourami (Trichogaster pectoralis) were thoroughly investigated. Shelf-life of the dried product was also evaluated during preservation (Minh et al., 2019).

In Vietnam, the snakeskin gourami P. elongatus is a high value species and has high potential for aquaculture in the Mekong Delta. P. elongatus aquaculture has developed rapidly to supply the high demand of domestic consumers. However there was not any research mentioned to the investigation of carrageenan coating to the dry-salted snakeskin gouramis (*Trichopodus pectoralis*) as well as the effect of storage temperature and packing procedure to oil rancidity and proteolytic activity. Purpose of this research was to demonstrate the feasibility of carrageenan coating, storage temperature and packaging procedure in prolonging product quality of dried snakeskin gouramis (*T. pectoralis*). Coatings can be considered as a barrier to protect against discoloration, degradation and oxidative rancidity.

MATERIAL AND METHODS

Material: Snakeskin gourami (*Trichopodus pectoralis*) were harvested from Ca Mau province, Vietnam. After harvesting, they were cooled below 4oC and moved to laboratory as soon as possible for further experiments. They were washed and sanitized under washing tank having 20 ppm per acetic acid with air bubble blowing to remove foreign matters. Besides snakeskin gourami we also used other material during the research such as per acetic, salt, carrageenan. Lab utensils and equipment included digital weight balance, Rotronic, stomacher, incubator, colony counter, and dry oven. Oil rancidity of dry-salted snakeskin gouramis was carried out by carrageenan coating during storage: They were soaked in 20 minutes with solution containing 4.0% salt, 1.0% sugar, 0.1% monosodium glutamate, 0.5% garlic extract, 0.5% honey. After that they were dried at 50°C to 18% moisture content. Various carrageenan concentrations were verified (0.5%, 0.75%, 1.0%, 1.25%, 1.5%) on

the dry-salted snakeskin gouramis. Oil rancidity was evaluated by Peroxide value (mEqO_2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg). All coated samples were periodically taken in 3 month-interval during 12 months of preservation at ambient temperature.

Proteolytic decomposition of dry-salted snakeskin gouramis by carrageenan coating during storage: Raw snakeskin gouramis (*Trichopodus pectoralis*) were soaked in 20 minutes with solution containing 4.0% salt, 1.0% sugar, 0.1% monosodium glutamate, 0.5% garlic extract, 0.5% honey. After that they were dried at 50oC to 18% moisture content. Various carrageenan concentrations were verified (0.5%, 0.75%, 1.0%, 1.25%, 1.5%) on the dry-salted snakeskin gouramis. Proteolytic decomposition was evaluated by the total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g). All coated samples were periodically taken in 3 month-interval during 12 months of preservation at ambient temperature.

Oil rancidity and proteolytic decomposition of dry-salted snakeskin gouramis was done by packaging during preservation: Raw snakeskin gouramis (*T. pectoralis*) were soaked in 20 minutes with solution containing 4.0% salt, 1.0% sugar, 0.1% monosodium glutamate, 0.5% garlic extract, 0.5% honey. After that they were dried at 50oC to 18% moisture content. 1.25% carrageenan coating and 4oC of storage were applied for all samples. Two packaging types (zipper, vacuum) were examined on the dry-salted snakeskin gouramis. Oil rancidity was estimated by Peroxide value (mEqO_2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg). Proteolytic decomposition was evaluated by the total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g). All coated samples were periodically taken in 3 month-interval during 12 months of preservation at ambient temperature. Chemical evaluation of dry-salted shrimp during storage: Peroxide value (mEqO_2/ kg) was evaluated by the CDR Food Lab® instrument. Thiobarbituric acid (mg maloaldehyde/ kg) was evaluated 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 utilized to determine 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. Statistical analysis was performed by the Statgraphics Centurion XVI.

RESULTS AND DISCUSSION

Oil rancidity of dry-salted *T. pectoralis* was carried out by carrageenan coating during storage. Seafood was highly perishable and has a short shelf-life. During storage many reactions occurred leading to changes in quality such as endogenous chemical and enzymatic reactions. The safety and shelf-life were related to the presence of food spoilage and pathogenic microorganisms. Edible coatings could improve the quality of fresh and frozen products by retarding microbial growth, reducing lipid oxidation and moisture loss, and functioning as a carrier of food additives (Dehghani et al., 2018). A barrier against both moisture and oxygen migration could be beneficial for seafood (Arfat et al., 2015; Li et al., 2013). Coatings could be used to provide physical protection to protect food products from mechanical damage, and from physical, chemical and microbiological activities (Min et al., 2005). Carrageenan was generally very hydrophilic, an anionic linear polysaccharide that is derived from red seaweed (Bourtoom, 2008). There were three kinds of carrageenan such as kappa, iota and lambda with different numbers and positions of sulfate groups on the galactose dimer (Lin et al., 2018).Carrageenan was formed by gelation through a process of moderate drying. After evaporation of solvent, the polysaccharide double helices will form a three-dimensional network, which subsequently forms a solid film (Karbowiak et al., 2007). It retarded moisture loss from the food products by adding additional moisture on the surface.

Table 1. Peroxide value (mEqO2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg), Total volatile base (TVB-N, mg N/100 g) nitrogen content and Trimethylamine (TMA, mg N/100 g) in dry-salted *Trichopodus pectoralis* by various carrageenan concentrations of coating (0.5%, 0.75%, 1.0%, 1.25%, 1.5%) after 3 months of storage

Carrageenan concentration	Peroxide value (mEqO_2/ kg)	Thiobarbituric acid (mg maloaldehyde/ kg)	TVB-N (mg N/100 g)	TMA (mg N/100 g)
Control	1.65±0.00^{a}	0.97±0.00^{a}	49.83±0.02^{a}	42.09±0.02^{a}
0.5%	0.89±0.02^{b}	0.54±0.01^{b}	37.69±0.01^{b}	31.65±0.01^{b}
0.75%	0.64±0.01^{bc}	0.41±0.03^{bc}	34.74±0.03^{bc}	28.74±0.00^{bc}
1.0%	0.51±0.03^{c}	0.32±0.02^{c}	30.48±0.02^{c}	26.57±0.03^{c}
1.25%	0.43±0.01^{cd}	0.29±0.00^{cd}	28.65±0.01^{cd}	24.12±0.00^{cd}
1.5%	0.38±0.00^{d}	0.25±0.03^{d}	27.17±0.00^{d}	22.04±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%).

In this research, raw snakeskin gouramis were soaked in 20 minutes with solution containing 4.0% salt, 1.0% sugar, 0.1% monosodium glutamate, 0.5% garlic extract, 0.5% honey. After that they were dried at 50oC to 18% moisture content. Various carrageenan concentrations were verified (0.5%, 0.75%, 1.0%, 1.25%, 1.5%) on the dry-salted snakeskin gouramis. Oil rancidity was evaluated by Peroxide value (mEqO_2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg). All coated samples were periodically taken in 3 month-interval during 12 months of preservation at ambient temperature. Results from table 1 and 2 emphasize that 1.25% carrageenan coating would be adequate to assure oil rancidity in dry-salted snakeskin gouramis at the lowest level during preservation.

Proteolytic decomposition of dry-salted snakeskin gouramis by carrageenan coating during storage: Owing to the reaction of internal enzymes existing in fish products or microbial activities, nitrogen compounds such as trimethylamine-N-oxide (TMAO) are decomposed 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). Raw snakeskin gouramis were soaked in 20 minutes with solution containing 4.0% salt, 1.0% sugar, 0.1% monosodium glutamate, 0.5% garlic extract, 0.5% honey. After that they were dried at 50oC to 18% moisture content. Various carrageenan concentrations were verified (0.5%, 0.75%, 1.0%, 1.25%, 1.5%) on the dry-salted snakeskin gouramis. Proteolytic decomposition was evaluated by the total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g). All coated samples were periodically taken in 3 month-interval during 12 months of preservation at ambient temperature.

Results from table 3 and 4 emphasized that storage at temperature 4oC could slow down the oil rancidity in dry-salted snakeskin gouramis to utmost level during 12 months of preservation. Minh et al. (2019) proved that 40% ethanol at ratio 20:80 for primary treatment; 2.0% of salt soaking; 1.0% of sorbitol addition; 46oC of drying were appropriate to maintain water activity (aw=0.65).

Oil rancidity and proteolytic decomposition of dry-salted *T. pectoralis* by packaging procedure during preservation: The edible muscle tissues of fish are liable to react with O2 in the presence of air. An increase in free fatty acid (FFA) lipolysis resulting from the enzymatic hydrolysis of esterified lipids also occurred in fish tissue post-mortem (Dehghani et al., 2018). Raw snakeskin gouramis (*T. pectoralis*) were soaked in 20 minutes with solution containing 4.0% salt, 1.0% sugar, 0.1% monosodium glutamate, 0.5% garlic extract, 0.5% honey. After that they were dried at 50oC to 18% moisture content. 1.25% carrageenan coating and 4oC of storage were applied for all samples. Two packaging types (zipper, vacuum) were examined on the dry-salted snakeskin gouramis. Oil rancidity was estimated by Peroxide value (mEqO_2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg). Proteolytic decomposition was evaluated by the total volatile base (TVB-N, mg N/100 g) nitrogen content and trimethylamine (TMA, mg N/100 g). All coated samples were periodically taken in 3 month-interval during 12 months of preservation at ambient temperature. Results from table 5 and 6 strongly emphasized that vacuum packaging could effectively limit the oil rancidity and proteolytic decomposition in dry-salted snakeskin gouramis during 12 month storage.

Drying enhanced fish quality by inactivating enzymes and decrease water activity to prevent bacterial and mold proliferation. Fatty fish cannot be dehydrated by normal drying procedure, and is not possible to preserve it in the normal way. Fat rancidity was one of the most obstacles in the dry-salted fish (Minh et al., 2018). This phenomenon negative affected the taste, odor and color of dry-salted fish. One method to prolonging the stability and quality of dry-salted fish was vacuum packaging. It is an effective strategy to slow down the lipid oxidation by limiting oxygen molecule (Taheri and Motellabi 2012). By preserving under vacuum in PA bag, the dry-salted snakeskin gourami still extended the product shelf-life for 12 months without any deterioration (Minh et al., 2019).

Table 2. Peroxide value (mEqO2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg), Total volatile base (TVB-N, mg N/100 g) nitrogen content and Trimethylamine (TMA, mg N/100 g) in dry-salted *T. pectoralis* by 1.255% carrageenan coating during 12 months of preservation

Storage (months)	Peroxide value (mEqO2/ kg)	Thiobarbituric acid (mg maloaldehyde/ kg)	TVB-N (mg N/100 g)	TMA (mg N/100 g)
3	0.43 ± 0.01^{b}	0.29 ± 0.00^{b}	28.65 ± 0.01^{b}	24.12 ± 0.00^{b}
6	0.49 ± 0.02^{ab}	0.32 ± 0.03^{ab}	29.04 ± 0.00^{ab}	24.38 ± 0.01^{ab}
9	0.53 ± 0.03^{ab}	0.35 ± 0.02^{a}	29.11 ± 0.02^{ab}	24.43 ± 0.03^{ab}
12	0.58 ± 0.01^{a}	0.36 ± 0.01^{a}	29.13 ± 0.01^{a}	24.45 ± 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%).

Table 3. Peroxide value (mEqO2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg), Total volatile base (TVB-N, mg N/100 g) nitrogen content and Trimethylamine (TMA, mg N/100 g) in dry-salted *T. pectoralis* by different concentration of preservation temperature (4°C, 12°C, 20°C, 28°C) after 3 months of preservation

Preservation temperature (°C)	Peroxide value ($mEqO_2$/ kg)	Thiobarbituric acid (mg maloaldehyde/ kg)	TVB-N (mg N/100 g)	TMA (mg N/100 g)
4	0.04 ± 0.00^{c}	0.05 ± 0.02^{c}	19.83 ± 0.03^{c}	17.63 ± 0.02^{c}
12	0.12 ± 0.01^{b}	0.14 ± 0.01^{b}	27.46 ± 0.02^{b}	21.08 ± 0.03^{b}
20	0.37 ± 0.03^{ab}	0.21 ± 0.00^{ab}	27.94 ± 0.01^{ab}	23.79 ± 0.01^{ab}
28	0.43 ± 0.01^{a}	0.29 ± 0.00^{a}	28.65 ± 0.01^{a}	24.12 ± 0.00^{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%).

Table 4. Peroxide value (mEqO2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg), Total volatile base (TVB-N, mg N/100 g) nitrogen content and Trimethylamine (TMA, mg N/100 g) in dry-salted *T. pectoralis* by preservation temperature (4°C) during 12 months of preservation

Storage (months)	Peroxide value (mEqO2/ kg)	Thiobarbituric acid (mg maloaldehyde/ kg)	TVB-N (mg N/100 g)	TMA (mg N/100 g)
3	0.04 ± 0.00^{a}	0.05 ± 0.02^{c}	19.83 ± 0.03^{c}	17.63 ± 0.02^{c}
6	0.07 ± 0.03^{ab}	0.09 ± 0.01^{b}	20.09 ± 0.01^{bc}	17.94 ± 0.00^{bc}
9	0.11 ± 0.02^{ab}	0.16 ± 0.03^{ab}	20.57 ± 0.03^{b}	18.15 ± 0.03^{b}
12	0.15 ± 0.01^{b}	0.22 ± 0.02^{a}	21.45 ± 0.00^{a}	19.49 ± 0.01^{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%).

Table 5. Peroxide value (mEqO2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg), Total volatile base (TVB-N, mg N/100 g) nitrogen content and Trimethylamine (TMA, mg N/100 g) in dry-salted *T. pectoralis* by two packaging procedure (zipper and vaccum) after 3 months of preservation

Packaging procedure	Peroxide value ($mEqO_2$/ kg)	Thiobarbituric acid (mg maloaldehyde/ kg)	TVB-N (mg N/100 g)	TMA (mg N/100 g)
Zipper	0.04 ± 0.00^{a}	0.05 ± 0.02^{a}	19.83 ± 0.03^{a}	17.63 ± 0.02^{a}
Vaccum	0.01 ± 0.03^{a}	0.03 ± 0.00^{a}	14.32 ± 0.01^{b}	13.04 ± 0.03^{b}

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 6. Peroxide value (mEqO_2/ kg), Thiobarbituric acid (mg maloaldehyde/ kg), Total volatile base (TVB-N, mg N/100 g) nitrogen content and Trimethylamine (TMA, mg N/100 g) in dry-salted *T. pectoralis* by vaccum during 12 months of preservation

Storage (months)	Peroxide value (mEqO2/ kg)	Thiobarbituric acid (mg maloaldehyde/ kg)	TVB-N (mg N/100 g)	TMA (mg N/100 g)
3	0.01±0.03b	0.03±0.00b	14.32±0.01b	13.04±0.03b
6	0.03±0.01ab	0.07±0.03ab	14.94±0.02ab	13.49±0.00ab
9	0.09±0.03ab	0.11±0.00ab	15.08±0.01ab	14.83±0.03ab
12	0.14±0.02a	0.16±0.01a	15.21±0.00a	14.91±0.02a

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%).

CONCLUSION

The snakeskin gourami has a high commercial meat yield and favouritely consumed as dried fish in Vietnam. Commercial farming of snakeskin gourami has been developed rapidly in recent years. Fat rancidity is responsible development of off-flavors, and the loss of fat-soluble vitamins and pigments especially in dry-salted snakeskin gourami fish. The reduction of oxygen to a low concentration can decrease oxidation. Our research demonstrated that fat oxidation and proteolytic decomposition create major changes in snakeskin gourami (*Trichopodus pectoralis*) quality. Carrageenan as edible coating will be an ideal approach to preserve dry-salted fish because it creates a good barrier against enzymatic decomposition, spoilage microbial as well as auto oxidation. The coating attributes prolong stability against physicochemical changes such as color, texture, and moisture. Our research successfully proved that 1.25% w/w carrageenan coating and 4oC in vaccum packaging was adequate for the dry-salted snakeskin gouramis for 12 months of preservation. Carrgeenan as edible coating should be verified on other valuable dry-salted fishes to improve their commercial value during distribution.

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