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RESEARCH ARTICLE

Efficacy of Edible Coatings on Enhancing the Shelf-Life of Fresh and Frozen Tilapia (*Oreochromis niloticus*) Fish

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Abstract

Tilapia (*Oreochromis niloticus*) is generally processed to fresh and frozen fillets for domestic consumption. Deterioration of fish quality in refrigerator storage has great impact on the nutritious value of fish and the health of consumers. Freezing and cold storage is an efficient method which preserves taste and nutritional value. Glazing is a process used to reduce undesirable drying or dehydration of fish during frozen or cold storage. Objective of the current research studied the feasibility of edible coating in maintaing and preserving the chemical, microbial and sensory attributes of fresh and frozen tilapia fillet. The fresh and frozen tilapia fillets were treated with different formulas (N_0: control; N_1: 1.5% w/w chitosan and peracetic acid; N_2: 1.5% w/w chitosan and gelatin; N_3: 1.5% w/w whey protein isolate and ethanol; N_4: 1.5% w/w xanthan gum). The chemical quality was based on the analyzed results of ammonia (µNH_3/g) and pH; biogenic amines such as putrescine (mg/kg), cadaverine (mg/kg), histamine (mg/kg), spermine (mg/kg), spermidine (mg/kg); lipid oxidation or malondialdehyde value (mg/kg); microorganism such as Total plate count (cfu/g), *Enterobacteriaceae (*cfu/g*), Coliform* (cfu/g), *E. coli* (cfu/g), *Staphylococus* (cfu/g), *Listeria* (cfu/g), *Salmonella* (cfu/g), *Vibrio* (cfu/g); sensory score. All treated fresh tilapia fillets were tested after 21 days of cool storage (4^{o}C). All treated frozen tilapia fillets were tested after 12 months of cold storage (-18^{o}C). Results revealed that the incorporation of 1.5% w/w chitosan and peracetic acid could inhibit microbial spoilage and lipid oxidation and therefore maintain the freshness of tilapia fillet for 21 days at 4^{o}C and 12 months at -18^{o}C storage. The study indicated that the combination treatment with edible coating could be commercially utilized to maintain the freshness and prolong the shelf-life of fresh and frozen tilapia fillet effectively.

Keywords: *Frozen, Tilapia fillet, Chitosan, Peractic acid, Gelatin, Whey protein isolate, Ethanol, Xanthan gum.*

Introduction

Tilapia *(Oreochromis niloticus*) is one of the most important economic freshwater fish cultivated in Mekong delta, Vietnam. *Oreochromis niloticus* considered one of an excellent quality fish characterized and rich vitamins with firmly textured rusticity and good sensorial properties of flesh making it more suitable and an appetizing fish to the consumers [1]. Marketing studies indicated that consumer's preferred the fresh fish than the frozen one. It is a fish from tropical climates that shows great robustness for cultivation, tasty meat, and white color, with a low fat percentage and an absence of Y-shaped bones [2]. Nile tilapia (*Oreochromis niloticus*) is an important aquaculture species, and like all fish, it deteriorates during chilled storage and also in the frozen state. Rodrigues et al [3].Evaluated changes in the sensory, physicochemical, and microbial counts of tilapias stored in ice for 28 days. The authors showed that the values for the volatile nitrogen bases (VNB) and trimethylamine (TMA) were within the legal limits for 22 days of storage (30 mg/100 g and 4 mg/100 g, respectively), and the microbial counts (7-log CFU/g) for up to 18 days, and concluded that tilapia could be stored in ice

for up to 18 days. In another study, Emire and Gebremariam [4] evaluated the influence of the storage period on changes in the proximate composition, pH, VNB, and microbiology of frozen stored Nile tilapia fillets for up to 90 days. Decreases in protein, moisture, and ash contents and an increase in fat content were found. The VNB and pH increased, and the total bacterial count decreased after 90 days of frozen storage. Fresh fish is extensively consumed and is one of the most-traded food commodities in the world.

Conventional preservation technologies include vacuum and modified atmosphere packaging, but they are costly since requires capital investment [5]. Fresh fish is a highly perishable product due to its high water activity, nutrient availability, nearly neutral-pH (factors that influence microbial growth) and the presence of autolytic enzymes; hence, it is susceptible to post-harvest losses (Makawa, Z. et al., 2014; Tesfay, S.; Teferi, M. et al., 2017).

The best method of preserving of seafood is freezing and storing at low temperatures. If properly frozen, seafood retains quality and flavour. Frozen seafood products are imperative since fish products are one of the most highly traded food commodities [6]. Freezing is a means to prevent fish quality deterioration during transportation, storage, retail display, or consumption.

The deterioration caused by microbial and enzyme activity can be limited effectively under frozen storage [7]. However, discoloration and destruction of texture caused by the denaturation of proteins and lipid oxidation still occurred during frozen storage [8, 9]. Upon thawing, there is a loss of fluid from the flesh of any fish product, which is explained by the denaturation of protein during the freezing process, which causes the protein to lose its water-binding capacity.

Drip loss, or the release at water during thawing, implies nutrient loss. Little drip loss occurs when the products are frozen quickly and stored properly, but if not, excessive drip loss can occur and render making the products unfit for consumption. One problem encountered by producers of both fresh and frozen seafood is dehydration of product and so the product must be protected from dehydration. Two protective methods are used, usually in combination: glazing and packaging. One particular established technology generally applied during freezing and frozen storage of seafood is the application of a layer of ice on the surface of a frozen product, referred to as ice-glazing [10]. Good packaging prevents the circulation of air over the surface of the product and protects the moisture in the surface layers of the product [11].

The final quality depends on the quality of seafood at the time of freezing as well as other factors during freezing, glazing, cold storage and distribution [12]. Biochemical reactions for example lipids oxidation, reactions due to activities of the fish's own enzymes, and the metabolic activities of microorganisms due to deterioration of food.

These activities cause to a short shelf life in fish and other seafood products. Edible coatings from polysaccharides, proteins, and lipids can increase the shelf life of the foods due to their functioning as solute, gas, and vapor barriers. Biofilm defined a thin continuous layer of polymers on food surfaces which protect food products against the many factors/located on food surface and makes them more difficult to decline.

There were several notable researches mentioned to application of coating in seafood. The effects of several natural chemicals incorporated alone or in combination in traditional water ice-glazing on the freshness and shelf-life of Pacific saury during frozen storage at -18 °C were investigated.

Pacific sauries were subjected to individual quick freezing followed immediately by dipping into cold tap water (control) or solutions containing nisin, chitosan, phytic acid (single-factor experiment) or their combinations for 10 s at 1 °C and then packaged in polypropylene bags before frozen storage at -18 °C. The storage duration tested was up to 12 months.

Ice-glazing treatments with individual chemicals could significantly ($P < 0.05$) inhibit the accumulation of thiobarbituric acid-reactive substances (TBARS), total volatile basic nitrogen (TVB-N) and histamine as well as the increase in bacterial total viable count (TVC) compared with controls, while the combination treatments gave even better effects [13]. The effect of an edible coating (EC) with 1.5% chitosan as an

additive, on common carp (Cyprinus carpio) fillet, was determined evaluating the biochemical, physicochemical, textural, microbiological, and nutritional characteristics periodically during its storage in the freezer (-18°C), observing a decrease in the rate of biochemical reactions related to degradation, hydroperoxides content (HPC) (0.8324 nM hydro peroxides/mg of protein versus 0.5540 nM/mg with regard to the EC sample), as well as protein carbonyl content (PCC) (0.5860 nM versus 0.4743 nM of reactive carbonyl groups/mg of protein of non-coated material), keeping properties for a longer period of time, and a lower protein solubility (7.8 mg of supernatant protein/mg of total protein versus 6.8 mg/mg) and less loss of moisture (8% less, with regard to EC); for the nutritional characteristics of the fillet, lysine is the limiting amino acid in the sample without EC, while leucine is the limiting amino acid for the EC sample.

According to microbial growth, the count was 2.2×10^5 CFU/g of sample in mesophiles versus 4.7×10^4 in the EC sample. The results indicate that the use of EC added with chitosan maintains the quality of the product regarding lipid and protein oxidation until fourth month of storage, maintaining moisture content without variation for at least 3 months, and inhibits microbial growth up to 2 logarithmic units, during five months of frozen storage [14]. A work aimed at evaluating the effect of water glazing and edible coatings of 0.5% w/v and 1.5% w/v chitosan on quality parameters of frozen fish. Samples coated with 1.5% w/v chitosan performed better in maintaining the color of the salmon and controlling microbial contamination of frozen and thawed samples [15].The aim of this work was to study the feasibility of edible coatings in maintain and preserving the chemical, microbial and sensory attributes of fresh and frozen tilapia fillet

Material and Method

Material

Frozen tilapia fillets were prepared as follow: Whole Alive Fish -> Slaughtering -> Blood Removing -> Filleting -> De-skinning -> Trimming (Fat Removing; Red Meat Removing; Belly-Flap Removing) -> Freezing -> Glazing -> Packing -> Cold Store. They were free of antibiotic residue to ensure food safety. Whole alive fish were collected from Dong Thap province, Vietnam.

Figure 1: Tilapia fillet

Researching Method

Effect of Edible Film Coating to Chemical Quality of Fresh and Frozen Tilapia Fillet during Storage

The fresh and frozen tilapia fillets were treated with different formulas (N_0: control; N_1: 1.5% w/w chitosan and peracetic acid; N_2: 1.5% w/w chitosan and gelatin; N_3: 1.5% w/w whey protein isolate and ethanol; N_4: 1.5% w/w xanthan gum). The chemical quality was based on the analyzed results of ammonia (μNH_3/g) and pH; biogenic amines such as putrescine (mg/kg), cadaverine (mg/kg), histamine (mg/kg), spermine (mg/kg), spermidine (mg/kg); lipid oxidation or malondialdehyde value (mg/kg). All treated fresh tilapia fillets were tested after 21 days of cool storage (4°C). All treated fresh tilapia fillets were tested after 21 days of cool storage (4°C). All treated frozen tilapia fillets were tested after 12 months of cold storage (-18°C).

Effect of Edible Film Coating to Microbial Quality of Fresh and Frozen Tilapia Fillet during Storage

The fresh and frozen tilapia fillets were treated with different formulas (N_0: control; N_1: 1.5% w/w chitosan and peracetic acid; N_2: 1.5% w/w chitosan and gelatin; N_3: 1.5% w/w whey protein isolate and ethanol; N_4: 1.5% w/w xanthan gum).

The microbial quality was based on the analyzed results of Total plate count (cfu/g), *Enterobacteriaceae (*cfu/g*), Coliform* (cfu/g), *E. coli* (cfu/g), *Staphylococus* (cfu/g), *Listeria* (cfu/g), *Salmonella* (cfu/g), *Vibrio* (cfu/g). All treated fresh tilapia fillets were tested after 21 days of cool storage (4^{o}C). All treated frozen tilapia fillets were tested after 12 months of cold storage (-18^{o}C).

Effect of Edible Film Coating to Sensory Score of Fresh and Frozen Tilapia Fillet during Storage

The fresh and frozen tilapia fillets were treated with different formulas (N_0: control; N_1: 1.5% w/w chitosan and peracetic acid; N_2: 1.5% w/w chitosan and gelatin; N_3: 1.5% w/w whey protein isolate and ethanol; N_4: 1.5% w/w xanthan gum). The sensory quality was based on the analyzed results of appearance or freeze-burn trace. All treated fresh tilapia fillets were tested after 21 days of cool storage (4^{o}C). All treated frozen tilapia fillets were tested after 12 months of cold storage (-18^{o}C).

Physico-chemical, Microbial and Sensory Evaluation of Fresh and Frozen Tilapia Fillet during Storage

Ammonia (μNH_3/g) was determined according to the colorimetric method. The pH values were measured by using a digital pH meter. Biogenic amines such as putrescine (mg/kg), cadaverine (mg/kg), histamine (mg/kg), spermine (mg/kg), spermidine (mg/kg) were determined based on reverse phase HPLC. Malondialdehyde value (mg/kg) was determined using the distillation method and a 2-thiobarbituric acid.

Microorganism such as Total plate count (cfu/g), *Enterobacteriaceae (*cfu/g*), Coli form* (cfu/g), *E. coli* (cfu/g), *Staphylococus* (cfu/g), *Listeria* (cfu/g), *Salmonella* (cfu/g), *Vibrio* (cfu/g) were determined by 3M-Petrifilm. The sensory score regarding to freeze-burn trace was carried out by selected panel of judges (9 members) rated on a nine point hedonic scale.

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 Stat graphics Centurion XVI.

Result & Discussion

Effect of Edible Film Coating to Chemical Quality of Fresh and Frozen Tilapia Fillet during Storage

Under normal refrigerated storage conditions, shelf-life of fresh fish is limited by the development of enzymatic (caused by endogenous or microbial enzymes) and chemical reactions. The main initial causative factor for fish spoilage is microbial growth and invasion, followed by the autolytic enzymes and then by chemical reactions, such as oxidation or hydrolysis [5].

The deterioration of fish meat during storage depends on many factors such as fish species, storage temperature, time, and enzymatic degradation [17, 18]. Denaturation and aggregation of muscle proteins during fresh and frozen storage are associated with the formation of disulfide and the interaction of lipid oxidation products with proteins [6].

Denaturation induced by fresh and frozen storage contributed to the decrease in the protein solubility of fish meat. The oxidation of lipid also takes place easily and limits the shelf life of fish during storage [9]. Lipid oxidation is of great concern to the food industries and consumers since it contributes to the development of poor color, odor, and texture as well as reduced nutritional value.

The lipid components of postmortem fish muscle tissue are prone to oxidation because fatty acids of fish lipids are much more unsaturated than those of mammals and birds. Quality changes and muscle discoloration related to lipid oxidation of some fish species have been reported [18, 19]. The fresh and frozen tilapia fillets were treated with different formulas (N_0: control; N_1: 1.5% w/w chitosan and peracetic acid; N_2: 1.5% w/w chitosan and gelatin; N_3: 1.5% w/w whey protein isolate and ethanol; N_4: 1.5% w/w xanthan gum).

The chemical quality was based on the analyzed results of ammonia (μNH_3/g) and pH; biogenic amines such as putrescine (mg/kg), cadaverine (mg/kg), histamine (mg/kg), spermine (mg/kg), spermidine (mg/kg); lipid oxidation or malondialdehyde value (mg/kg). All treated fresh tilapia fillets

were tested after 21 days of cool storage (4°C). All treated fresh tilapia fillets were tested after 21 days of cool storage (4°C). All treated frozen tilapia *s* fillets were tested after 12 months of cold storage (-18°C). Results revealed in Table 1 & 2. From Table 1 & 2, formula (N_1) gave the best result to maintain the chemical quality of frozen tilapia fillet during storage.

Table 1: Effect of edible film coating to chemical quality of fresh tilapia fillet in 4°C storage

Coating	Before storage	After 21 days of storage at 4°C				
		N_0	N_1	N_2	N_3	N_4
Ammonia (μNH_3/g)	0.17±0.03^{d}	0.81±0.03^{a}	0.21±0.01^{d}	0.26±0.02^{c}	0.32±0.01^{bc}	0.41±0.02^{b}
pH	6.16±0.00^{c}	6.28±0.02^{a}	6.16±0.00^{c}	6.19±0.01^{bc}	6.22±0.02^{b}	6.25±0.01^{ab}
Putrescine (mg/kg)	0.79±0.01^{d}	1.39±0.01^{a}	0.80±0.03^{d}	0.88±0.02^{bc}	0.83±0.00^{c}	0.91±0.03^{b}
Cadaverine (mg/kg)	0.31±0.02^{d}	1.21±0.00^{a}	0.31±0.02^{d}	0.40±0.01^{bc}	0.35±0.03^{c}	0.43±0.00^{b}
Histamine (mg/kg),	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Spermine (mg/kg)	0.42±0.02^{d}	1.29±0.00^{a}	0.43±0.01^{cd}	0.49±0.02^{bc}	0.46±0.01^{c}	0.64±0.01^{b}
Spermidine (mg/kg)	1.67±0.00^{d}	2.53±0.02^{a}	1.70±0.03^{cd}	1.92±0.00^{b}	1.81±0.01^{bc}	1.77±0.02^{c}
Malondialdehyde value (mg/kg)	1.23±0.01^{d}	2.75±0.02^{a}	1.25±0.02^{cd}	1.41±0.01^{bc}	1.52±0.03^{b}	1.37±0.01^{c}

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%). N0: control; N1: 1.5% w/w chitosan and peracetic acid; N2: 1.5% w/w chitosan and gelatin; N3: 1.5% w/w whey protein isolate and ethanol; N4: 1.5% w/w xanthan gum

Table 2: Effect of edible film coating to chemical quality of frozen tilapia fillet in -18°C storage

Coating	Before storage	After 12 months of storage in -18°C				
		N_0	N_1	N_2	N_3	N_4
Ammonia (μNH_3/g)	0.17±0.03^{d}	0.72±0.02^{a}	0.17±0.03^{d}	0.24±0.03^{c}	0.30±0.01^{bc}	0.33±0.03^{b}
pH	6.16±0.00^{c}	6.25±0.00^{a}	6.16±0.03^{c}	6.17±0.00^{bc}	6.23±0.02^{b}	6.21±0.01^{ab}
Putrescine (mg/kg)	0.79±0.01^{d}	1.22±0.01^{a}	0.79±0.02^{d}	0.82±0.01^{bc}	0.83±0.00^{c}	0.85±0.02^{b}
Cadaverine (mg/kg)	0.31±0.02^{d}	1.05±0.02^{a}	0.31±0.00^{d}	0.35±0.01^{bc}	0.33±0.03^{c}	0.37±0.01^{b}
Histamine (mg/kg),	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Spermine (mg/kg)	0.42±0.02^{d}	1.06±0.00^{a}	0.43±0.04^{cd}	0.47±0.03^{bc}	0.44±0.01^{c}	0.52±0.02^{b}
Spermidine (mg/kg)	1.67±0.00^{d}	2.24±0.00^{a}	1.69±0.02^{cd}	1.80±0.02^{b}	1.74±0.00^{bc}	1.71±0.00^{c}
Malondialdehyde value (mg/kg)	1.23±0.01^{d}	2.11±0.03^{a}	1.23±0.02^{d}	1.29±0.00^{bc}	1.32±0.02^{b}	1.25±0.01^{c}

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%). N0: control; N1: 1.5% w/w chitosan and peracetic acid; N2: 1.5% w/w chitosan and gelatin; N3: 1.5% w/w whey protein isolate and ethanol; N4: 1.5% w/w xanthan gum

Total volatile base nitrogen (TVB-N) is one of the most widely used fish spoilage indicator [20]. It represents the sum of ammonia, methylamine, dimethylamine, trimethylamine, and other basic nitrogenous volatile compounds resulted from fish degradation the thiobarbituric acid reactive substances (TBARS) assay is commonly used to evaluate malondialdehyde (MDA) content.

MDA is one of the most significant products of lipid damage [21]. A study investigated the effects of whey protein coating on the quality of common kilka during frozen storage period.

Chemical factors consisting of humidity, protein, lipid, ash and calorie were higher in the test samples compared to the control samples. Peroxide value, free fatty acids, thiobarbitoric acid, TVB-N and pH were lower in the test samples. Sensory tests had a better quality in the test samples compared to the control samples [22]. Biogenic amines are formed in fish after their death, due to decarboxylation of amino acids. Those reactions are mediated by exogenous decarboxylation enzymes, produced by bacteria growing in the fish flesh or through endogenous decarboxylation enzymes, which occur naturally in fish muscle [23, 24]. The biogenic amines intoxication is mostly related to histamine poisoning. The symptoms of such intoxication usually occur shortly after consumption of meal and last for few hours. The most common clinical manifestations of histamine poisoning are diarrhea, nausea, vomiting, rash, headaches, edema, hypotension, flushing and palpitations [25].

Although the histamine formation is more closely related to scombroid fish species, the research performed on fish fillets acquired from restaurants in Czech Republic showed that the hazard of histamine poisoning associated with consumption of *Pangasius* fillets imported to European Union is real [26].

A study evaluated the chemical quality parameters regarding frozen *Pangasius hypophthalmus* specimens from Vietnam. The proximate composition, pH, ammonia, biogenic amines (BAs), total mercury (Hg), malondialdehyde (MDA), and polyphosphate were determined.

The moisture, protein, lipid and ash values were between 83.83-85.59, 12.51-14.52, 1.09-1.65, and 0.76-2.38 g 100 g-1, respectively. Fraud by excessive polyphosphate addition was detected in 30% of the samples whereas Hg above the recommended limit was observed in 50% of the samples. With regard to compounds from the degradation process, low concentrations of individual BAs and pH values were found in this study and ranged from 5.88 to 6.18, except for samples with polyphosphate >1 g 100^{-1} (pH > 7.00) were observed in the present study.

However, ammonia concentration indicated that a degradation process initiated in 80% of the samples (0.12-0.34 NH3 g^{-1}) and 20% of the samples (1.87-1.94 µg NH3 g^{-1}) were in an advanced deterioration process. Furthermore, MDA values (1.21-7.88 mg kg^{-1}) suggested some failures, mainly during transportation and/ or storage [27].

Guimarães et al [28]. Evaluated the chemical quality parameters regarding frozen P. hypophthalmusfillets from Vietnam. The authors reported presence of mercury (Hg) and malondialdehyde (MDA) hazardous compounds in more than 50% of studied samples along with detrimental compounds. Suggested the need for quality control points in production chain to promote product standardization for safer food supply chain. Mohan et al [29]. Assessed the effectiveness of O_2 scavenger on shelf life of *Pangasius* catfish steaks during chilled storage.

Results showed that O_2 scavenger reduced the oxygen content by 99.58% within 24h in packages. O_2 scavenger packaged steaks maintained chemical (pH, TVB-N, TMA-N, TBA and PV), physical (drip loss and water holding capacity), microbial (total mesophilic and psychrotrophic counts), sensory qualities and lipid oxidation was reduced significantly, which extended the shelf life of steaks upto 20 days in comparison to air pack which had half the life 10 days.

The effect of chitosan on shelf life of restructured fish products from *Pangasius* (*P. hypophthalmus*) surimi during chilled storage were studied by Jeyakumari et al [30]. In which restructured products were prepared from *Pangasius* surimi by incorporation of chitosan subjected to qualities analysis under chilled storage and results from the study indicated that chitosan coating could be used as a natural ingredient to prevent lipid oxidation in surimi based food systems for the development of novel healthy fish products. Physicochemical changes of Basa fish (*Pangasius* bocourti) fillet during frozen storage at -20°C for 0-20 weeks were studied.

The content of thiobarbituric acid reactive substances (TBARS) of fish samples suddenly increased when the samples were stored longer than 8 weeks. The increase in TBARS value of the fish fillet was concomitant with the increase in value (yellow color). Marked decreases in Ca^{2+}-ATPase activity, sulfhydryl content, and protein solubility of the fish fillet after 8 weeks of storage were observed. Those decreasing values were well correlated with the increasing of disulfide bond content and surface hydrophobicity content. Increases in sheer force of fish meat during storage were also observed. The results indicated that frozen storage at -20°C affected on Basa fillet qualities, especially after 8 weeks of storage [31].

Effect of Edible Film Coating to Microbial Quality of Fresh and Frozen Tilapia Fillet during Storage

Fresh fish spoils due to the action of a group of microorganisms, the so-called specific spoilage organisms. These organisms have the ability to dominate the fish flora and produce metabolites that directly affect the sensory properties of the product resulting in its rejection by consumers [32].During storage, the micro flora changes owing to different capacities of the microorganisms to tolerate the preservation conditions [33].

Under aerobic iced storage, the flora of fish is composed almost exclusively of *Pseudomonas* spp. and *Shewanella putrefaciens* (SSOs) regardless of whether it was caught or harvested in temperate or sub-tropical and tropical waters. At ambient temperature (25 ◦C), micro flora is dominated by mesophilic *Vibrionaceae*, and, particularly if the fish is caught in polluted waters, by mesophilic *Enterobacteriaceae* [34].

Microbial spoilage is due to the proliferation of microorganisms after the death of fish as a result of the immune system collapsing, followed by the microbial invasion of the fish body through the skin. Fish have a unique osmoregulatory mechanism to avoid

dehydration in marine environments and water logging of tissue in freshwater; it contains osmoregulatory compounds, like trimethylamine oxide (TMAO) and urea [35]. Microbial enzymes that are present in fish can break down TMAO to trim ethylamine (TMA) and urea to ammonia, volatile organic compounds associated with microbial spoilage.

Many other volatile compounds can be formed by microbial enzymatic degradation of other substrates, such as hydrogen sulphide (from cysteine), methanethiol and methyl sulphide (from methionine), histamine (from histidine), acetate, carbon dioxide and water (from carbohydrates and lactate), hypoxanthine (from inosine and inosine-50 -monophosphate), esters, ketones, aldehydes (from amino acids, like glycine, serine, and leucine), as well as ammonia (from amino acids and urea). These molecules are responsible for sweet, fruity, ammonia-like, putrid, and sulphuric off-flavours in spoiled fish [36]. Edible coatings can improve the quality of fresh, frozen, and processed seafood products [37]. In the current research, the fresh and frozen tilapia fillets were treated with different formulas (N_0: control; N_1: 1.5% w/w chitosan and peracetic acid; N_2: 1.5% w/w chitosan and gelatin; N_3: 1.5% w/w whey protein isolate and ethanol; N_4: 1.5% w/w xanthan gum).

The microbial quality was based on the analyzed results of Total plate count (cfu/g), *Enterobacteriaceae (*cfu/g*), Coli form* (cfu/g), *E. coli* (cfu/g), *Staphylococus* (cfu/g), *Listeria* (cfu/g), *Salmonella* (cfu/g), *Vibrio* (cfu/g). All treated fresh tilapia fillets were tested after 21 days of cool storage (4°C). All treated frozen tilapia fillets were tested after 12 months of storage. Results revealed in table 3 & 4. From table 3 & 4, formula (N_1) also gave the best result to maintain the microbial quality of frozen tilapia fillet during storage.

Table 3: Effect of edible film coating to antimicrobial capacity of fresh tilapia fillet in 4°C storage

Coating	Before storage	After 21 days of storage in 4°C				
		N_0	N_1	N_2	N_3	N_4
Total plate count (cfu/g)	$2.0x10^3$ $\pm0.00^d$	$7.9x10^4$ $\pm0.04^a$	$1.1x10^3$ $\pm0.02^e$	$1.5x10^3$ $\pm0.01^{bc}$	$1.9x10^3$ $\pm0.01^b$	$1.2x10^3$ $\pm0.03^c$
*Enterobacteriaceae (*cfu/g*)*	$2.5x10^1$ $\pm0.03^d$	$8.4x10^2$ $\pm0.00^a$	$2.3x10^1$ $\pm0.03^e$	$4.6x10^1$ $\pm0.00^{bc}$	$4.8x10^1$ $\pm0.02^b$	$4.1x10^1$ $\pm0.00^c$
Coliform (cfu/g)	$1.9x10^1$ $\pm0.02^d$	$6.9x10^2$ $\pm0.03^a$	$1.7x10^1$ $\pm0.01^e$	$2.7x10^1$ $\pm0.03^{bc}$	$2.9x10^1$ $\pm0.00^b$	$2.3x10^1$ $\pm0.01^c$
E. coli (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Staphylococus (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Listeria (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Salmonella (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Vibrio (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

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%). N0: control; N1: 1.5% w/w chitosan and peracetic acid; N2: 1.5% w/w chitosan and gelatin; N3: 1.5% w/w whey protein isolate and ethanol; N4: 1.5% w/w xanthan gum

Table 4: Effect of edible film coating to antimicrobial capacity of frozen tilapia fillet in -18°C storage

Coating	Before storage	After 12 months of storage in -18°C				
		N_0	N_1	N_2	N_3	N_4
Total plate count (cfu/g)	$2.0x10^3$ $\pm0.00^d$	$3.8x10^4$ $\pm0.03^a$	$0.2x10^3$ $\pm0.02^e$	$2.4x10^3$ $\pm0.01^{bc}$	$2.8x10^3$ $\pm0.03^b$	$2.3x10^3$ $\pm0.02^c$
*Enterobacteriaceae (*cfu/g*)*	$2.5x10^1$ $\pm0.03^d$	$6.2x10^2$ $\pm0.01^a$	$0.5x10^1$ $\pm0.00^e$	$2.9x10^1$ $\pm0.01^{bc}$	$3.1x10^1$ $\pm0.01^b$	$2.7x10^1$ $\pm0.01^c$
Coliform (cfu/g)	$1.9x10^1$ $\pm0.02^d$	$2.9x10^2$ $\pm0.00^a$	$0.2x10^1$ $\pm0.02^e$	$2.2x10^1$ $\pm0.03^{bc}$	$2.5x10^1$ $\pm0.00^b$	$2.1x10^1$ $\pm0.02^c$
E. coli (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Staphylococus (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Listeria (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Salmonella (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Vibrio (cfu/g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

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%). N0: control; N1: 1.5% w/w chitosan and peracetic acid; N2: 1.5% w/w chitosan and gelatin; N3: 1.5% w/w whey protein isolate and ethanol; N4: 1.5% w/w xanthan gum

Microbiological contamination is one of the most important spoilage mechanisms in fish deterioration. The research performed by Tong Thi, et al [38].Showed that frozen fillets from *Pangasius* processed in Vietnam can be contaminated with *Escherichia coli, Staphylococcus aureus* and even *Vibrio cholera*, despite the fact, that the processing plant had implemented food safety management systems, such as HACCP, BRC and IFS.

Some research indicated that those bacteria can also be present in *Pangasius* flesh [39, 40]. Kumari et al [41].Developed *Pangasius* steaks by sous-vide technology with its process optimization. Results suggested suitable combination of chitosan and spices. This enhanced antimicrobial and oxidative stability.

Chitosan concentration of 1.08 %, temperature of 70. 93º C and cooking time of 16.48 min as optimized conditions for sousvide processing of *Pangasius* steaks. On grass carp fillets, Yu et al [42].Have evaluated the efficacy of coatings based on 2% chitosan incorporated with different concentrations of glycerol monolaurate (0.1% and 0.3%) against *Pseudomonas* spp. and H2S producing bacteria. In a study on pike-perch fillets, Shokri & Ehsani [43] have tested the efficacy of coatings based on 10% whey protein isolate incorporated with 2.5% lactoperoxidase, 1.5% and 3.0% α-tocopherol, respectively, combinations of lactoperoxidase and α-tocopherol (2.5%/1.5% and 2.5%/3.0%) against Pseudomonas spp. and H2S producing bacteria. Gómez-Estaca et al [44].

Have tested the efficacy of edible films based on 8% gelatin and 8% gelatin/chitosan, both incorporated with 7.5% clove EO on salmon fillets, in vitro against Listeria innocua and Escherichia coli, then in situ against total viable organisms. Han et al [45].

Have investigated the efficacy of films based on 6.75% (w/w) gelatin, with and without nisin-incorporated, against Listeria monocytogenes in rainbow trout fillets that were challenged with 2 log CFU/g inoculums before and after coating. The edible film incorporated with 18 µg/cm2 nisin, applied before inoculation, showed the highest inhibitory effect on Listeria monocytogenes. The efficacy of gelatin coatings containing different concentrations of oregano EO (0.5%, 1.0%, and 2.0% v/v) was also investigated by Min and Oh [46], in catfish fillets that were inoculated with Salmonella typhimurium and Escherichia coli O157:H7. The coating based on 3% (w/v) gelatin containing 2% oregano EO exhibited the best inhibitory effect on both bacteria. Edible coatings based on chitosan [46] applied to salmon fillets, respectively chitosan-gelatin to golden pomfret fillets exhibited an antimicrobial effect compared to uncoated controls. When tested against total yeasts and moulds, the edible coating based on 0.4% chitosan and 3.6% gelatin applied to golden pomfret fillets was the most effective among all formulations [47].

A study investigated the effects of whey protein coating on the quality of common kilka during frozen storage period. Results showed that total bacterial counts and Staphylococcus bacteria count were lower in the test samples compared to the control samples. Coliform, Escherichia coli and Pseudomonas bacterial contamination were negative till the end of the storage period in the covered samples [22].

Effect of Edible Film Coating to Sensory Score of Frozen Tilapia Fillet during Storage

Edible coatings can help prevent physical damage, enhance appearance, and reduce microbial growths so that they can be a cost-effective alternative to the modified atmosphere packaging [48]. The fresh and frozen tilapia fillets were treated with different formulas (N_0: control; N_1: 1.5% w/w chitosan and peracetic acid; N_2: 1.5% w/w chitosan and gelatin; N_3: 1.5% w/w whey protein isolate and ethanol; N_4: 1.5% w/w xanthan gum).

The sensory quality was based on the analyzed results of appearance or freeze-burn trace. All treated fresh tilapia fillets were tested after 21 days of cool storage (4^{o}C). All treated frozen tilapia fillets were tested after 12 months of storage. Results revealed in table 5 & 6. From Table 5 & 6, formula (N_1) also gave the best result to maintain the sensory attribute of frozen tilapia fillet during storage.

Table 5: Effect of edible film coating to sensory attribute of fresh tilapia fillet in 4°C storage

Coating	Before storage	After 21 days of storage in 4°C				
		N_0	N_1	N_2	N_3	N_4
Sensory score	8.63±0.01[a]	8.17±0.03[c]	8.59±0.00[a]	8.44±0.02[ab]	8.41±0.01[ab]	8.34±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%). N0: control; N1: 1.5% w/w chitosan and peracetic acid; N2: 1.5% w/w chitosan and gelatin; N3: 1.5% w/w whey protein isolate and ethanol; N4: 1.5% w/w xanthan gum

Table 6: Effect of edible film coating to sensory attribute of frozen tilapia fillet in -18°C storage

Coating	Before storage	After 12 months of storage in -18°C				
		N_0	N_1	N_2	N_3	N_4
Sensory score	8.63±0.01[a]	8.25±0.01[c]	8.62±0.01[a]	8.47±0.02[ab]	8.43±0.00[ab]	8.37±0.01[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%). N0: control; N1: 1.5% w/w chitosan and peracetic acid; N2: 1.5% w/w chitosan and gelatin; N3: 1.5% w/w whey protein isolate and ethanol; N4: 1.5% w/w xanthan gum

Even frozen, seafood remains a sensitive product that loses its quality during storage due to the exudate, lipid oxidation, and cold burn. In order to reduce these problems, processing industries use technological procedures, such as the glazing process [49]. Glazing is a widespread method that wraps the seafood in a thin layer of ice, protecting the product from oxygen and dehydration, and promoting an increase in shelf life [50, 10, 51, 52].

A research compared the effect of chitosan solutions on frozen salmon preservation with that of water glazing. For this purpose, three chitosan solutions (0.25%, 0.50% and 0.75% w/v) and water were applied in different amounts (6%, 8% and 11% of coated fillet weight) directly on the surface of frozen salmon. In order to accelerate the deterioration processes, salmon was stored during 14 weeks at 5 C. Microbial and chemical indices were used to assess deterioration during storage and the coating stability was evaluated through weight loss measurements.

The results obtained showed that chitosan coatings can be a good barrier to protect frozen fish from deterioration. Microbial growth, assessed by total viable counts (TVC), and total volatile basic nitrogen (TVB-N) were maintained below the maximum limits recommended which are 5 10^5 CFU/g and 35 mg nitrogen/100 g fish, respectively.

The use of 0.50% and 0.75% chitosan solutions generally demonstrated to be more efficient in preventing salmon weight loss [15]. When applied to salmon fillets, the film based on 8% gelatin/chitosan, 3:1 incorporated with 7.5% clove EO [49] and coatings based on 1%, 1.5%, and 2% chitosan [47] enhanced the shelf-lives by 6 days.

The study of Shokri & Ehsani [43] on pike-perch fillets show a shelf-life prolongation by 8 days when a packaging material based on 10% whey protein isolate incorporated with 2.5% lactoperoxidase was used for coating. Another study, carried out by Qiu et al [53].Has shown increased storage stability (from 8 to 12 days) of Japanese sea bass fillets coated with a solution containing 1.5% chitosan and 0.5% citric acid.

The coating formulation of Li et al [54].Also based on 1.5% chitosan but incorporated with 0.2% tea polyphenols, prolonged the microbiological shelf-life of red drum fillets by 8 days. A study investigated the effects of whey protein coating on the quality of common kilka during frozen storage period. Sensory tests had a better quality in the test samples compared to the control samples [22]. In another study, freezing of fish muscle at -12±2 °C causes comparatively lesser spoilage than chilling at 4±1°C.

Freezing of fish creates unfavourable environmental conditions which slow down the bacterial growth and biochemical decomposition of fish muscle, thereby increasing the shelf life; while chilling at 4±1oC allows the comparatively rapid proliferation of bacteria, protein denaturation, lipid hydrolysis and oxidation; thereby reducing the shelf life [55].

Conclusion

Tilapia fish has been playing an important role in addressing nutritional and livelihood security of people in the developing countries.

Besides, it is good source of polyunsaturated fatty acids (PUFA's), protein, minerals and vitamins which are vital to our health. Although fish is highly nutritious, yet it is one of the most rapid perishable foods because of its short shelf life. The extension of shelf life can be achieved by freezing, chilling. Post-harvest losses of fresh fish due to microbial spoilage are a matter of great importance to the fishing industry. So, specific requirements and preservation techniques are needed to minimize the activity of spoilage bacteria.

Fresh fish products are presently stored on ice or under refrigeration during their distribution and marketing. The present study showed that 1.5% w/w chitosan and peracetic acid was appropriated for coating of fresh and frozen tilapia fillet for 21 days in cool storage (4°C) and and 12 months cold storage (-18°C) respectively.

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