

A Seminar Paper
on
Chitosan Coating Enriched with Natural Extracts
Improve the Quality and Shelf Life of Fish Fillets during
Frozen Storage

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on
Chitosan Coating Enriched with Natural Extracts Improve the Quality and Shelf Life of Fish Fillets during Frozen Storage¹

by
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Abstract

Fish fillet is one kind of fishery product prepared from fresh fish after removal of scale, gut, head and tail of the fish. It is popular all over the world due to easy for cooking. Fish fillets can be stored at frozen temperature to keep fresh and retain the quality of the product. However, the loss of quality is inevitable and eventually the fish fillets will become unfit for consumption for a very short period during frozen storage. With the increasing demand of frozen fresh fish fillets, it is of interest to find new methods to reduce the quality loss and extend the shelf life of frozen fish fillets. Currently, natural antioxidant and antimicrobial ingredients have been used to preserve the frozen fish fillets instead of synthetic additives to meet consumers' requirements. Chitosan is a natural cationic polysaccharide made from crustacean shells and has been reported to have antioxidant and antimicrobial activities. It can be coated to fish, fruits and vegetables, meat and many other foods to improve the quality and extend the shelf life as a protective edible film. Edible films and coatings are possible opportunities to prolong the shelf life of perishable food products such as fish fillets. Chitosan alone or in combination with biologic or not biologic materials are good candidates for this purpose. Chitosan based edible coating provides excellent oxygen barrier properties along with its antimicrobial activity. Many natural extracts such as ginger, orange, pomegranate, cinnamon, thyme can be used along with chitosan coating to increase the positive effect of chitosan towering increase the shelf life of frozen fillets. They have been found to be nontoxic, biodegradable, biofunctional and biocompatible biopolymer. This paper reviews about the preservative action of chitosan coating combined with natural extracts on fish fillets during frozen storage. Chitosan coating along with natural extracts promote long shelf life of frozen fillets without hampering the quality of the products.

Keywords: Fish fillet; frozen storage; chitosan; natural extracts: antioxidant; antimicrobial properties.

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Chapter I

Introduction

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. Shelf life of fish products is defined as the storage time until spoilage. The point of spoilage may be defined by a certain maximum acceptable level of the microbiological and physico-chemical indicators and sensory requirements. It has been reported that the spoilage of fish muscle is a combination of different spoilage mechanisms including lipid oxidation, microbial and endogenous enzymes activities as well as enzymatic browning. These events lead to a decrease in the shelf life of fish meat and other seafood products (Arashisar et al., 2004). Consumers usually buy fish in bulk and store in refrigerator. Deterioration of fish quality in frozen storage have great impact on the nutritious value of fish and the health of consumers. Fish flesh quality is an indispensable factor for marketing. Considerable research has focused on improving fillet quality, such as high-pressure treatment (Ko et al., 2006), salting (Hong et al., 2011), partial freezing (Song et al., 2012), and modified atmosphere (Genc et al., 2013). Fish fillets is very popular all over the world specially Salmon, Tuna, Trout, Carp etc. Many food items like fish burger, fish finger, fish fry are made quickly from fillets thus increases its demand day by day. Though fish fillet is high marketable and demandable fishery product, its shelf life is lower than fish. Several recent studies have focused on using natural ingredients to enhance fillet quality during cold storage (Fan et al., 2008). In recent years, new techniques have been tried by many researchers to prolong the shelf life of food products. Among different applied methods, application of bio-based films and coatings was the most promising technique (Georgantelis et al., 2010).

Nanotechnology research is entirely multidisciplinary and the results of such research can be applied very quickly to improve fresh products (Clapper et al., 2008). Increasing consumer demands for high quality and microbiologically safer foods, together with longer product shelf life, are continuously forcing researchers and the industry to develop new food preservative strategies. Natural preservatives, such as chitosan, has been widely used in the food industry because of their good preservative effect.

Chitosan [b-(1, 4)-2-amino-2-deoxy-D-glucopyranose], which is mainly made from crustacean shells, is the second most abundant natural polymer in nature after cellulose (Shahidi et al., 1999). Chitosan have been used in foods as a food additives, as a clarifying agent in apple juice (Boguslawski et al., 1990), and antimicrobial and antioxidant in muscle foods (Gómez-Estaca et al., 2007). Furthermore, chitosan also has potential for food packaging, especially as edible films and coatings (Subramaniam et al., 2007). The preservative effect of chitosan is mainly due to the inhibition of some enzymes' activities, as well as the free radical scavenging ability and therefore, prevention of lipid oxidation. Due to its non-toxic nature, antibacterial and anti-oxidative activity, film-forming property, biocompatibility and biodegradability, chitosan has attracted much attention as a natural food additive (Majeti et al., 2000). Moreover, applications of chitosan for the improvement of quality and shelf life of various foods from agriculture, poultry, and seafood origin were discussed by No et al. (2007).

Spices and herbs have been used in many cuisines to impart flavor, aroma and pungency to food (Kanatt et al., 2008). Several studies have shown that the antimicrobial and antioxidative effect of chitosan was greatly enhanced by the addition of chitosan to essential oils (Georgantelis et al., 2008). Nature is abundant with many precious herbs, trees, spices which have lots of beneficial effects. Development of natural preservative coatings or films with high antioxidant, antibacterial activities that prolong the shelf life of fish and fish products is desirable. As consumers are increasingly aware of the risk for health because of the presence of chemical substances added to food for their preservation and antimicrobial properties, herbal extracts and essential oils are gaining interest for their application in food preservation. Combinations of chitosan and herbal extracts and essential oils such as cinnamon oil (Ojagh et al., 2010), tea polyphenols (Li et al., 2012) and rosemary extract (Li et al., 2012) have been used previously for extending the shelf life of fresh fish samples.

Objectives

After completing this article, readers will be able:

- ✓ To highlight the anti-microbial and anti-oxidantal properties of chitosan and natural extracts
- ✓ To review the preservative effects of chitosan combined with natural extracts coating on frozen fish fillets
- ✓ To explore long shelf life of frozen fish fillets

Chapter II

Methodology

Scientific approach requires a close understanding of the subject matter. This paper mainly depends on the secondary data. Different published reports of different journals mainly supported in providing data in this paper. This paper is completely a review paper. Therefore, no specific method has been followed in preparing this paper. It has been prepared by browsing internet, studying comprehensively various articles published in different journals, books, proceedings, dissertation available in the libraries of BSMRAU and personal communication. The author would like to express her deepest sense of gratitude to her major professor and course instructors for their efficient and scholastic guidance, precious suggestions to write this manuscript from its embryonic stage. All the information collected from the secondary sources have been compiled systematically and chronologically to enrich this paper.

Chapter III

Review of Major Findings and Discussion

3.1 Frozen Storage

Freezing is one kind of preservation technique that have been widely used is fish storage. Usually frozen temperature is -18 to -40 °C. Freezing at -18 °C inactivates any microbes such as bacteria, yeasts and molds present in food. Once thawed, these microbes can again become active, multiplying under the right conditions to levels that can lead to foodborne illness. Temperature should be maintained in freezer unless product will be deteriorated. However, it is the long storage technique of fish and fishery products. Fish can be stored 2-3 month in freezer with intact quality. Technology has been employed and research have been investigated to increase the shelf life of frozen fish and fishery products.

3.2 Fish Fillet

Fish fillet is one kind of fishery product produced from fish. The flesh of a fish which has been cut or sliced away from the bone by cutting lengthwise along one side of the fish parallel to the backbone. In preparation for filleting, any scales on the fish is removed. The contents of the stomach also are removed from the fillet. Because fish fillets do not contain the larger bones running along the vertebrae, they are often said to be "boneless". However, some species, such as the common carp, have smaller intramuscular bones called pins within the fillet. The skin present on one side may or may not be stripped from the fillet.



Fig. 1: Fish filleting

Source: (<https://www.dfw.state.or.us/resources/fishing>)

3.3 Properties of Chitosan

Chitosan is derived from chitin, a natural biopolymer found in the shells of crustaceans and cell walls of fungi. Chitin is the second most available biopolymer on earth (Shahidi et al., 1999). Chitin is mainly composed of poly b-(1-4)-2-acetamido-D-glucose, while chitosan is a copolymer that contains b-(1-4)-2-acetamido-D-glucose and b-(1-4)-2-amino-D-glucose units (Elsabee et al., 2013). Chitosan is typically produced from the partial deacetylation of chitin with sodium hydroxide. At a degree of deacetylation of over 50%, chitin becomes soluble in acidic solutions and can then be classified as chitosan. The degree of deacetylation of chitosan, in addition to molecular weight, strongly influences its antimicrobial activity (Tsai et al., 2002). Some inherent characteristics of chitosan, such as its large particle size (Qi et al., 2004) and high viscosity in solution (Jo et al., 2001), may limit its penetration into shrimp muscle tissues.

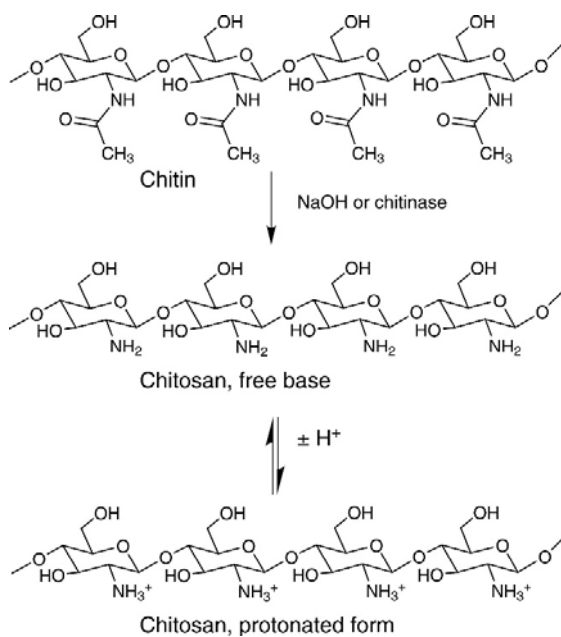


Fig. 2: Deacetylation of chitin to chitosan and acid-base equilibrium of chitosan.

(Source: Mendel et al., 2010)

A number of functional properties including antioxidant, antimicrobial, and oxygen barrier properties have been reported for chitosan film (Jeon et al., 2002). Chitosan film also exhibits a very good barrier to oxygen (Butler et al., 1996). The oxygen permeability of edible film is one of the key factors that limit the shelf life of packaged or coated products. During the frozen storage, oxidation occurs and changes color, and flavor of the fish. It is very important to limit oxygen transport from the storage environment to the fish. Chitosan is a widely used

polysaccharide in edible films and coatings due to its non-toxicity, antimicrobial activity, and antioxidant properties (Elsabee et al., 2013).

Table 1. Antioxidative and antimicrobial activities of chitosan in fish

Target	Chitosn type used	Assay	Microbes inhibited	Mechanism	Outcome
Cod	Chitosan of different molecular weights	Mixing with chitosan	None	Inhibition of lipid oxidation	Prevent off-flavor
Lingcod	Chitosan with fish oil coatings	Storage of coated lingcod for 3 weeks	Psychrotrophic organisms	Antioxidative effect	Extend shelf life, omega-3 fatty acids have added benefit
Herring	Chitosan coatings	Coating of fish flesh	None	Inhibition of oxidation	Hydroperoxide were reduced during storage
Atlantic Cod	Chitosan coatings	Coating of seafood	None	Antioxidative effect	Enhanced quality during storage
Trout	2% chitosan plus 1.5% cinnamon oil coatin solution	Pour plate method using plate count agar	Total viable count; psychrotrophic count	Growth inhibition of spoilage organisms	Improved shelf life

(Source: Mendel et al., 2010)

3.4 Properties of Natural Extracts

As consumers are increasingly aware of the risk for health because of the presence of chemical substances added to food for their preservation and antimicrobial properties, herbal extracts and essential oils are gaining interest for their application in food preservation. Incorporation of natural extracts into chitosan films or coatings may not only enhance the film's antimicrobial and antioxidant properties but also reduce water vapor permeability and slow lipid oxidation of the product on which the film is applied (Yanishlieva et al., 1999). Combinations of chitosan and herbal extracts such as cinnamon oil (Ojagh et al., 2010), tea polyphenols (Li et al., 2012) and rosemary extract (Li et al., 2012) have been used previously for extending the shelf life of fresh fish samples. It has been found that 6-gingerol extracted from ginger rhizome has medicinal properties like antioxidant, anti-inflammatory, antifungal, antiviral, and anticancer activities. *Cinnamomum zeylanicum* commonly known as cinnamon is rich in cinnamaldehyde as well as β -caryophyllene, linalool and other terpenes. Cinnamaldehyde is the major constituent of cinnamon leaf oil and provides the distinctive odor and flavor associated with cinnamon. It has anti-microbial activities which increase shelf life of fish. Chemical constituents and their bioactivities in peel, seed, leaf, pulp and juice of different fruits such as pomegranate (*Punica granatum*) and orange (*Citrus sinensis*) have been investigated. A range of polyphenols and antioxidants has been found in orange and pomegranate peels, juices, pulps and seeds (Bocco et al., 1998). Licorice root extract is from the licorice plants called *Glycyrrhiza glabra* and *Glycyrrhiza uralensis*. It contains glycyrrhizin, a very sweet syrup or powder which has medicinal properties. Licorice has long been used in China as a traditional herbal medicine because of its anti-inflammatory, antiviral, anti-allergic, antioxidant and anticancer properties (Jiang J et al., 2013). Licorice extract has also been applied to the preservation of meat products for its antioxidant and antimicrobial activities (Zhang HY et al., 2009).

3.5 Freshness Indicators

3.5.1 pH: Generally, pH values of fresh fish muscle range from 6.0 to 7.0 (Li et al., 2012). The reduced pH value during the initial storage could be explained by the accumulation of lactic acid resulting from glycogen consumption in fish muscle (Cai et al., 2014), while the subsequent increase of pH value may be due to the formation of volatile basic compounds, such as trimethylamine and ammonia that resulted from either microbial or endogenous enzymes (Duman et al., 2015).

3.5.2 TVB-N (Total Volatile Basic Nitrogen): TVB-N is a group primarily composed of primary, secondary, and tertiary amines which are used as indicators of meat deterioration; the increase in these is related to the activity of endogenous enzymes and bacteria. TVB-N is often used to evaluate fish spoilage (Erkan et al., 2007). TVB-N mainly includes ammonia, trimethylamine, and dimethylamine and results from the degradation of protein and non-protein nitrogen compounds caused by microbial activity and endogenous enzymatic action (Liu et al., 2013). Gimenez et al. (2002) proposed that the maximum level of TVB-N indicating good quality of the fish muscle was 25-40 mg N/100 g.

3.5.3 TBARS (Thiobarbituric Acid Reactive Substances): Thiobarbituric acid (TBA) is a widely used indicator for the assessment of degree of lipid oxidation (Ibrahim, 2007). The level of tissue malondialdehyde, a degradation product of lipid, is often measured in order to assess the extent of lipid peroxidation that has occurred in biological systems. It has been proposed that the maximum level of TBA value indicating good quality of the fish (frozen, chilled or stored with ice) is 5 mg of malonaldehyde equivalents/kg of tissue, while the fish may be consumed up to the level of 8 mg of malonaldehyde equivalents/ kg of tissue (Ibrahim, 2007).

3.5.4 K-Value: The K-value, an indicator of ATP degradation, is recommended as the most effective and reliable evaluation index for fish freshness. For freshly caught fish, initial K-value reported generally do not exceed 10%, and the value of 60% is regarded as its rejection threshold (Cai et al., 2015).

3.5.5 Peroxide Value: When meat and meat products are stored under frozen conditions, microbial spoilage may be delayed, but fat deterioration occurs and the meat constituents may be oxidized. Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. The peroxide value is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil. A suggested limit of peroxide value for quality and acceptability of oils for human consumption is 8 meq/kg (Boran et al., 2006).

3.5.6 Microbiological Analysis: In a living fish, bacteria are generally present in the skin, gills and gut, but they are prevented from entering the muscle. Once a fish dies, autolysis begins and bacteria can enter and decompose the muscle (Li et al., 2012). Total viable count (TVC) indicates whether a food product satisfies or exceeds the acceptable standards for

freshwater fish. A TVC value as high as 10^7 cfu/g indicates that the fish has been spoiled (Huang et al., 2008). Changes in TVC and psychrotrophic counts (PTC) of fish samples during frozen storage are also analyzed to determine the quality.

3.5.7 Changes in Free Fatty Acids (FFA): Free fatty acids are the products of enzymatic or microbial degradation of lipids. Fish lipids undergo hydrolysis and release FFA as a result of enzymatic reactions during frozen storage. FFA oxidize more readily than esterified lipid to produce low-molecular-weight compounds that can cause the rancid off-flavor of fish products. FFA also promote protein denaturation. Determination of FFA gives information about stability of lipid during storage. Increasing FFA indicates spoilage of frozen storage of fish.

3.5.8 Drip Loss: Drip loss can cause nutrition loss and reduction in weight of products, resulting in a loss of quality. The increase of drip loss in the fish fillet samples is caused by the accretion of ice crystals formed in fish tissues during frozen storage. The ice crystals deform and rupture adjacent cells and thus increase the release of cell constituents to form drip losses (Mackie 2009). Drip loss may also be caused by myosin aggregation, which could lead to the toughening of fish muscle and a reduction in water-holding capacity. Drip loss is measured for determining the storage quality of frozen fish.

3.5.9 Sensory Evaluation: Sensory evaluation is one of the most important parameters use to determine the quality of frozen fish. Score are given according to odor, taste, texture, color of storage of raw fish.

3.6 Effect of Chitosan (1.5%, w/v) Coating on the Quality of Common Carp (*Cyprinus carpio*) Fillets during Frozen Storage for 5 Months

Changes in Moisture

During its storage (5 month) under freezing, a significant ($p < 0.05$) decrease in moisture in both treatments is observed (Figure 3). For the carp with the coating, the decrease is observed until the fourth month; this can be due to the chitosan coating, which promote crosslinking in the gelatin, thus diminishing the free volume of the polymeric matrix. It reduces the diffusion rate of the water molecules through the coating film. According to Dutta et al., this is a desirable characteristic in coatings and is not observed in the control sample of this study, since a loss in moisture occurs ($p < 0.05$) during all of its storage period. After five months of

storage, in Figure 3, it is found that chitosan coating was showed less moisture loss than the sample without coating.

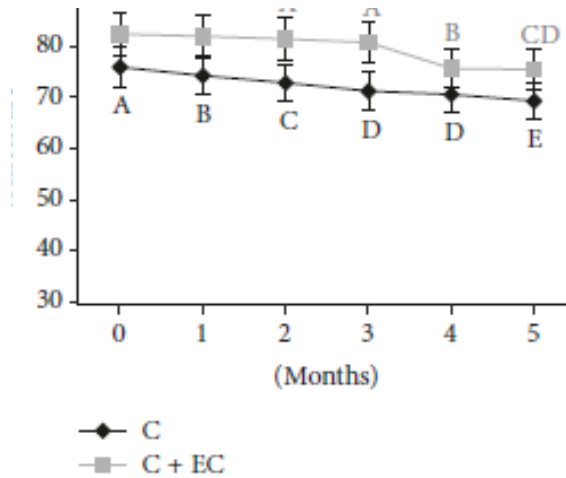


Fig. 3: Changes in moisture of common carp fillets stored at $-18\text{ }^{\circ}\text{C}$

The results are the mean of three replications. C: fillet carp without coating; C + EC: fillet carp with edible coating. (Source: Ana et al., 2017)

Changes in pH

A significant decrease ($p < 0.05$) in pH is observed in Figure 4 at the end of both treatments. pH is increased from second to third month of the frozen storage. The greatest variation in percentage at the end of storage is corresponded to the non-coated sample. A lower pH in the sample with the coating can bolster microbial inhibition and contribute to the preservation of the samples inhibiting the endogenous proteases.

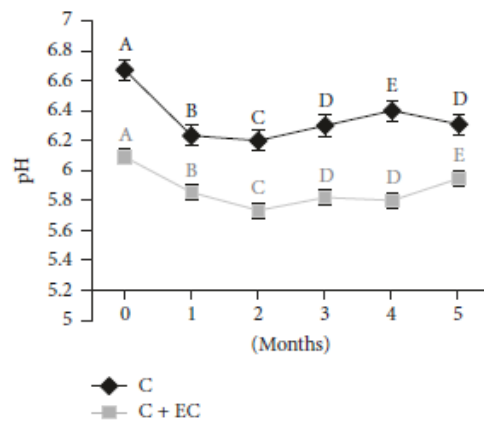


Fig. 4: Changes in pH of common carp fillets stored at $-18\text{ }^{\circ}\text{C}$

The results are the mean of three replications. C: fillet carp without coating; C + EC: fillet carp with edible coating. (Source: Ana et al., 2017)

Changes in Total Volatile Basic Nitrogen (TVB-N)

In Figure 5, it is demonstrated that; the concentration of TVB-N is greater in the control sample in each stage of storage; this may be due to the fact that the presence of chitosan helps in reducing the capacity of bacteria for oxidative deamination of non-protein nitrogenated compounds. Increasing TVB-N indicates decreasing quality of frozen stored fish. The amount TVB-N is high in uncoated sample.

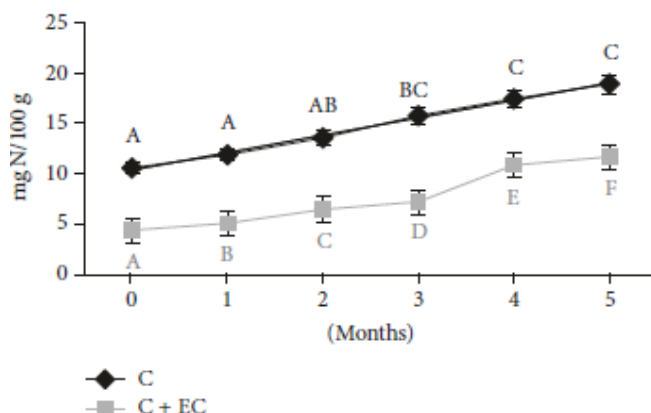


Fig. 5: Changes in TVB-N of common carp fillets stored at -18°C

The results are the mean of three replications. C: fillet carp without coating; C + EC: fillet carp with edible coating. (Source: Ana et al., 2017)

Changes in Lipid Oxidation Product

In storage at freezing temperature (-18°C), oxidation is the most important factor in deterioration, even over microbial activity. The concentration of primary products of oxidation can be measured by the content of peroxides. In Figure 6 it is showed that, the carp with coating shows a significant increase ($p < 0.05$) in peroxides by the fourth month and presents a final value of 0.55nM HPOx/mg of protein while the control sample presents an increase by the first month, having an increase of 59% with regard to the sample with coating. Chitosan-added coatings act as excellent barriers to the permeability of oxygen, once they are applied directly over the meat's surface, retarding the diffusion of oxygen.

Changes in TBARS Value

TBARS quantifies the compounds responsible for the loss of flavor and scent and is also important in the stages of deterioration of foods. In Figure 6, it is showed that; the values of TBA of both treatment samples presented a significant increase ($p < 0.05$) by the second and

forth months; however, by the fourth month the control sample presented a 39% increase with regard to a 6% increase in the sample with coating; this is due to the absence of chitosan in the control sample's coating. The antioxidative mechanism of the chitosan is due to the fact that its primary amino groups form a stable fluorosphere with volatile aldehydes such as malondialdehyde, derived from the rupture of fats during oxidation. The results in Figure 6 indicates that the chitosan employed in a 1.5% chitosan coating preserves the fish fillet through reduction of lipid oxidation.

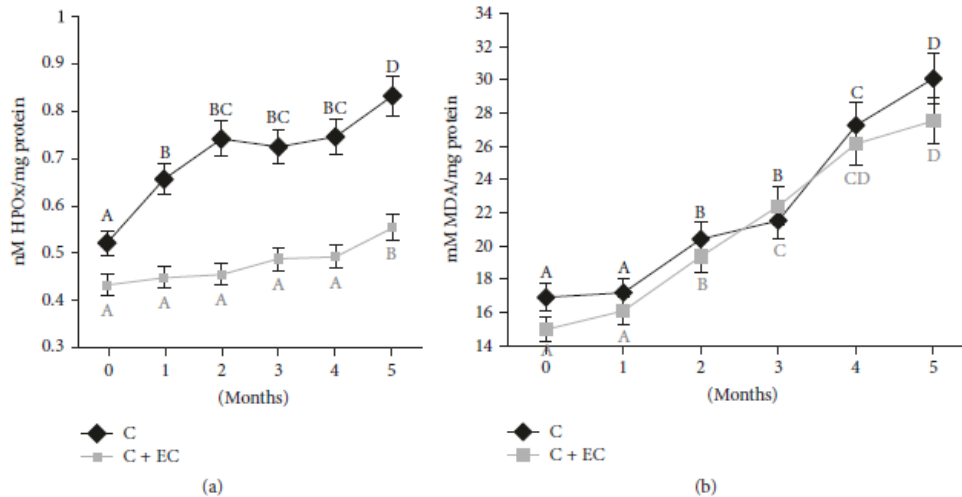


Fig. 6: Changes in HPOx content (a) and MDA content (b) values of common carp fillets stored at -18°C

The results are the mean of three replications. C: fillet carp without coating; C + EC: fillet carp with edible coating. (Source: Ana et al., 2017)

Microbiological Analysis

From Figure 7, it is demonstrated that the initial value for the carp without the coating is $2.3 \log 10^4$ CFU/g and $1.1 \log 10^4$ CFU/g for the carp with the coating; these values depend on the environment from which the fish is obtained as well as postmortem conditions. The evolution of the aforementioned is detailed in Figure 7, observing significant differences ($p < 0.05$) by the second month, obtaining a time-dependent increase in both treatments. The properties of the chitosan added to the coating had an inhibitory effect, thereby obtaining $4.7 \log 10^4$ CFU/g, while, for the control, a value of $2.2 \log 10^6$ CFU/g was obtained after five months of storage. Figure 7 also demonstrated that chitosan inhibits microbial growth up to 2 logarithmic units, decreasing reactions involved in deterioration by microorganisms during its storage in the freezer.

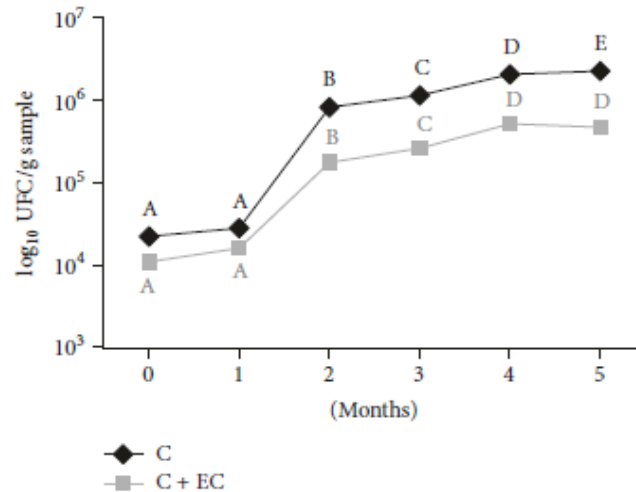


Fig. 7: Changes in Total viable counts of common carp fillets stored at -18°C . The results are the mean of three replications. C: fillet carp without coating; C + EC: fillet carp with edible coating. (Source: Ana et al., 2017)

3.7 Effect of Chitosan (2%, w/v) Coating Combined with Cinnamon Oil (1%, w/v) on the Quality of Frozen Rainbow Trout Fillets during Frozen Storage for 3 Months

Changes in Peroxide Value (PV)

In Figure 8 the effect of coating on the changes of PV of fish lipids is depicted. The PV value is increased significantly ($p < 0.05$) in all samples during storage. Control samples show the highest rate of increase in PV, while the Ch+C coated samples show the slowest. The lowest rate in PV indicated that chitosan coating enriched with cinnamon oil is highly effective in lipid oxidation inhibition. Chitosan may retard lipid oxidation by chelating ferrous ions present in the fish, thus eliminating oxidant activity of ferrous ions or preventing their conversion to ferric ion. Xue et al. (1998) reported that the antioxidant mechanism of chitosan could be by chelant action of ion metals and/or the combination with lipids. The protective action of chitosan is also effective when it is applied as a protective film or coating, where it retards lipid oxidation and microbial spoilage by acting as a barrier against oxygen (No et al., 2007). In Figure 8, the Ch+C coated samples show a lower PV in comparison with Ch coated samples on rainbow trout fillets. Adding cinnamon oil to chitosan coating, therefore, probably have a synergistic effect.

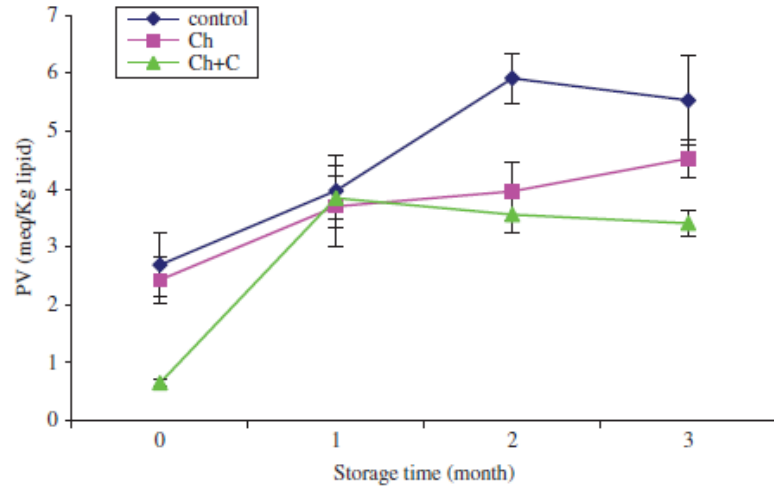


Fig. 8: Changes in PV of rainbow trout fillets during frozen storage at $-18\text{ }^{\circ}\text{C}$ (Source: Seyed et al., 2014)

Changes in TBARS Value

In Figure 9, the TBA values for the different treatments during storage are presented. The TBA values (mg MA per kg of fish sample) is increased up to Month 2 for all samples. Control samples show the highest value of TBA, and a significant decline ($p < 0.05$) is observed after Month 2. The TBA content is lower in coated samples than in control samples. The antioxidant and gas barrier properties of chitosan are crucial for prolonging the shelf life of fish and seafood products (Shahidi et al., 1999). Inhibition of lipid oxidation resulting from the addition of cinnamon oil in Ch+C coated samples is observed.

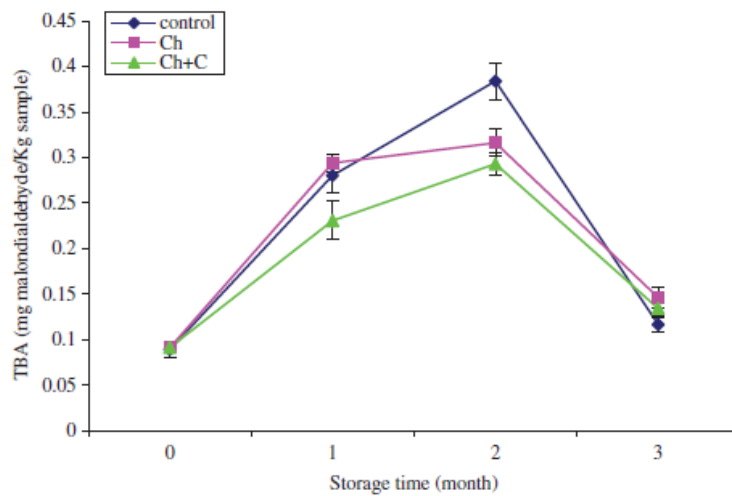


Fig. 9: Changes in TBA values of rainbow trout fillets during frozen storage at $-18\text{ }^{\circ}\text{C}$ (Source: Seyed et al., 2014)

Changes in Free Fatty Acids (FFA)

Figure 10 presents the FFA values for the different treatments during storage. The FFA is increased significantly ($p < 0.05$) in all samples during storage up to 2 months; then in the 3rd month, a significant decline is observed in FFA content. This may have been due to depletion of substrate or oxidation of the FFA (Namulema et al., 1999). Generally, the control samples significantly ($p < 0.05$) show a higher FFA in comparison with Ch+C coated samples during the same period, indicating extensive hydrolysis of lipids. The quality of rainbow trout fillet coated with chitosan and chitosan enriched with cinnamon oil is showed better than without coating as coating retard lipid oxidation.

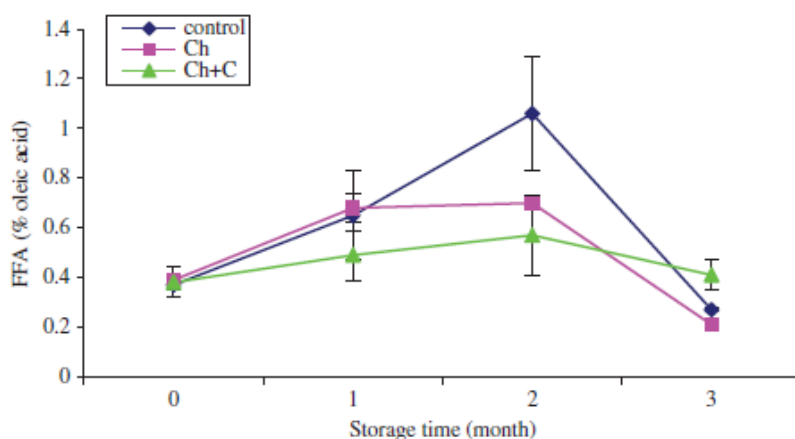


Fig. 10: Changes in FFA values of rainbow trout fillets during frozen storage at -18°C (Source: Seyed et al., 2014)

Sensory Evaluation of Cooked Fish Fillet

Table 2 represents the sensory analysis on cooked rainbow trout fish fillet. Results obtained from uncoated controls, Ch coated, and Ch+C coated samples indicates that the coatings are imperceptible; and the coating treatments, at the concentrations used, do not produce undesirable sensory properties. However, there is significant difference in texture, off-flavor, taste, and overall acceptability of fresh fish and fish stored (uncoated and coated fish) at -18°C . Frozen fish scores lower than the fresh fish in all parameters, except for appearance for which there was no significant difference. Chitosan coating and chitosan combined with cinnamon oil score are higher than uncoated fish fillets. Differences in the texture of frozen samples from the fresh sample may be attributed to denaturation of proteins, particularly the myofibrillar proteins that contribute greatly to the textural properties of fish (Namulema et

al., 1999). Changes in flavor of frozen samples may be due to the formation of low molecular weight compounds from lipid oxidation.

Table 2: Changes in attributes scores of fish samples during frozen storage at $-18\text{ }^{\circ}\text{C}$

Treatment	Texture	Off-flavor	Taste	Appearance	Overall acceptability
Fresh	5.00 ± 0.00^a	0.00 ± 0.00^b	5.00 ± 0.00^a	4.66 ± 0.21^a	8.66 ± 0.21^a
Uncoated	4.16 ± 0.21^b	0.83 ± 0.30^{ab}	4.16 ± 0.21^b	4.33 ± 0.21^a	6.83 ± 0.30^b
Chitosan	4.16 ± 0.21^b	1.00 ± 0.36^{ab}	4.33 ± 0.21^{ab}	4.33 ± 0.21^a	7.00 ± 0.25^b
Chitosan + cinnamon	4.16 ± 0.2^b	1.00 ± 0.25^a	3.83 ± 0.21^b	4.83 ± 0.16^a	7.00 ± 0.25^b

^{a,b,c} Means with different small letters in the column represent significant difference at $p < 0.05$.

The attributes are texture (0 = very rubbery to 5 = smooth fresh fish texture), appearance (1 = very poor to 5 = excellent), off-flavor (0 = absent to 5 = extremely strong), taste (0 = very poor to 5 = excellent), and overall acceptability (1 = dislike extremely to 9 = like extremely). (Source: Seyed et al., 2014)

3.8 Effect of Chitosan (1.5%, w/v) Coating Combined with Citric Acid (1%, w/v) or Licorice (1%, w/v) Extract on the Quality of Ovate Pompano (*Trachinotus Ovatus*) Fillets during Frozen Storage for 6 Months

Changes in pH

Figure 11 shows that the pH values of treatment samples are lower initially than the control samples. The pH values of the chitosan and citric acid solution, chitosan solution, and chitosan and licorice extract solution are 4.1, 4.7 and 4.8, respectively. The pH of the control samples is dropped on month 2 and slowly increased to 6.86 during 6 months of frozen storage. The pH of all the other treatments samples is increased slightly before month 3 and then decreased slightly, and ended with a higher value at the end of storage ($P < 0.05$). All the treatment samples have a lower pH than the control during the later storage period ($P < 0.05$).

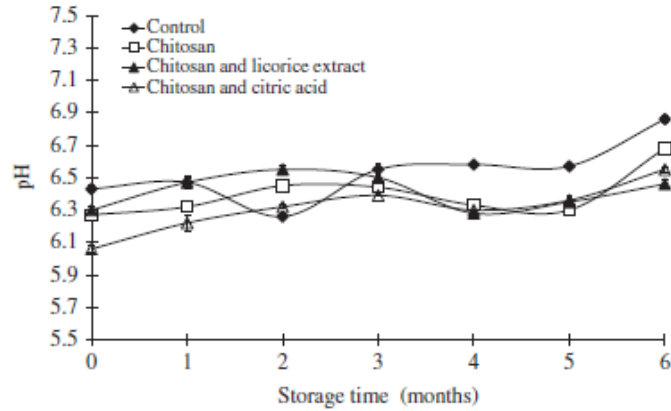


Fig. 11. Changes in pH of ovate pompano fillets during storage at -18 °C. (Source: Xujian et al., 2016)

Changes in PV

In Figure 12, PV is increased in all samples during 6 months of frozen storage ($P < 0.05$). PV is increased quickly during the first month of storage, then increased dramatically again after 4 months. It indicates that, during the whole frozen storage period treatment samples with chitosan, chitosan and citric acid or licorice extract combination have a lower PV than the control sample. The inhibition effects are clearly shown during later storage, with chitosan and licorice extract treatment the most effective, followed by chitosan and citric acid treatment, and chitosan treatment alone ($P < 0.05$). At the end of storage, chitosan, chitosan and citric acid, chitosan and licorice extract reduce PV to 2.96, 2.58, and 2.18 meq active oxygen kg^{-1} lipid respectively in the treatment samples, while the PV value of the control is 4.31 meq active oxygen kg^{-1} lipid. Chitosan may chelate ferrous ions in fish proteins and thus eliminate their pro-oxidant activity or their conversion to ferric ions. Licorice extract can have strong hydrogen peroxide scavenging activity as it represents better result than others.

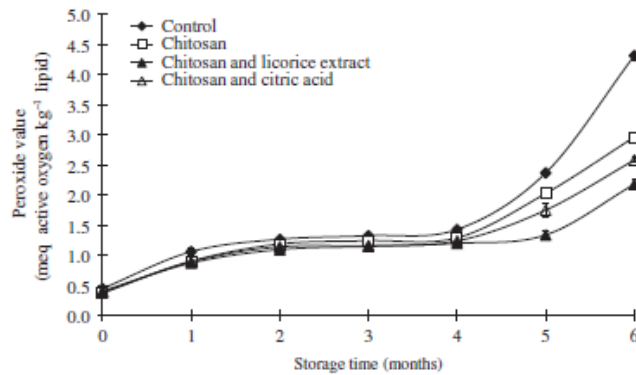


Fig. 12: Changes in peroxide value (PV) of ovate pompano fillets fish fillets during storage at -18 °C. (Source: Xujian et al., 2016)

Changes in TBARS Value

In Figure 13, the TBARS value in the controlled sample is increased dramatically from 0.22 to 3.05mg MA eq kg⁻¹ during 6 months of storage, suggesting significant lipid oxidation. For all the treatments samples, TBARS values do not increase until month 4. Starting from month 1, all treatments significantly inhibits the TBARS values compared to the control ($P < 0.05$). At the end of storage, chitosan, chitosan plus citric acid, and chitosan plus licorice extract reduce TBARS to 1.56, 1.35 and 1.11mg MA eq kg⁻¹ in the treatment samples, whereas the TBARS of the control is 3.05mg MA eq kg⁻¹. Chitosan coating can serve as a good barrier to oxygen permeation and thus inhibit lipid oxidation. It is found that polyphenols in the licorice may act as a strong radical scavenger or may reduce the potency of pro-oxidative metal ions in meat. The combination of chitosan and citric acid or licorice extract may offer synergistic antioxidant effects owing to their different antioxidant mechanisms.

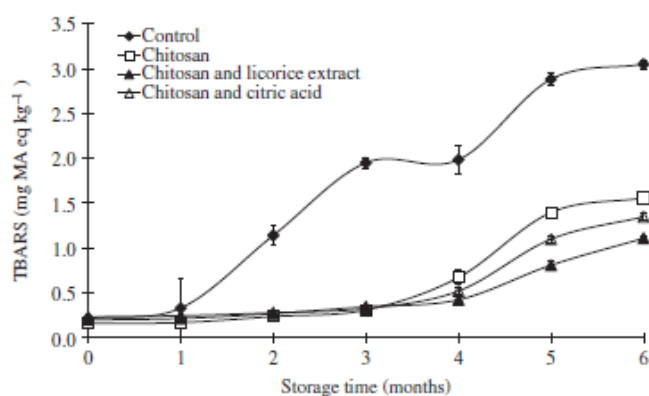


Fig. 13: Changes in thiobarbituric acid reactive substances (TBARS) of ovate pompano fish fillets during storage at -18°C . (Source: Xujian et al., 2016)

Changes in Free Fatty Acid (FFA)

In Figure 14, the changes in FFA content fish lipid extracted from different treatment samples during storage are shown. Significant lipid hydrolysis is occurred in storage for all treatments ($P < 0.05$). Lipid hydrolysis development is higher in control sample than other three treatment samples after 2 months of frozen storage ($P < 0.05$). On month 5, chitosan combined with citric acid or licorice extract show a significant drop in FFA compared to chitosan alone ($P < 0.05$). At the end of storage, chitosan and licorice extract treatment sample have the lowest FFA among all the treatment samples.

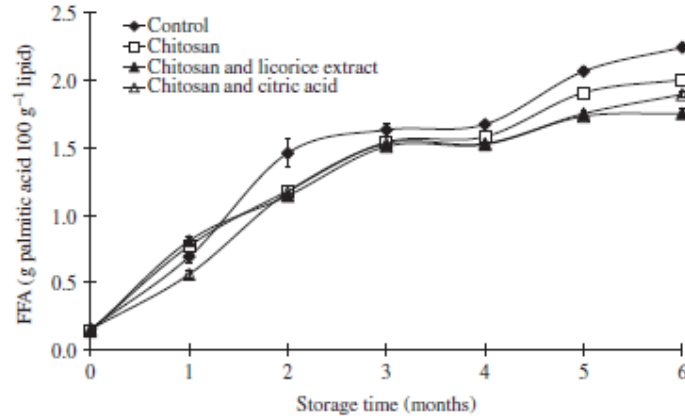


Fig. 14: Changes in free fatty acid (FFA) of ovate pompano fillets during storage at $-18\text{ }^{\circ}\text{C}$ (Source: Xujian et al., 2016)

Drip Loss

In Figure 15, it is showed that drip loss is increased with time for both the control and treatment samples during frozen storage. All treatments have lower drip loss compared to the control from month 3 to the end of storage ($P < 0.05$). At the end of storage, chitosan plus licorice extract, chitosan plus citric acid, and chitosan treatment samples reduce the drip loss to 7.41%, 8.51% and 9.08%, respectively, in the treatment samples, while the drip loss of the control is 11.80%. Although citric acid does not show much additional effect on reduction of drip loss, licorice extract enhances the reduction of drip loss from month 4 to month 6 ($P < 0.05$).

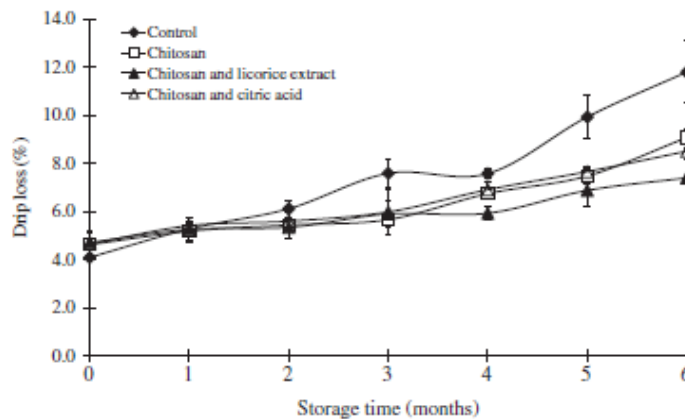


Fig. 15: Changes in drip loss of ovate pompano fillets during storage at $-18\text{ }^{\circ}\text{C}$. (Source: Xujian et al., 2016)

Chapter IV

Conclusions

Fish fillet is very demandable fishery product all over the world. Many food items like fish burger, fish finger, fish fry are prepared from fish fillets. The shelf life of fish fillets is not satisfactory comparing with whole fish. The quality of fish fillets gets degraded due to microbial attack, lipid oxidation and protein denaturation. Most studies on freezing have been focusing on increased shelf life and quality changes of fish fillets during storage. Freezing is done commercially for the storage of fish fillets but it is not enough for long time storage. Long time freezing degrades the quality of fish. The Many research has been conducted to increase the shelf life of frozen fish fillets with retaining proper quality.

Development of natural preservative coatings or films with high antioxidant, antibacterial activities that prolong the shelf life of fish fillets is desirable. Many natural herbs, spices, essential oils, peptides have been incorporated to increase products quality. In this sense, chitosan and natural extracts has attracted much attention. and hence improve overall fish quality and extent its shelf-life.

Food packaging is an important sector of the industry where nanotechnology applications are beginning to live up to their promise. The role of chitosan on frozen storage of fish has attracted the attention of scientists, food professionals, entrepreneurs and environmentalists. Chitosan coating acts upon lipid oxidation and decrease free fatty acid formulation. It diminishes peroxide formation rate, malondialdehyde formation that prevents spoilage of frozen fish. It also acts as a barrier for microbial attack. Natural extracts such as cinnamon oil, licorice extract have showed anti-microbial activity and anti-oxidant activity upon frozen storage of fish fillets. Chitosan combined with natural extracts that having anti-oxidant, antimicrobial effect boosts the preservative action towards frozen products. Chitosan combined with natural extracts coating have showed better results than uncoated or only chitosan coated frozen fish fillets. It can be applied in commercially for prolonging shelf life of frozen fish fillets with ensured better quality.

Chapter V

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