DEVELOPMENT OF SMOKED AND GELATIN-BASED

PRODUCTS FROM CATFISH

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DEVELOPMENT OF SMOKED AND GELATIN-BASED PRODUCTS FROM CATFISH

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PRODUCTS FROM CATFISH

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Mingkang Jiang, son of Lianmin Jiang and Qingzhi Sun, was born on June 19, 1979, in Yantai, China. He attended Dalian Fisheries University in Dalian, Liaoning, in 1998, and graduated with a Bachelor's degree in Aquaculture in 2002. In summer 2004, he came to Auburn University and worked as graduate research assistant under Dr. William H. Daniels with the Department of Fisheries and Allied Aquacultures. After graduating with a Master's degree in Aquaculture in fall 2005 he continued his Ph.D. study on the seafood processing under Dr. Yifen Wang with the Department of Biosystems Engineering in Auburn University. He married Yinfeng Zhang in summer 2005. They have a son whose name is Austin.

DISSERTATION ABSTRACT

DEVELOPMENT OF SMOKED AND GELATIN-BASED

PRODUCTS FROM CATFISH

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Catfish is the most important aquaculture species in the southern United States. A major part of the catfish production is processed and sold in raw (fresh or frozen) form. There is a lack of catfish product forms in the market which can potentially add value to the existing products and create more job opportunities.

The potential of hot-smoked catfish fillets was studied as a value-added and ready-to-eat (RTE) catfish product. Textural properties play an important role in the quality control and acceptability of both raw and processed products. Consequently, textural properties of raw and smoked catfish fillets were measured by “finger” or “tooth” methods together with a novel sampling technique. The “finger” method was proven to

be a better method for texture measurement on catfish fillets. The sampling technique was rapid and applicable to most irregular fillet shapes, including catfish and other fish species. Brining of fish is one of the critical control points (CCPs) in the hot-smoked fish products. Brining behavior of fresh catfish fillets was studied. Salt content of smoked catfish was determined by both the traditional titration method and novel near infrared spectroscopy (NIR) methods. Nonlinear and multiple linear regression (MLR) models were developed to predict the catfish brining behavior. In order to evaluate products' shelf-life, hot-smoked catfish fillets were individually packaged in film with different oxygen transmission rates (OTR, 0, 4,000, and 10,000 $cm^3/m^2/24h/atm$ at 20 °C and 0%RH) and stored at either 4 or 25 °C. Shelf-life of 53-, 35-, and 33-day was observed for samples stored at 4 °C and packaged with 0, 4,000, and 10,000 OTR films, respectively. Shelf-life of 3 to 5-day was observed for samples stored at 25 °C.

The utilization of catfish processing by-product was also studied. Gelatin was produced from catfish skin by thermal extraction. Gelatin film was prepared with different levels of triacetin in the film forming solutions. Structures of the films were examined using a transmission X-ray microscope (TXM). Addition of triacetin caused decreased tensile strength (TS) and increased percent elongation (E%), water solubility, UV and visible light barrier properties of the film.

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I. INTRODUCTION

Statement of the problem

Catfish is the most important freshwater aquaculture species in the southern part of United States. Over 90% of the total catfish production comes from four states: Mississippi, Alabama, Arkansas, and Texas. Every year about 95% of the total catfish produced are processed into different forms of products, such as dressed, fillets, and nuggets. According to catfish processing data reported by NASS, USDA (Feb 18, 2009), farm-raised catfish processed during 2008 totaled 2.31×10^5 metric tons (510 million pounds). The average price paid to producer in 2008 was $1.71 per kilogram ($0.77 per pound). After processing, about 1.14 × 105 metric tons (251 million pounds) of catfish were sold. The average price sold for processed catfish was $5.38 per kilogram ($2.44 per pound). However, profit margin of the catfish is restricted by its limited product forms. Value-added products from catfish are scarce in the market indicating a great need to fill the gap.

Suppose there is a value-added catfish processing plant with a processing capacity of 453.6 kilograms (1,000 pounds) per day. The plant would require 1.95×10^5 kilograms of raw material if it runs 280 days per year, which is only 0.17% of the total amount of catfish sold after being processed. Given a 65% yield of the value-added catfish product and a wholesale price of $17.64 per kilogram ($8.00 per pound), it would

have a market of $2.24 million. The profit would be $1.19 million, if the raw material cost ($1.05 million) is deducted.

Of all the existing processing techniques, smoking has great potential due to its low initial investment cost and ease of manipulation. Smoking is an ancient preservation method for fish and other meats. Fish is typically salted before being dried and smoked. Both salting and drying lower the water activity (a_w) in the fish. In addition, smoking introduces antioxidant and bacteriostatic effects to the fish thus extending its shelf-life.

Typically, smoking of fish starts with receiving the raw material (fresh or frozen), (thawing if frozen), cleaning, brining, drying, smoking, cooling, packaging, and storage. Standard sanitation operation procedures (SSOPs) and standardized, safe processing or quality assurance (QA) procedures are required for commercial food products, including fish. Few studies have been carried out on quality control aspects of smoked catfish processing. Therefore, this study included quality control of hot-smoked catfish products such as textural properties, brine control, and shelf-life studies.

Texture is an important quality indicator for fish products. Textural properties of fish are mainly determined by its moisture and lipid content. Textural properties of fish are normally conducted through sensory evaluation which is a subjective process. Currently, there is no standard method for objective measurement of fish texture.

Fish has to be salted (brined) before smoking. Salt not only brings flavor to the fish, but also lowers water activity (a_w) and creates an unfavorable environment for most bacteria. Most bacteria vegetative cells can be destroyed during the smoking process. However, their spores may be hard to eliminate. The current FDA guidelines for hot-smoked fish products in reduced oxygen packaging requires a minimum of 3.5% water

phase salt (WPS) in fish muscle (without other preservatives) so that toxin production by *Clostridium botulinum* can be inhibited. The only time salt can be introduced into the fish muscle is during brining at which time fish are submerged under a saline solution for a fixed time period. Control of the brining will be a critical control point (CCP) in fish smoking.

Hot-smoked catfish is still perishable due to its high moisture content. Retort pouch packaging can destroy all the bacteria including their spores. Unfortunately, the cost of retorable pouch is high and this process often negatively influences fish texture. Vacuum packaging and refrigerated storage are normal methods to extend the shelf-life of fish. However, vacuum packaging creates a favorable living condition for facultative bacteria and anaerobic bacteria such as *C. botulinum*. Vacuum packaging film with certain oxygen permeability may prolong the shelf-life of hot-smoked catfish and inhibit the germination of *C. botulinum* spores at the same time.

In addition to value-added strategies, there are a number of by-products that are underutilized. During the processing of catfish, the catfish skin is normally discarded as a processing waste. Catfish skin, however, accounts for about 6% of processed fish weight. As a rich source of collagen, catfish skin can be an alternative source for gelatin. Extraction of gelatin from catfish skin involves acid or alkaline pretreatments followed by thermal extraction and filtering. Traditionally, gelatin is obtained from bones and hides of mammals (mainly beef and pork). However, gelatin from these two sources may not be acceptable for kosher (Jewish) and halal (Muslim) foods or for people concerned about bovine spongiform encephalopathy (BSE) or foot-and-mouth disease (FMD).

Gelatins extracted from fish products, including catfish skin gelatin, have received a lot of attention in recent years.

Research objectives

The main purpose of this study was to develop profitable value-added hot-smoked catfish product which could provide additional revenue. Several quality-related issues had to be studied to make this product a high quality and shelf-life stable product. Thus, the objectives of this study are as follows:

1. To develop a reliable instrumental texture measurement method with an easy and novel sampling technique for both raw and smoked irregular shape catfish fillets.
2. To investigate the brine behaviors of fresh catfish fillets.
3. To develop and standardize the procedure to produce hot-smoked catfish.
4. To evaluate the shelf-life of hot-smoked catfish.
5. To extract gelatin from catfish skin and use it as primary material to make edible films.

II. LITERATURE REVIEW

Global seafood production increased steadily and reached a record 143.6 million metric tons in 2006 according to a United Nations' Food and Agriculture Organization report published (Table 1, FAO, 2009). In general, seafood refers to edible seawater finfish, mollusks, crustaceans and seaweeds. An extended definition includes edible freshwater species. Hence, the most common definition includes all animals living in water. Given that water covers about 71% surface of our globe, there is a great deal of diversity of seafood. There are more than 20,500 known fish species, which outnumber all the other vertebrates on earth (Eyo, 2001). According to the FDA database, there are over 1500 accepted species of imported and domestic finfish and shellfish in the U.S market (http://vm.cfsan.fda.gov/~frf/seaintro.html).

Per capita consumption of seafood in the U.S has steadily increased up to 7.25 kilograms (16 lbs) annually (Table 2, National Fisheries Institute). Seafood has more unique features compared to those of land animals. This is primarily due to properties of the aquatic environment in which they are living. The amount of protein in seafood varies from species to species and even within species. Normally an edible finfish fillet contains about 18 to 22 percent protein (wet base). The protein content of crustaceans (crabs, shrimp) and mollusks (oysters, clams, scallops, etc.) can be higher or lower, respectively (Dean, 1990). Seafood protein supplies all the essential amino acid needed for human tissue building and repair and is of high biological value (Huss, 1988). Seafood protein

Table 1. World fisheries and aquaculture production from 2002-2006.

Production	2002	2003	2004	2005	2006
	(million metric tons)				
Inland					
Capture	8.7	9	8.9	9.7	10.1
Aquaculture	24	25.5	27.8	29.6	31.6
Total inland	32.7	34.4	36.7	39.3	41.7
Marine					
Capture	84.5	81.5	85.7	84.5	81.9
Aquaculture	16.4	17.2	18.1	18.9	20.1
Total marine	100.9	98.7	103.8	103.4	102
Total capture	93.2	90.5	94.6	94.2	92
Total aquaculture	40.4	42.7	45.9	48.5	51.7
Total world fisheries	133.6	133.2	140.5	142.7	143.6

Table 2. Top 10 U.S. per capita seafood consumption by species in pounds from 2002-2007.

	2007		2006		2005	
Rank	Species	Lbs	Species	Lbs	Species	Lbs
1	Shrimp	4.1	Shrimp	4.4	Shrimp	4.1
2	Canned tuna	2.7	Canned tuna	2.9	Canned tuna	3.1
3	Salmon	2.4	Salmon	2.0	Salmon	2.4
4	Pollock	1.7	Pollock	1.6	Pollock	1.5
5	Tilapia	1.1	Tilapia	1.0	Catfish	1.0
6	Catfish	0.9	Catfish	1.0	Tilapia	0.8
7	Crab	0.7	Crab	0.7	Crab	0.6
8	Cod	0.5	Cod	0.5	Cod	0.6
9	Clams	0.4	Clams	0.4	Clams	0.4
10	Flatfish	0.3	Scallops	0.3	Flatfish	0.4
	Total all species	16.3		16.5		16.2
Rank	2004		2003		2002	
	Species	Lbs	Species	Lbs	Species	Lbs
1	Shrimp	4.2	Shrimp	4.0	Shrimp	3.7
2	Canned tuna	3.3	Canned tuna	3.4	Canned tuna	3.1
3	Salmon	2.2	Salmon	2.2	Salmon	2.0
4	Pollock	1.3	Pollock	1.7	Pollock	1.6
5	Catfish	1.1	Catfish	1.1	Catfish	1.1
6	Tilapia	0.7	Cod	0.6	Cod	0.7
7	Crab	0.6	Crabs	0.6	Clams	0.6
8	Cod	0.6	Clams	0.5	Crabs	0.5
9	Clams	0.5	Tilapia	0.5	Flatfish	0.4
10	Flatfish	0.3	Scallops	0.3	Tilapia	0.3
	Total all species	16.6		16.3		15.6

is also especially digestible because it has very little connective tissue, which is also the reason that seafood does not develop tenderness during cooking (Nettleton, 1987).

Seafood is also rich in unsaturated fat while the total fat content is relatively low (about 5%). Meats from most animals are rich in saturated fats, the fats that raise blood cholesterol levels and cause clogging of blood vessel. Seafood is low in such saturated fats and rich in long chain omega-3 polyunsaturated fatty acids (PUFA), which includes alpha-linolenic acid (ALA, C18:3) and its longer-chain metabolites: eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). Beneficial health effects of omega-3 PUFA, especially long chain EPA and DHA, are well demonstrated, mainly in the prevention of cardiovascular diseases (Brunner & Iso, 2008).

The unsaturated fats in seafood, however, make it susceptible to oxidation after harvest. Other factors, such as reactions caused by the activities of the fish's own enzymes and the metabolic activities of micro-organisms, contribute to the spoilage of fish and shellfish as well (Ashie, Smith, & Simpson, 1996). Because seafood is highly perishable, a considerable effort has been directed to extend the shelf-life of seafood using preservation and processing techniques, such as refrigeration, freezing, canning, smoking, salting, and drying. What’s more, some of these techniques can also be used to enhance the value of seafood. Smoking seafood is the one of them.

Fish smoking

Smoking is a method that utilizes smoke to introduce flavor, taste, and preservative ingredients into the food. It is one of the oldest methods that has been used to process and preserve food (Dore, 1993). The drying effects during smoking, together with the antioxidant and bacteriostatic effects of the smoke, allow smoked products to

have extended shelf-life (Eyo, 2001; Hall, 1997). Smoking is commonly applied to fish (Cardinal, Cornet, Serot, & Baron, 2006; Varlet et al., 2007) and meat products (Poligne, Collignan, & Trystram, 2002; Modi, Mahendrakar, Sachindra, & Rao, 2004) but also to other food categories, such as cheese (Suchanova, Hajslova, Tomaniova, Kocourek, & Babicka, 2008) and mushroom (Eissa, Fouad, & Shouk, 2008). Developments of modern food preservation technology, such as pasteurization, cooling/refrigeration, deep-freezing, and vacuum packaging, have eclipsed the preserving functions of many traditional methods including smoking. Nowadays, the main purpose of smoking has been shifted for sensory quality rather than for its preservative effect.

Depending upon how the smoke is delivered into the food and smoking temperature, four basic types of smoking can be defined: hot smoking, cold smoking, liquid smoking, and electrostatic smoking (Wheaton & Lawson, 1985). Hot smoking is the traditional smoking method using both heat and smoke, which usually occurs at temperatures above 70 °C. For smoked fish and fisheries products, a minimum thermal process of 30 min at or above 145 °F (62.8 °C) is required by FDA (2001). Therefore, after hot smoking, products are fully cooked and ready for consumption.

Torry smoking kiln was introduced in the early 1960s by United Kingdom's Torry Research Station. The Torry smoking kiln is considered as a model for the modern smokers/smokehouses by enabling the precise controls of the heating temperature, air ventilation, and smoke density. Some recently designed smokehouse may also be equipped with more precise time and temperature controls, humidity control, and product internal temperature monitor probes. Thus, the products produced by the modern smokehouses are much more uniform than those produced with traditional smokers. Hot

smoking is typically not a single process. Several other steps such as brining, drying and smoking are also involved to produce a product of good quality. A detailed hot smoking process will be explained later.

Fish can also be subjected to cold smoking. Temperatures of cold smoking typically do not exceed 30 °C. Thus, cold smoked products are not cooked and typically heavily salted. Compared to the traditional hot smoking, cold smoking runs longer, has a higher yield and retains the original textural properties much better than the hot-smoked ones. Cold smoking of varied fish species has been reported, including rainbow trout (Lyhs, Bjorkroth, Hyytia, & Korkeala, 1998), cod (Dillon & Patel, 1993), tuna (Emborg & Dalgaard, 2006), and salmon (Joffraud et al., 2006).

Liquid smoke is smoke condensate that is dissolved in a solvent, such as water or oil (Maga, 1988). Liquid smoke can be used directly on products by dipping or spraying. It is rapid and much easier to achieve a uniform smoke flavor than traditional cold and hot smoking processes, although the flavor and color from the traditional smoking can not be exactly duplicated (Varlet et al., 2007). Higher processing yield and similar textural properties to traditional smoked salmon were reported (Birkeland & Skara, 2008). Some potential harmful ingredients (e.g. polycyclic aromatic hydrocarbons, PAHs) in the nature smoke can be separated out and excluded from the liquid smoke (Chen & Lin, 1997). Other advantages of liquid smoke include easy modification, application to food items that traditionally are not smoked, lower operation cost, and less environmental pollution (Simon et al., 2005; Abu-Ali & Barringer, 2007). However, the application of liquid smoking may be expensive compared to other methods (Varlet et al., 2007). Liquid smoking of fish species had been reported on swordfish (Muratore & Licciardello, 2005),

salmon (Martinez, Salmeron, Guillen, & Casas, 2007), and rainbow trout (Visciano, Perugini, Conte, & Arnorena, 2008).

Electrostatic smoking is another rapid way to smoke. In the electrostatic smoking, fish are sent into a tunnel where an electrostatic field is created. Smoke particles are given a positive charge and deposit onto the surface of the fish which are negative charged. Although this procedure will change the composition of the smoke, the efficiency of smoking is still higher than that of the traditional smoking. It can also be operated continuously. Similar textural properties of salmon to the hot smoking could be obtained from electrostatic smoking (Sigurgisladottir, Sigurdardottir, Torrissen, Vallet, & Hafsteinsson, 2000b). The smoke compound ratio in the vapor phase may be modified by the electrostatic field, which results in increased level of carbonyl compounds (Ruiter, 1979). Factors that may influence the electrostatic smoking operation include the skin thickness, presence of scales, and subcutaneous fat amount (Maga, 1988). This operation may present safety problems to employees. Applications of electrostatic smoking have been reported mainly in salmon (Cardinal et al., 2001; Montero, Gomez-Guillen, & Borderias, 2003) and herring (Cardinal et al., 2006).

Hot smoking of fish

Good smoked products can only be obtained from good raw material (Dore, 1993). In addition, control of the smoking procedures play an equal importance in the production of good products. From raw material preparation to final product storage, smoking includes several operations, such as brining, drying, smoking, packaging and storage.

Brining

This is the stage when the flavors and spices are introduced into the fish. Cleaned fish are submerged under a prepared brine solution for a certain amount of time. A brine time less than 12 hours at 3.3 °C (38 °F) is recommended to minimize the possible spoilage in the fish (Lee, 1977). Salt is an important ingredient to be delivered into the fish tissue at this stage as well as a key hazard analysis and critical control point (HACCP) preventive measure for smoked fish. Not only does it bring the taste but also reduces the water activity (a_w) in the product, so that bacterial growth can be inhibited in the smoked fish.

Of all the bacteria that can exist in fish products, *Clostridium botulinum* is a major concern for vacuum or reduced packaged fish products. *C. botulinum* is a strictly anaerobic, gram positive bacillus bacterium. It is ubiquitous and its spores are naturally present in soil, as well as both fresh-and salt-water mud (Dunbar, 1990). Although food illness caused by botulinum neurotoxins is rare, its mortality rate is high. Botulinum toxin is one of the most toxic compounds known. A dosage as little as 0.3 ng/kg can cause fatality in mice. Human lethal dosage is believed to be 0.2 to 2.0 ug/kg (Sharma & Whiting, 2005). Typical clinical illness is characterized by vomiting, constipation, respiratory difficulties or heart failure in humans (Adam & Moss, 2000). There were 962 recorded botulism outbreaks in the United States from 1899 to 1990, which resulted in 2320 cases and 1036 deaths (Solomon & Lilly, 2001). The number of cases dropped to 263 from 160 food borne botulism events between the year 1990 to 2000 (Sobel, Tucker, Sulka, McLaughlin, & Maslanka, 2004). The fatality rate was also decreased to 4%. Based on the serological properties of the toxins that were produced, there are seven

types of *C. botulinum* numbered from A through G (Lietzow, Gielow, Le, Zhang, & Verhagen, 2008). Humans are only sensitive to type A, B, E and F (Ball et al., 1979; Hambleton, 1992; Sharma et al., 2005; Dahlsten, Korkeala, Somervuo, & Lindstrom, 2008). Type C and D are responsible for animal and avian botulism (Yoneyama et al., 2008). Type A, B, E, and F can be further classified into two groups: proteolytic (types A, B, and F) and nonproteolytic group (types B, E, and F). The proteolytic group is mesophilic and grows at temperatures above 10 °C and optimally at 35-37 °C. The nonproteolytic group is psychrotrophic and can grow at temperatures as low as 3 °C and optimally at 26-30 °C (Lindstrom, Kiviniemi, & Korkeala, 2006). Foodborne botulism outbreaks related to fishery products are largely caused by the nonproteolytic type E strains of *C. botulinum* because the type E is an aquatic organism (Huss, 1980; Korkeala et al., 1998).

The vegetative cells and their neurotoxins can be easily destroyed by heat (less than five minutes) at 85 °C. However, their spores are very resistant to heat and can survive for up to 2 hours at 100 °C (Caya, 2001). Thus, prevention of botulism from hot smoked fish products depends on the destruction of all *C. botulinum* spores or inhibition germination of the spores that may be present in the products.

Water phase salt (WPS) is used to measure the amount of salt in the fish products. The WPS is calculated as (FDA, 2001):

$$WPS = \frac{\%Salt}{\%Salt + \%Moisture} \times 100 \quad (1)$$

The higher the WPS value, the less the availability of the water. When sodium chloride is the only major humectant in the cured food, the relationship between the a_w and WPS can be express as (Ross & Dalgaard, 2004):

$$a_w = 1 - 0.0052471 \cdot WPS\% - 0.00012206 \cdot (WPS\%)^2 \quad (2)$$

or

$$WPS\% = 8 - 140.07 \cdot (a_w - 0.95) - 405.12 \cdot (a_w - 0.95)^2 \quad (3)$$

Current regulations require at least 3.5% WPS in the loin muscle of the vacuum packaged smoke products; at least 3.0% WPS if at least an additional 100 ppm nitrite exists in the vacuum packaged product; air packaged smoked fish products must contain at least 2.5% WPS (FDA, 2001).

Several salting methods are available to deliver the salt into the fish. The most common techniques used by the industry are dry and brine salting (Yanar, Celik, & Akamca, 2006; Aursand, Gallart-Jornet, Erikson, Axelson, & Rustad, 2008). Dry salting is widely used in low fat fish. Basically, fish are put into layers with dry salt separating each layer. Water removed by salt is allowed to drain away. Periodical reshuffling of the layers may be necessary to make sure all the fish get uniform salting and pressure. Muscle fiber shrinks more during dry salting than brine salting (Sigurgisladottir et al., 2000b). Thus, dry salting of fish typically results in over-dried fish and low yield. A better quality and higher yield is usually obtained from brine salting (Beraquet, Iaderoza, Jardim, & Lindo, 1983).

Fish are brine salted by completely being covered in a prepared brine solution for a certain time period. The brine solution can have a salt concentration from relatively low to saturated levels. Brine salting is also used widely for most fatty fish since oxygen can not oxidize the fish fat easily. Some modern processors inject the brine to speed up the process, therefore lowering the cost and minimizing the chance of fish deterioration (Birkeland, Akse, Joensen, Tobiassen, & Skara, 2007; Akse, Birkeland, Tobiassen,

Joensen, & Larsen, 2008). Salt is distributed evenly in the fish when injection brine is used. A higher brine yield can be obtained through injection brine as compared to brine or dry salting (Rora, Furuhaug, Fjæra, & Skjervold, 2004). Flavor ingredients can also be incorporated into the injection solution. However, the injecting brine operation has to be carefully controlled to avoid contamination delivered by the needles into the previously sterile flesh (Dore, 1993). Brine salting is still one of the most widely used salting methods for smoked fish. Efficiency of salt penetration into the fish tissue is affected by several factors, such as species (Gallart-Jornet, Barat, Rustad, Erikson, Escriche, & Fito, 2007a), physiological state of fish (rigor), fish quality (fresh/frozen) fish dimension (thickness), brine concentration, brine time, brine to fish ratio, brine temperature, fat content, texture, etc. (Jittinandana, Kenney, Slider, & Kiser, 2002).

Rigor mortis is used to measure the postmortem change in fish. Fish muscles are totally relaxed immediately after death, which means that they are soft and pliable (pre-rigor). Shortly after that, the muscle will begin to contract and become hard and stiff, which is called “in-rigor”. Finally, the muscles return to a relaxed state again, which is the “post-rigor” stage (Huss, 1988).

Very limited research has been conducted on the influence of postmortem state to the salt uptake of fish. A finite difference model was developed to simulate the salt infusion into the Atlantic salmon muscle. It was found that the pre-rigor salmon had the lowest salt concentration and also less uniform salt distribution inside the salmon than the in-rigor and post-rigor fish. The in-rigor fish also responded better to the salt concentration increases than the other states (Wang, Tang, & Correia, 2000). A lower salt uptake rate and equilibrium concentration was reported in pre-rigor salmon fillet than

those of in-rigor and post-rigor fillets when they were subjected to a 20% (w/v) sodium chloride brine at 10 °C (Wang, Tang, Correia, & Gill, 1998). Similar results were also reported in cod (*Gadus morhua* L.) by Lauritzsen, Akse, Johansen, Joensen, Sorensen, & Olsen (2004) when a lower salt uptake and higher moisture loss were found in pre-rigor fish than those of post-rigor fish.

Freezing of fresh raw material or smoked product is common in the seafood supply chain to meet market need and make more profits (Sigurgisladottir, Ingvarsdottir, Torrissen, Cardinal, & Hafsteinsson, 2000a). Whether fish was previously frozen or fresh affects the salt migration rate into the muscle greatly. The water in the fish cells is not pure, but most of them will be changed into ice crystal in the temperature range -5 to -1 °C. At the beginning of ice formation, tiny ice crystals are formed around numerous nucleuses that are randomly distributed in the cell fluid. These ice crystals will then aggregate and grow into larger size crystal until all the available free water is converted into ice, which is called recrystallization. This is a typical ice formation procedure under normal pressure and freezing conditions. There will be a 9% increase in the volume after the water is changed from liquid to solid phase (Ice density= 0.9167 g/cm^3 at 0 °C). Although the cell walls may be elastic enough to endure the change in volume, there are possibilities that some may rupture during this process and the intercellular fluid will run off. Additinally, the shape of the ice crystal may be too sharp for the cell wall, thus damaging to the cell wall which will result in more fluid running out the cells. Some inevitable results from freezing are the change in texture of the fish tissue (Sigurgisladottir et al., 2000a; Zhou & Li-Chan, 2009) as well as the ability to uptake salt.

Studies showed that previously frozen fish take up salt more quickly than fresh fish due to the damage to the muscle cells after the freezing (Deng, 1977; Stefansson et al., 2000).

Brining of fish always starts with the preparation of brine solution. A handy instruction table by Hilderbrand (1999) can be used to facilitate this process and make reliable brine solutions.

Brine concentration affects the rate of salt diffusion and also the equilibrium salt content in the muscle. A positive correlation can be found between the brine concentration and the salt diffusion rate (Poernomo, Fawzya, & Ariyani, 1992; Lawrie, 1998). Previous research conducted on yellowtail (*Trachurus mccullochi* Nichols), which was brined in 5, 10, 15, 21% and saturated brine solutions, showed that fish brined in higher brine concentrations absorbed salt more rapidly and reached a higher equilibrium salt content than those brined in lower concentrations (Berhimpon, Souness, & Edwards, 1990; Birkeland, Akse, Joensen, Tobiassen, & Skara, 1991). However, a higher brine concentration (17.4%) may also cause more moisture loss from the fish than a lower brine concentration (8.7%), resulting in lower moisture content of the brined and cooked fish (Jittinandana et al., 2002; Gallart-Jornet, Barat, Rustad, Erikson, Escriche, & Fito, 2007b). This is because a high brine solution may cause the denaturation of muscle protein which reduces its water holding capacity and results in releasing water into the brine (Nketsia-Tabiri & Sefa-Dedeh, 1995). There are studies showing that denaturation of protein might occur at brine concentration above 9-10% (Sigurgisladottir et al., 2000b; Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2004).

The decreased moisture content caused by increased brine concentration may further influence textural properties of the fish products since moisture is a major factor

determining the fish texture (Dunajski, 1979). When salmon fillets were treated with a series of brine solution (4%, 10%, 15%, 18%, 25% and dry salting) hardness of the fillet increased and elasticity decreased with the increasing brine concentration (Gallart-Jornet et al., 2007b). Jittinandana et al. (2002) also reported increased shear force on hot-smoked rainbow trout fillets previously brined at higher brine concentrations due to the lower moisture content.

It takes time for the fish to attain a certain salt content in the brine solution. Fish and brine will eventually reach an equilibrium salt content if they were left for an extended period of time. Not only will fish dehydrate more over the increased brining time, but the solubility of both myosin and actin will also increase in rainbow trout fillets (Jittinandana et al., 2002). Increasing salting time may also negatively affect color in some fish. Birkeland & Bjerkeng (2005) reported that the content of the carotenoid pigment astaxanthin, which made the main contribution to the attractive pink color of the salmon flesh, decreased with increasing salting time. Production yield may also be affected by the elongated brine time due to increased moisture loss from fish.

Brine temperature also influences the rate of brine uptake. The rate of salt diffusion is believed to be temperature dependent and increases with increasing temperature (Diaz, Wolf, Kostaropoulos, & Spiess, 1993; Chiralt et al., 2001; Corzo & Bracho, 2004). Other factors that may affect the brining results are brine to fish ratio, fish dimension (size, thickness), existence of skin, and fat content of the fish muscle. Fat has been reported as a blocking factor for salt and water diffusion during the salting due to its hydrophobic nature (Jason, 1965). Only a few studies (Wang et al., 2000; Cardinal et al., 2001; Rora et al., 2004; Gallart-Jornet et al., 2007b), have tried to identify the effect of

fat on the behavior of fish flesh during salting. Cardinal et al., (2001) reported that the water diffusion rate is faster in the flesh of lean fish than that in fat fish, which leads to more rapid drying and lower yield. In another study by Gallart-Jornet et al. (2007b) a lower salt uptake rate was also observed in salmon, which had a fat content of 12% compared to cod with a 0.5% fat content in the muscle and a higher salt uptake rate. After brining, fish have to be rinsed with clean water to remove the brine solution on its surface because a harsh, salty flavor can develop due to residues of brine solution.

Drying

It is widely know that reducing the water activity (a_w) will result in a reduction of microbial activity. The a_w is defined as:

$$a_w = p / p_0 \quad (4)$$

where p is the vapor pressure of the product, and p_0 is the vapor pressure of pure water at the same temperature (Olley, Doe, & Heruwati, 1989). For ideal solutions (real solutions at low concentrations), water activity can be calculated from the formula:

$$a_w = n_1 /(n_1 + n_2) \quad (5)$$

where n_1 is the number of moles of solvent, and n_2 is the number of moles of the solute. This relationship may become complex due to the interactions between moisture and the fish tissue and also the relatively high solute concentration involved in cured fish. Drying of the fish can still be simulated with the formula in a way that drying the fish will cause a decrease in n_1 and an increase in n_2, which finally decreases the a_w.

A certain amount of moisture has to be lost from fish after brining; so that water activity (a_w) can be decreased and a good texture can be obtained at the end of the smoking process. Drying of fish occurs at the early stage of smoking process. An air flow

is applied on the fish; so that moisture in the fish tissue can migrate to the surface and leave the fish by evaporation. The temperature, relative humidity and velocity of the air flow are keys to the rate of drying. Drying with a low relative humidity air at high velocity may not drive the moisture out of the fish fast. If the temperature are too high fish surface may be hardened at the beginning of drying resulting in a blocking layer to the inside moisture migration. The hardened surface may also prevent smoke penetrating into the tissue, which decreases the preservative effects of the smoke. Tissues under the hardened surface will tend to spoil from inside.

Drying at temperatures below 70 to 80 °C was recommended to minimize the damage to protein quality in fish (Opstvedt, 1989). Drying also influences the quality of finished smoked fish product. When trout were dried at 20 °C for different periods (2, 4, 6, 8, 16 and 24 hr) prior to liquid smoking, the samples that received 16 or 24 hr drying time was favored by the sensory evaluation as more brown colored and smoke flavored, although no statistical difference was observed between the samples among each group (Siskos, Zotos, & Taylor, 2005).

Smoking

Smoke is generated from the incomplete combustion of wood at certain temperatures followed by thermal disintegration or pyrolysis of high molecular organic compounds into volatile lower molecular mass (Eyo, 2001). Smoke is composed of two phases: a particulate or dispersed phase and a gaseous or dispersing phase. The major parts of dispersed phase are particles in the droplet form having an average diameter of 0.196 to 0.346 μm (Maga, 1988; Wheaton & Lawson, 1985). These particles are mainly tars, wood resins, and compounds with high or low boiling points. The dispersed phase is

the visible part of the smoke. The dispersing phase is responsible for flavoring, coloring, antioxidative, and bacteriostatic roles of the smoke (Hall, 1997). The composition of the dispersing smoke phase is complicated, many of which have yet been identified. More than 200 components have been identified. The most abundant chemicals found in smoke are carbonyls, organic acids, phenols, alcohols, and hydrocarbons.

Quality and composition of the smoke are affected by several factors, such as combustion temperature, wood type, moisture content of wood, air ventilation rate, and wood size (Jonsdottir, Olafsdottir, Chanie, & Haugen, 2008).

The combustion temperature is the most important factor that influences the composition of generated smoke. The temperature at which smoked is generated greatly influences its composition. After the initial drying period, the thermal decomposition of hemicellulose happenes at early stages of smoking (200-260 °C), followed by the cellulose (260-310 °C) and lignin (310-500 °C). Most woods will be largely pyrolyzed at temperatures over 500 °C (Maga, 1998).

Studies showed that temperatures of 350 to 500 °C produces more smoke at a higher rate. However, it did not significantly change the smoke composition but reduced the concentration of active components by about 35%. The antioxidative effect and antimicrobial effect of the smoke were not influenced by the increased smoke generation temperature (Fretheim, Granum, & Vold, 1980). Guillen & Ibargoitia (1996) reported that more acidity and flavor components could be obtained from smoke generated at a high temperature using *Vitis vinifera* L. shoot sawdust. Similar results were also reported by Maga & Chen (1985) with preference of a high generation temperature (from 290 to 450 °C) in producing hickory wood smoke.

Both hardwoods and softwoods can be used to generate smoke. Hardwoods are woods that typically produce broad or bladelike, woody, two-seed leaves (dicotyledenous) with their seeds enclosed in a seed case. Softwoods are woods that typically produce needle-shaped leaves and naked seeds. Their name does not always match their actual physical hardness properties. For example, hardwoods like aspen and cottonwood are actually very soft, while softwoods like Douglas fir are firm in structure (Maga, 1988).

Cellulose, hemicellulose and lignin are three main components in wood and their contents and compositions vary in different types of wood. Cellulose levels are fairly consistent among different species. Softwoods have higher lignin content than hardwoods. Lignin is a complex, polyphenolic molecule with a high molecular weight. Hardwoods typically contain more hemicellulose than softwoods. The hemicellulose in hardwoods is largely pentosan-based. The hemicellulose in the softwoods is mostly hexosan-based. Decomposition of hemicellulose happens at the early stage of smoking as mentioned before and produces furan and its derivatives as well as aliphatic carboxylic acids, which drops the pH in the smoked product. Softwoods also contain more resin acids than hardwoods, which typically introduces unpleasant flavor to the fish. Hardwoods, such as hickory, oak, cherry, apple and beech, are preferred in most situations over the softwoods for smoke generation. This is because hardwoods tend to produce more phenols and organic acids which contribute to the flavor and preservation effect of smoking (Hall, 1997).

The amount and type of volatile compounds in the smoke is influenced by the initial moisture content of the wood. Gorbatov et al. (1971) evaluated the influence of wood moisture contents (1.8, 21.5, 24.5, and 31.2%) on the chemical composition and

sensory properties of the smoke and found that low moisture wood produces more phenols, acids, and carbonyls, but the total smoke condensation production was lower than that of wood with high moisture content. The sensory test favored smoke produced by wood with medium moisture content (21.5 and 24.5%), possibly, because the formation of some flavor compounds are moisture dependent (Jakab, Liu, & Meuzelaar, 1997). Research showed that the duration of smoke production increased and the maximum temperature reached decreased in beech wood with higher moisture content, which further changed the acidity and composition of the liquid smoke (Guilln & Ibargoitia, 1999). More smoke was produced in low moisture treatment at a faster speed. More studies on this topic are needed to identify which compounds are preferentially produced at different moisture levels.

The amount of air present during the production of smoke also influences the results of wood pyrolysis. Lower temperature and less air produce a smoke with more flavoring and preserving substances. While a higher temperature and more air burn the woods into carbon dioxide and water. Maga et al. (1985) studied the effect of air on the smoke composition of hickory sawdust and found lack of air resulted in an increase of most smoke compounds and an increase in total smoke production from 41.03 to 51.33 mg/100 g of wood. A study by Wasserman & Fiddler (1965) also showed that the phenolic compounds and carbonyls in the smoke increased significantly at decreased oxygen level (10%) compared to other oxygen levels (0, 20, 30, 40, and 50%).

Smoke production can be influenced by the size of wood. Wood can be used as chunks, chips or sawdust forms. However, their combustion rates will vary if same ventilation rate is used. Sawdust produces more smoke than chunks or chips due to its

self-smoldering effect, which blocks the access of oxygen. Fish is also more likely to be charred with less smoke when chunks or chips are used. Most modern smokers use continuously fed sawdust to maintain a consistent production of smoke.

Although people like the flavor and taste of the smoked product, there are concerns about the negative side of smoked products, which are mainly focused on the carcinogenic substances found in the smoke: the polynuclear aromatic hydrocarbons (PAHs). PAHs are composed of multiple fuzed benzene rings. It can be thermally produced by either high temperature pyrolysis or from the incomplete combustion of materials containing carbon and hydrogen. Up to 100 PAHs compounds have been either identified or detected (Maga, 1988). Some of these compounds are considered as highly carcinogenic according to laboratory animals tests and are also implicated in breast, lung and colon cancers in human. They can form some covalent adducts with protein and nucleic acids, thus, initiating cell mutation and eventual malignancy in vivo (Simko, 2002; 2005). The level of PAHs can be reduced by decreasing the combustion temperature since the PAHs content was found to change linearly from 5 to 20 μg/100g in temperature range 400 to 1000 °C (Eyo, 2001). Indirect smoking like liquid and electrostatic smoking also significantly reduces the PAHs amount.

As it was mentioned earlier, the major difference between hot and cold smoking is temperature. Hot-smoked fish receives a smoke at temperatures above 70 °C. Internal temperature of the fish (at the thickest part) must also be maintained for 30 min to make sure the fish is fully cooked after smoking. Fish protein quality can be affected by hot smoking. The carbonyls in the smoke react with lysine, which occurs in a similar way to Maillard reaction and reduces protein quality (Opstvedt, 1989). Extractability of

myofibril proteins can also be decreased by high smoking temperature (Hultmann, Rora, Steinsland, Skara, & Rustad, 2004).

During the cold-smoking of fish, which normally occurs below 30 °C, Sigurgisladottir et al. (2000b) found that texture of smoked salmon was not affected by the smoking temperature (20 and 30 °C) when the shear force was used as a textural properties indicator. Astaxanthin in salmon was not affected by the low cold-smoking temperature (Birkeland, Haarstad, & Bjerkeng, 2004). Rora, Birkeland, Hultmann, Rustad, Skara, & Bjerkeng (2005) studied the effects of cold-smoking temperature on the quality characteristics of farmed Atlantic salmon (*Salmo salar*) and found that appearance of the cold-smoked salmon was not affected by the smoking temperature. Processing yield, i.e. liquid holding capacity, decreased with increased smoking temperature. At the same time total phenol content, fat loss during storage, and firmness increased with increasing temperature. In another study by Birkeland et al. (2005) process yield was not found to be affected by the smoking temperature which happened at lower degree (4 and 10-12 °C).

Packaging and storage

Several packaging methods are available for fish and fishery products, such as controlled-atmosphere packaging (CAP), modified-atmosphere packaging (MAP), and vacuum packaging (VP) (Hall, 1997). Controlled-atmosphere packaging (CAP) refers to packaging in an atmosphere where the composition of gases is continuously monitored and controlled during the storage period. This technique is primarily used for bulk storage of products. Previous study showed that rainbow trout fillets stored under controlled atmosphere had better color than those stored under modified atmosphere after four

weeks' storage at 4 °C (Choubert & Baccaunaud, 2006). Compared to ice storage, black spot development was delayed for deepwater pink shrimp (*Parapenaeus longirostris*) stored in controlled atmosphere at refrigeration temperature (Martinez-Alvarez, Gomez-Guillen, & Montero, 2005). The principle of modified-atmosphere packaging (MAP) is the replacement of air in the package with a different fixed gas mixture. No further control is applied on the introduced gas mixture and the composition will inevitably change. MAP in combination with refrigeration has proven to be an effective preservation method for the extension of shelf-life of fresh fish and fish products (Stammen, Gerdes, & Caporaso, 1990; Sivertsvik, Jeksrud, & Rosnes, 2002). The shelf-life of fish products in MAP can be extended, depending on raw materials, temperature, gas mixtures and packaging materials (Farber, 1991). The percentage increase of shelf-life in MAP ranges from 0 to 280% when compared with aerobic storage (Reddy, Armstrong, Rhodehamel, & Kauter, 1992).

The three major gases used commercially in MAP are carbon dioxide (CO_2), Nitrogen (N_2), and oxygen (O_2). CO_2 is the most important gas used in MAP of fish. Carbon dioxide is both water and lipid soluble. It inhibits growth of many spoilage bacteria. As many as four mechanisms of CO_2 effect on micro-organisms have been identified (Daniels, Krishnamurthi, & Rizvi, 1985; Dixon & Kell, 1989; Farber, 1991): 1. alteration of cell membrane functions; 2. inhibition of enzyme reactions; 3. changes of intracellular pH; 4. changes in proteins properties.

The inhibition effects of carbon dioxide are increased with increased CO_2 concentration in the atmosphere (Sivertsvik et al., 2002). Water solubility of the carbon dioxide also increases greatly with decreased temperature. Its solubility in water is 1.73 g

CO_2/kg H_2O at 20 °C and 1 atmosphere and 3.38 g CO_2/kg H_2O at 0 °C and 1 atmosphere (Knoche, 1980). Therefore, the effectiveness of the gas is always conditioned by the storage temperature with increased inhibition of bacterial growth as temperature is decreased (Haines, 1933; Gill & Tan, 1980; Ogrydziak & Brown, 1982).

Nitrogen is an inert tasteless gas, which has low solubility in water and lipid. It is used primarily to replace oxygen in packs; so that oxidative rancidity can be delayed and growth of aerobic micro-organisms can be inhibited. Oxygen generally causes oxidative rancidity in fatty fish. Thus, it is usually excluded from these fish species. Oxygen also stimulates the growth of aerobic bacteria and inhibits the growth of obligate anaerobic bacteria.

The extension of the shelf-life of fish products in MAP is dependent on raw material, temperature, gas mixtures and packaging materials (Davis, 1993). Besides these, some new techniques were also combined with the MAP to extend the stability of the products, such as essential oil (Goulas & Kontominas, 2007), ozone (Hovda, Sivertsvik, Lunestad, & Rosnes, 2007), superchilled storage (Wang, Sveinsdottir, Magnusson, & Martinsdottir, 2008) and irradiation (Robertson et al., 2006; Reale et al., 2008).

If the air is totally evacuated from the package, it is called vacuum packaging (VP). No further control is performed on the inside condition either. The residue gaseous atmosphere inside the package will change during the storage due to the metabolism of the product or micro-organism activities. The effects of VP on products are similar to those by MAP, but the packaging volume is decreased and some deleterious effects that occur sometimes after a prolonged exposure of products to CO_2 can be avoided (Dalgaard, Gram, & Huss, 1993; Masniyom, Benjakul, & Visessanguan, 2002; 2005).

Ozogul, Taylor, Quantick, & Ozogul (2000) compared the quality of Atlantic herring (*Clupea harengus*) stored in ice-free boxes under VP and MAP at 2±2 °C and found that the herring shelf-life was extended by 10 and 8 days, for MAP and vacuum packaging, respectively, when compared to the ice stored herring. However, effects of VP and MAP may also vary for different species as Sanguandeekul, Siripatrawan, & Narakaew (2008) reported that the sensory quality of MAP abalone was acceptable up to 15 days compared to 3 days for atmospheric and vacuum-packaged treatments when they were all stored at 2 °C.

Fish processing by-product utilization

The fishery processing industries produce large amounts of by-products. Most of these by-products are disposed into land fills or the ocean. However, they can also be valuable bio-resources if used properly since they are rich source of bio-materials, such as protein, lipids, and chitin (Wang & Hwang, 2001). Various methods had been used to recover those valuable parts including composting as fertilizer (Liao, Chen, Vizcarra, & Lo, 1994), fish feed (Goddard, Al-Shagaa, & Ali, 2008), minced fish (Kasapis, 2009), fish oil (Aidos, Van der Padt, Boom, & Luten, 2001), and fish gelatin (Zhou, Mulvaney, & Regenstein, 2006).

Gelatin is a soluble polypeptide derived from the insoluble parent protein collagen (Wrolstad et al., 2005) by procedures involving the destruction of crosslinkages between polypeptide chains of collagen along with some amount of breakage of polypeptide chain bonds. It is the only food material that gels and melts reversibly below human body temperature (37 ^{o}C). Because of its unique and outstanding functional properties, along with its reasonable cost, gelatin is the most widely used food and

pharmaceutical ingredient by weight. Most commercial gelatin is made from mammals, mainly from pork skin, bovine hide, and bovine bone. Less than 1% of the gelatin in the world market is obtained from fish and other species (Table 3). In recent years, due to food-safety concerns and the preferences of specific religious groups, research on the production of gelatin from fish processing by-products has attracted extensive attention from various industry and research groups.

Gelatin from fish skins may provide an alternative to cattle and pork gelatin. Extraction of fish gelatin had been reported for various fish species. Some of them are cod (Gudmundsson & Hafsteinsson, 1997), hake (Montero, Gomez-Guillen, & Borderias, 1999), blue shark (Yoshimura, Terashima, Hozan, & Shirai, 2000), tilapia (Choi & Regenstein, 2000; Jamilah & Harvinder, 2002), yellowfin tuna (Cho, Gu, & Kim, 2005), Alaska pollock (Zhou et al., 2006), horse mackerel (Badii & Howell, 2006), skate (Cho, Jahncke, Chin, & Eun, 2006), and catfish (Yang, Wang, Jiang, Oh, Herring, & Zhou, 2007a).

Processing of catfish in the U.S. was about 2.25×10^5 metric tons in 2007 (NASS, 2008). During the processing of catfish, about 40% of the weight of catfish goes to waste and catfish skin accounts for about 6% of processed fish weight (Lovell, 1980), which is about 13.5×10^3 ton waste annually. It appears that commercial fish production may be able to supply an alternate source of gelatin for food and pharmaceutical uses for American consumers, and, possibly, for export. Extraction of catfish gelatin had been reported by Yang et al. (2007a) as well as studies on its physical properties (Yang, Wang, Regenstein, & Rouse, 2007b; Yang, Wang, Zhou, & Regenstein, 2008).

Table 3. World gelatin market data from 2001 to 2003.

(Unit in metric tons. Gelatin Manufacturers of Europe 2006).

	2001	2002	2003
	Production (percentage)		
Pork skin	110,400 (41.0%)	113600 (41.7%)	117950 (42.4%)
Bovine hides	77,200 (28.6%)	77500 (28.4%)	81650 (29.3%)
Bones	80,800 (30.0%)	79600 (29.2%	76750 (27.6%)
Fish and others	1,000 (0.4%)	1800 (0.7%)	1950 (0.7%)
Total	269,400	272,500	278,300

References:

Abu-Ali, J. M. & Barringer, S. A. (2007). Color and texture development of potato cylinders with liquid smoke during baking, frying and microwaving. *Journal of Food Processing and Preservation*, *31*, 334-344.

Adams, M. R. & Moss, M. O. (2000). Bacterial agents of foodborne illness. In *Food Microbiology, 2nd editon*. Pp. 200-211. London, UK: Royal Society of Chemistry.

Aidos, I., Van der Padt, A., Boom, R. M., & Luten, J. B. (2001) Upgrading of maatjes herring byproducts: production of crude fish oil. *Journal of Agricultural and Food Chemistry, 49,* 3697-3704.

Akse, L., Birkeland, S., Tobiassen, T., Joensen, S., & Larsen, R. (2008). Injection-salting and cold-smoking of farmed Atlantic cod (*Gadus morhua* L.) and Atlantic salmon (*Salmo salar* L.) at different stages of rigor mortis: effect on physical properties. *Journal of Food Science*, *73,* E378-E382.

Ashie, I. N. A., Smith, J. P., & Simpson, B. K. (1996). Spoilage and shelf-life extension of fresh fish and shellfish. *Critical Reviews in Food Science and Technology, 36,* 87-121.

Aursand, I. G., Gallart-Jornet, L., Erikson, U., Axelson, D. E., & Rustad, T. (2008). Water distribution in brine salted cod (*Gadus morhua*) and salmon (*Salmo salat*): A low-field H-1 NMR study. *Journal of Agricultural and Food Chemistry, 56*, 6252-6260.

Badii, F. & Howell, N. K. (2006). Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. *Food Hydrocolloids, 20*, 630-640.

Ball, A. P., Hopkinson, R. B., Farrell, I. D., Hutchison, J. G., Paul, R., Watson, R. D., Page, A. J., Parker, R. G., Edwards, C. W., Snow, M., Scott, D. K., Leone-Ganado, A., Hastings, A., Ghosh, A. C., & Gilbert, R. J. (1979). Human botulism caused by *Clostridium botulinum* type E: the Birmingham outbreak. *Quarterly Journal of Medicine*, *48*, 473-491.

Beraquet, N. J., Iaderoza, M., Jardim, D. C. P., & Lindo, M. K. K. (1983). Salting of mackerel (*Scomber japoonicus*) II. Comparison between brining and mixed salting in relation to quality and salt uptake. *Coletaneado Instituto de Tecnologia de Alimentos*, *13,* 175-198.

Berhimpon, S., Souness, R. A., Driscoll, R. H., Buckle, K. A., & Edwards, R. A. (1991). Salting behavior of yellowtail (*Trachurus mccullochi* Nichols). *Journal of Food Processing and Preservation, 15,* 101-114.

Berhimpon, S., Souness, R. A., & Edwards, R. A. (1990). The effect of brine concentration on the salting and drying of yellowtail (*Trachurus mccullochi*). *FAO Fisheries Report*, 153-159.

Birkeland, S., Akse, L., Joensen, S., Tobiassen, T., & Skara, T. (2007). Injection-salting of prerigor fillets of Atlantic salmon (*Salmo salar). Journal of Food Science*, *72,* E29-E35.

Birkeland, S. & Bjerkeng, B. (2005). The quality of cold-smoked Atlantic salmon (*Salmo salar*) as affected by salting method, time and temperature. *International Journal of Food Science and Technology*, *40*, 963-976.

Birkeland, S., Haarstad, I., & Bjerkeng, B. (2004). Effects of salt-curing procedure and smoking temperature on astaxanthin stability in smoked salmon. *Journal of Food Science, 69*, E198-E203.

Birkeland, S. & Skara, T. (2008). Cold smoking of Atlantic salmon (*Salmo salar*) fillets with smoke condensate-an alternative processing technology for the production of smoked salmon. *Journal of Food Science, 73,* S326-S332.

Brunner, E. & Iso, H. (2008). Fish oil and secondary prevention of cardiovascular disease. *British Medical Journal, 337*, a2541.

Chen, B. H. & Lin, Y. S. (1997). Formation of polycyclic aromatic hydrocarbons during processing of duck meat, *Journal of Agricultural and Food Chemistry, 45*, 1394-1403.

Cardinal, M., Cornet, J., Serot, T., & Baron, R. (2006). Effects of the smoking process on odour characteristics of smoked herring (*Clupea harengus*) and relationships with phenolic compound content. *Food Chemistry*, *96*, 137-146.

Cardinal, M. Knockaert, C. Torrissen, O., Sigurgisladottir, S., Morkore, T., Thomassen, M., & Vallet, J. L. (2001). Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). *Food Research International*, *34*, 537-550.

Caya, J. G. (2001). *Clostridium botulinum* and the ophthalmologist: a review of botulism, including biological warfare ramifications of botulinum toxin. *Survey of Ophthalmology, 46*, 25-34.

Chiralt, A., Fito, P., Barat, J. M., Andres, A., Gonzalez-Martinez, C., Escriche, I., & Camacho, M. M. (2001). Use of vacuum impregnation in food salting process. *Journal of Food Engineering, 49,* 141-151.

Cho, S. M., Gu, Y. S., & Kim, S. B. (2005). Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocolloids, 19,* 221-229.

Cho, S. H., Jahncke, M. L., Chin, K. B., & Eun, J. B. (2006). The effect of processing conditions on the properties of gelatin from skate (*Raja Kenojei*) skins. *Food Hydrocolloids, 20,* 810-816.

Choi, S. S. & Regenstein, J. M. (2000). Physicochemical and sensory characteristics of fish gelatin. *Journal of Food Science, 65*, 194-199.

Choubert, G. & Baccaunaud, M. (2006). Colour changes of fillets of rainbow trout (*Oncorhynchus mykiss* W.) fed astaxanthin or canthaxanthin during storage under controlled or modified atmosphere. *LWT - Food Science and Technology, 39,* 1203-1213.

Corzo, O. & Bracho, N. (2004). Effects of brine concentration and temperature on equilibrium distribution coefficients during osmotic dehydration of sardine sheets. *LWT - Food Science and Technology, 37,* 475-479.

Dahlsten, E., Korkeala, H., Somervuo, P., & Lindstrom, M. (2008). PCR assay for differentiating between Group I (proteolytic) and Group II (nonproteolytic) strains of *Clostridium botulinum*. *International Journal of Food Microbiology*, *124*, 108-111.

Dalgaard, P., Gram, L., & Huss, H. H. (1993). Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres, *International Journal of Food Microbiology, 19,* 283-294.

Daniels, J. A., Krishnamurthi, R., & Rizvi, S. S. H. (1985). A review of effects of carbon dioxide on microbial growth and food quality. *Journal of Food Protection, 48,* 532-537.

Davis, H. K. (1993). Fish. In: R.T. Parry, Editor, *Principles and Applications of Modified Atmosphere Packaging of Foods*, Blackie Academic & Professional, Glasgow, Pp. 189-228.

Dean, L. M. (1990). Chapter 16: Nutrition and preparation. In *the Seafood Industry*, edited by Martin, R. E. and Flick, G. J. An Osprey Book Published by Van Nostrand Reinhold. New York, U.S.A.

Deng, J. C. (1977). Effect of freezing and frozen storage on salt penetration into fish muscle immersed in brine. *Journal of Food Science, 42*, 348-351.

Diaz, G., Wolf, W., Kostaropoulos, A. E., & Spiess, W. E. L. (1993). Diffusion of low-molecular compounds in food model systems. *Journal of Food Processing and Preservation, 17,* 437-454.

Dillon, R. & Patel, T. (1993). Effect of cold smoking and storage temperatures on *Listeria monocytogenes* in inoculated cod fillets (*Gadus morhus*). *Food Research International*, *26*, 97-101.

Dixon, N. M. & Kell, D. B. (1989). The inhibition of CO_2 of the growth and metabolism of micro-organisms. *Journal of Applied Bacteriology, 67,* 109-136.

Dore, I. (1993). Chapter one: Basic technology. In *The Smoked and Cured Seafood Guide*. Urner Barry Publications, Inc., NJ.

Dunajski, E. (1979). Texture of fish muscle. *Journal of Texture Studies*, *10*, 301-317.

Dunbar, E. M. (1990). Botulism. *Journal of Infection*, *20,* 1-3.

Eissa, H. A., Fouad, G. M., & Shouk, A. E. A. (2008). Effect of some thermal and hemical pretreatments on smoked Oyster mushroom quality. *International Journal of Food Science and Technology, 43,* 1088-1098.

Emborg, J. & Dalgaard, P. (2006). Formation of histamine and biogenic amines in cold-smoked tuna: An investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisoning. *Journal of Food Protection*, *69*, 897-906.

Eyo, A. A. (2001). Fish smoking. In *Fish Processing Technology in the Tropics*, Pp. 153-192. New Bussa, Nigeria: NIFFR.

FAO. (2009). *The State of World Fisheries and Aquaculture*. Food an Agriculture Organization of the United Nations. Rome, Italy.

Farber, J. M. (1991). Microbiological aspects of modified-atmosphere packaging technology -a review. *Journal of Food Protection, 54,* 58-70.

FDA. (2001). Food and Drug Administration, Chapter 13: *Clostridium botulinum* toxin formation. In *Fish and fisheries products hazards and control guide*, *3rd edition.* Office of Seafood, Food and Drug Administration, Department of Health and Human Services, US Government Printing Office, Washington, DC.

Fretheim, K., Granum, P. E., & Vold, E. (1980). Influence of generation temperature on the chemical composition, antioxidative, and antimicrobial effects of wood smoke. *Journal of Food Science*, *45*, 999-1002.

Gallart-Jornet, L., Barat, J. M., Rustad, T., Erikson, U., Escriche, I., & Fito, P. (2007a). A comparative study of brine salting of Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*). *Journal of Food Engineering*, *79,* 261-270.

Gallart-Jornet, L., Barat, J. M., Rustad, T., Erikson, U., Escriche, I., & Fito, P. (2007b). Influence of brine concentration on Atlantic salmon fillet salting. *Journal of Food Engineering*, *80,* 267-275.

Gill, C. O. & Tan, K. H. (1980). Effect of carbon dioxide on growth of meat spoilage bacteria. *Applied and Environmental Microbiology, 39,* 317-319.

Goddard, S., Al-Shagaa, G., & Ali, A. (2008). Fisheries by-catch and processing waste meals as ingredients in diets for Nile tilapia, *Oreochromis niloticus*. *Aquaculture Research, 39,* 518-525.

Gomez-Estaca, J., Gomez-Guillen, M. C., & Montero, P. (2007). High pressure effects on the quality and preservation of cold-smoked dolphinfish (*Coryphaena hippurus*) fillets. *Food Chemistry*, *102,* 1250-1259.

Gomez-Estaca, J., Montero, P., Gimenez, B., & Gomez-Guillen, M. C. (2007). Effect of functional edible films and high pressure processing on microbial and oxidative spoilage in cold-smoked sardine (*Sardina pilchardus*). *Food Chemistry, 105,* 511-520.

Gorbatov, V. M., Krylova, N. N., Volovinskaya, V. P., Lyaskovskaya, Y. N., Bazarova, K. Y., Khlamova, R. Y., & Yakovleva, G. Y. (1971). Liquid smokes for use in cure meats. *Food Technology*. *25,* 71-77.

Goulas, A. E. & Kontominas, M. G. (2007). Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes. *Food Chemistry, 100,* 287-296.

Gudmundsson, M. & Hafsteinsson, H. (1997). Gelatin from cod skins as affected by chemical treatment. *Journal of Food Science, 62,* 37-47.

Guillen, M. D. & Ibargoitia, M. L. (1996). Relationships between the maximum temperature reached in the smoke generation processes from *Vitis vinifera* L shoot sawdust and composition of the aqueous smoke flavoring preparations obtained. *Journal of Agricultural and Food Chemistry, 44,* 1302-1307.

Guillen, M. D. & Ibargoitia, M. L. (1999). Influence of the moisture content on the composition of the liquid smoke produced in the pyrolysis process of *Fagus sylvatica* L. wood. *Journal of Agricultural and Food Chemistry. 47,* 4126-4136.

Haines, R.B. (1933). The influence of carbon dioxide on the rate of multiplication of certain bacteria, as judged by viable counts. *Journal of the Society of Chemical Industry, 52,* 13T-17T.

Hall, G. M. (Editor). (1997). *Fish Processing Technology, 2nd edition*. Chapman and Hall, New York.

Hambleton, P. (1992). *C. botulinum* toxins: a general review of involvement in disease, structure, mode of action and preparation for clinical use. *Journal of Neurology, 239,* 16-20.

Hilderbrand, K. S. Jr. (1999). *Preparation of salt brines for the fishing industry*. ORESU-H-99-0002 (formerly SG 22).

Hilderbrand, K. S. Jr. (2001) (revised). *Fish smoking precedures for forced convection smokehouses*. Oregon State University Extention Sea Grant Program. ORESU-I-01-001.

Hovda, M. B., Sivertsvik, M., Lunestad, B. T., & Rosnes, J. T. (2007). Microflora assessments using PCR-Denaturing gradient gel electrophoresis of ozone-treated and modified-atmosphere-packaged farmed cod fillets. *Journal of Food Protection, 70,* 2460-2465.

Hultmann, L., Rora, A. M. B., Steinsland, I., Skara, T., & Rustad, T. (2004). Proteolytic activity and properties of proteins in smoked salmon (*Salmo salar*)-effects of smoking temperature. *Food Chemistry*, *85*, 377-387.

Huss, H. H. (1980). Distribution of *Clostridium botulinum*. *Applied and Environmental Microbiology, 39*, 764-769.

Huss, H. H. (1988). *Fresh fish-quality and quality changes*. A training manual prepared for the FAO/DANIDA training programme on fish technology and quality control. FAO. Rome. Italy.

Jakab E., Liu K., & Meuzelaar, H. L. C. (1997). Thermal decomposition of wood and cellulose in the presence of solvent vapors. *Industrial and Engineering Chemistry Research. 36,* 2087-2095.

Jamilah, B. & Harvinder, K.G. (2002). Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chemistry, 77,* 81-84.

Jason, A.C. (1965). Effects of fat content on diffusion of water in fish muscle, *Journal of Science and Food Agriculture, 16,* 281-288.

Jittinandana, S. Kenney, P. B., Slider, S. D., & Kiser, R. A. (2002). Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. J*ournal of Food Science, 67,* 2095-2099.

Joffraud, J. J., Cardinal, M., Cornet, J., Chasles, J. S., Leon, S., Gigout, F., & Leroi, F. (2006). Effect of bacterial interactions on the spoilage of cold-smoked salmon. *International Journal of Food Microbiology, 112,* 51-61.

Jonsdottir, R., Olafsdottir, G., Chanie, E., & Haugen, J.-E. (2008). Volatile compounds suitable for rapid detection as quality indicators of cold smoked salmon (*Salmo salar*). *Food Chemistry, 109,* 184-195.

Kasapis, S. (2009). Developing minced fish products of improved eating quality: an interplay of instrumental and sensory texture. *International Journal of Food Properties, 12,* 11-26.

Knoche, W. (1980). Chemical reactions of CO_2 in water. In *Biophysics and Physiology of Carbon Dioxide,edited by . Bauer, C., Gros, G., and Bartels, H.* Pp. 3-11. Springer-Verlag, Berlin.

Korkeala, H., Stengel, G., Hyytia, E., Vogelsang, B., Bohl, A., Wihlman, H., Pakkala, P., & Hielm, S. (1998). Type E botulism associated with vacuum-packaged hot-smoked whitefish. *International Journal of Food Microbiology, 43,* 1-5.

Lauritzsen, K., Akse, L., Johansen, A., Joensen, S., Sorensen, N. K., & Olsen, R. L. (2004). Physical and quality attributes of salted cod (*Gadus morhua* L.) as affected by the state of *rigor* and freezing prior to salting. *Food Research International*, *37,* 677-688.

Lawrie, R. A. (1998). The storage and preservation of meat II. Moisture control. In *Lawrie's Meat Science, 6th edition*, 191-194. Cambridge, UK: Woodhead Publishing Limited.

Lee, J. S. (1977). *Hazard analysis and critical control point applications to the seafood industry*. Oregon State University Sea Grant College Program. Publication No. ORESU-H-77-001.

Liao, P. H., Chen, A., Vizcarra, A. T., & Lo, K. V. (1994). Evaluation of the maturity of compost made from salmon farm mortalities. *Journal of Agricultural Engineering Research, 58,* 217-222.

Lietzow, M. A., Gielow, E. T., Le, D., Zhang, J., & Verhagen, M. F. (2008). Subunit stoichiometry of the *Clostridium botulinum* type A neurotoxin complex determined using denaturing capillary electrophoresis. *Protein Journal, 27,* 420-425.

Lindstrom, M., Kiviniemi, K., & Korkeala, H. (2006). Hazard and control of group II (non-proteolytic) *Clostridium botulinum* in modern food processing. *International Journal of Food Microbiology*, *108,* 92-104.

Lovell, R. (1980). *Utilization of Catfish Processing Waste*. Bulletin 521. Agriculture Experiment Station, Auburn, AL.

Lyhs, U., Bjorkroth, J., Hyytia, E., & Korkeala, H. (1998). The spoilage flora of vacuum-packaged, sodium nitrite or potassium nitrate treated, cold-smoked rainbow trout stored at 4 degrees C or 8 degrees C. *International Journal of Food Microbiology, 45,* 135-142.

Maga, J. A. 1988. Smoke in Food Processing. CRC Press, Inc. Boca Raton, FL.

Maga, J. A. & Chen, Z. (1985). Pyrazine composition of wood smoke as influenced by wood source and smoke generation variables. *Flavour* and *Fragrance Journal, 1*, 37-42.

Martinez, O., Salmeron, J., Guillen, M. D., & Casas, C. (2007). Textural and physicochemical changes in salmon (*Salmo salar*) treated with commercial liquid smoke flavourings. *Food Chemistry*, *100,* 498-503.

Martinez-Alvarez, O., Gomez-Guillen, M. C., & Montero, P. (2005). Role of sulfites and 4-hexylresorcinol in microbial growth and melanosis prevention of deepwater pink shrimp (*Parapenaeus longirostris*) using a controlled atmosphere. *Journal of Food Protection, 68,* 98-104.

Masniyom, P., Benjakul, S., & Visessanguan, W. (2002). Shelf-life extension of refrigerated seabass slices under modified atmosphere packaging, *Journal of the Science of Food and Agriculture, 82,* 873-880.

Masniyom, P., Benjakul, S., & Visessanguan, W. (2005). Collagen changes in refrigerated sea bass muscle treated with pyrophosphate and stored in modified-atmosphere packaging. *European Food Research and Technology, 220,* 322-325.

Modi, V. K., Mahendrakar, N. S., Sachindra, N. M., & Rao, D. N. (2004). Quality of nuggets prepared from fresh and smoked spent layer chicken meat. *Journal of Muscle Foods*, *15,* 195-204.

Montero, P., Gomez-Guillen, M. C., & Borderias, A. J. (1999). Functional characterization of muscle and skin collagenous material from hake (*Merluccius merluccius L.*). *Food Chemistry, 65*, 55-59.

Montero, P., Gomez-Guillen, M. C., & Borderias, A. J. (2003). Influence of salmon provenance and smoking process on muscle functional characteristics. *Journal of Food Science, 68,* 1155-1160.

Muratore, G. & Licciardello, F. (2005). Effect of vacuum and modified atmosphere packaging on the shelf-life of liquid-smoked swordfish (*Xiphias gladius*) slices. *Journal of Food Science*, *70,* C359-C363.

NASS. (2008). *Catfish Processing*. USDA Economics, Statistics and Market Information System. Publication date: 02.21.2008. Washington D.C.

Nettleton, J. A. 1987. *Seafood and Health*. Osprey books, New York.

Nketsia-Tabiri, J. & Sefa-Dedeh, S. (1995). Optimization of process conditions and quality of salted dried tilapia (*Oreochromis niloticus*) using response surface methodology. *Journal of the Science of Food and Agriculture*, *69,* 117-127.

Ogrydziak, D. M. & Brown, W. D. (1982). Temperature effects in modified-atmosphere storage of seafoods. *Food Technology, 36,* 86-96.

Olley, J., Doe, P. E., & Heruwati, E. S. (1989). Chapter 1: The influence of drying and smoking on the nutritional properties of fish: an introductory overview. in *Fish drying and smoking. The effect of smoking and drying on the nutritional properties of fish*, edited by Burt, J. R. Elsevier Applied Science, London and New York.

Opstvedt, J. (1989). Influence of drying and smoking on protein quality, in *Fish drying and smoking. The effect of smoking and drying on the nutritional properties of fish*, edited by Burt J. R. Elsevier Applied Science, London and New York.

Ozogul, F., Taylor, K. D. A., Quantick, P., & Ozogul, Y. (2000). Chemical, microbiological and sensory evaluation of Atlantic herring (*Clupea harengus*) stored in ice, modified atmosphere and vacuum pack. *Food Chemistry, 71,*267-273.

Poernomo, A., Fawzya, Y. N., & Ariyani, F. (1992). Salting and drying of mackerel. *ASEAN Food Journal, 7,* 141-146.

Poligne, I., Collignan, A., & Trystram, G. (2002). Effects of salting, drying, cooking, and smoking operations on volatile compound formation and color patterns in pork. *Journal of Food Science*, *67,* 2976-2986.

Reale, A., Sorrentino, E., Iaffaldano, N., Rosato, M. P., Ragni, P., Coppola, R., Capitani, D., Sobolev, A. P., Tremonte, P., Succi, M., & Mannina, L. (2008). Effects of ionizing radiation and modified atmosphere packaging on the shelf life of aqua-cultured sea bass (*Dicentrarchus labrax*). *World Journal of Microbiology & Bibthchnology, 24,* 2757-2765.

Reddy, N. R., Armstrong, D. J., Rhodehamel, E. J., & Kauter, D. A. (1992). Shelf-life extention of haddock in carbon dioxide-oxygen atmospheres with and without potassium sorbate. *Journal of Food Quality, 5***,** 285-300.

Robertson, C. B., Andrews, L. S., Marshall, D. L., Coggins, P., Schilling, M. W., Martin, R. E., & Collette, R. (2006). Effect of X-ray irradiation on reducing the risk of listeriosis in ready-to-eat vacuum-packaged smoked mullet. *Journal of Food Protection, 69,* 1561-1564.

Rora, A. M. B., Birkeland, S., Hultmann, L., Rustad, T., Skara, T., & Bjerkeng, B. (2005). Quality characteristics of farmed Atlantic salmon (*Salmo salar*) fed diets high in

soybean or fish oil as affected by cold-smoking temperature. *LWT-Food Science and Technology, 38,* 201-211.

Rora, A. M. B., Furuhaug, R., Fjæra, S. O., & Skjervold, P. O. (2004). Salt diffusion in pre-rigor filleted Atlantic salmon. *Aquaculture, 232,* 255-263.

Ross, T. & Dalgaard, P. (2004). Chapter 3: Secondary models. in *Modeling Microbial Responses in Foods,* edited by McKellar, R. C. and Lu, X. CRC Series in Contemporary Food Science. CRC Press. Boca Raton. FL. U.S.A.

Ruiter, A. (1979). Color of smoked foods. *Food Technology, 33,* 54-63.

Sanguandeekul, R., Siripatrawan, U., & Narakaew, V. (2008). Changes in the quality of abalone (*Haliotis asinina* Linnaeus) packaged under atmospheric air, vacuum and modified atmosphere. *Packaging Technology and Science, 21,* 159-164.

Sharma, S. K. & Whiting, R. C. (2005). Methods for detection of *Clostridium botulinum* toxin in foods. *Journal of Food Protection*, *68,* 1256-1263.

Sigurgisladottir, S., Ingvarsdottir, H., Torrissen, O. J., Cardinal, M., & Hafsteinsson, H. (2000a). Effects of freezing/thawing on the microstructure and the texture of smoked Atlantic salmon (*Salmo salar*). *Food Research International*, *33,* 857-865.

Sigurgisladottir, S., Sigurdardottir, M. S., Torrasen, O., Vallet, J. L., & Hafsteinsson, H. (2000b). Effects of different salting and smoking processes on the microstructure, the texture, and yield of Atlantic salmon (*Salmo salar*) fillets. *Food Research International, 33*, 847-855.

Simko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of Chromatography B, 770,* 3-18.

Simko, P. (2005). Factors affecting elimination of polycyclic aromatic hydrocarbons from smoked meat foods and liquid smoke flavorings. *Molecular Nutrition & Food Research*, *49,* 637-647.

Simon, R., de la Calle, B., Palme, S., Meier, D., & Anklam, E. (2005). Composition and analysis of liquid smoke flavouring primary products. *Journal of Separation Science*, *28*, 871-882.

Siskos, I., Zotos, A., & Taylor, K. D. A. (2005). The effect of drying, pressure and processing time on the quality of liquid-smoked trout (*Salmo gairdnerii*) fillets. *Journal of the Science of Food and Agriculture, 85,* 2054-2060.

Sivertsvik, M., Jeksrud, W. K., & Rosnes, J. T. (2002). A review of modified atmosphere packaging of fish and fishery products-significance of microbial growth activities and safety, *International Journal of Food Science and Technology, 37,* 107-127.

Sobel, J., Tucker, N., Sulka, A., McLaughlin, J., & Maslanka, S. (2004). Foodborne botulism in the United States, 1990-2000. *Emerging Infectious Diseases, 10,* 1606-1611.

Solomon, H. M. & Lilly, T. Jr. (2001). Chapter 17: *Clostridium botulinum*. in *Bacteriological Analytical Manual.* U.S. Food and Drug Administration. Washington D. C.

Stammen, K., Gerdes, D., & Caporaso, F. (1990). Modified atmosphere packaging of seafood, *CRC Critical Reviews in Food Science and Nutrition, 29,* 301-331.

Stefansson, G., Nielsen, H. H., Skara, T., Schubring, R., Oehlenschlager, J., Luten, J., Derrick, S., & Gudmundsdottir, G. (2000). Frozen herring as raw material for spice-salting. *Journal of the Science of Food and Agriculture, 80,* 1319-1324.

Suchanova, M., Hajslova, J., Tomaniova, M., Kocourek, V., & Babicka, L. (2008). Polycyclic aromatic hydrocarbons in smoked cheese. *Journal of the Science of Food and Agriculture, 88,* 1307-1317.

Thorarinsdottir, K. A., Arason, S., Bogason, S. G., & Kristbergsson, K. (2004). The effects of various salt concentrations during brine curing of cod (*Gadus morhua*). *International Journal of Food Science and Technology, 39,* 79-89.

Varlet, V., Serot, T., Knockaert, C., Cornet, J., Cardinal, M., Monteau, F., Bizec, B. L., & Prost, C. (2007). Organoleptic characterization and PAH content of salmon (*Salmo salar*) fillets smoked according to four industrial smoking techniques. *Journal of the Science of Food and Agriculture, 87,* 847-854.

Visciano, P., Perugini, M., Conte, F., & Arnorena, M. (2008). Polycyclic aromatic hydrocarbons in farmed rainbow trout (*Oncorhynchus mykiss*) processed by traditional flue gas smoking and by liquid smoke flavourings. *Food and Chemical Toxicology*, *46,* 1409-1413.

Wang, S. L. & Hwang, J.R. (2001). Microbial reclamation of shellfish wastes for the production of chitinases. *Enzyme and Microbial Technology, 28,* 376-382.

Wang, T., Sveinsdottir, K., Magnusson, H., & Martinsdottir, E. (2008). Combined application of modified atmosphere packaging and superchilled storage to extend the shelf life of fresh cod (*Gadus morhua*) loins. *Journal of Food Science, 73,* S11-S19.

Wang, D., Tang, J., & Correia, L. R. (2000). Salt diffusivities and salt diffusion in farmed Atlantic salmon muscle as influenced by rigor mortis. *Journal of Food Engineering*, *43,* 115-123.

Wang, D., Tang, J., Correia, L. R., & Gill, T. A. (1998). Postmortem changes of cultivated Atlantic salmon and their effects on salt uptake. *Journal of Food Science*, *63,* 634-637.

Wasserman, A. E. & Fiddler, W. (1965). Natural smoke: composition and properties. *Proceedings* of the *Meat Industry Research Conference.*163. Chicago. IL.

Wheaton, F. W. & Lawson, T. B. (1985). Chapter 11: Other preservation methods. in *Processing Aquatic Food Products*, Pp. 273-327. New York, Wiley.

Wrolstad, R. E., Acree, T. E., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., Shoemaker, C. F., Smith, D., & Sporns, P. (2005).Chapter H: Texture/Rheology. in *Handbook of Food Analytical Chemistry*, Hoboken, N. J., John Wiley & Sons, Inc. 363-456.

Yanar, Y., Celik, M., & Akamca, E. (2006). Effects of brine concentration on shelf-life of hot-smoked tilapia (*Oreochromis niloticus*) stored at 4 °C. *Food Chemistry, 97,* 244-247.

Yang, H., Wang, Y., Jiang, M., Oh, J. H., Herring, J., & Zhou, P. (2007a). 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. *Journal of Food Science, 72,* C188-C195.

Yang, H., Wang, Y., Regenstein, J. M., & Rouse, D. B. (2007b). Nanostructural characterization of catfish skin gelatin using atomic force microscopy. *Journal of Food Science, 72,* C430-C440.

Yang, H., Wang, Y., Zhou, P., & Regenstein, J. M. (2008). Effects of alkaline and acid pretreatment on the physical properties and nanostructures of the gelatin from channel catfish skins. *Food Hydrocolloids, 22,* 1541-1550.

Yoneyama, T., Miyata, K., Chikai, T., Mikami, A., Suzuki, T., Hasegawa, K., Ikeda, T., Watanabe, T., Ohyama, T., & Niwa, K. (2008). *Clostridium botulinum* serotype D neurotoxin and toxin complex bind to bovine aortic endothelial cells via sialic acid. *FEMS Immunology & Medical Microbiology*, *54,* 290-298**.**

Yoshimura, K., Terashima, M., Hozan, D., & Shirai, K. (2000). Preparation and dynamic viscoelasticity characterization of alkali-solubilized collagen from shark skin. *Journal of Agricultural and Food Chemistry, 48,* 685-690.

Zhou, L. S. & Li-Chan, E. C. Y. (2009). Effects of Kudoa spores, endogenous protease activity and frozen storage on cooked texture of minced Pacific hake (*Merluccius productus*). *Food Chemistry*, *113,* 1076-1082.

Zhou, P., Mulvaney, S. J., & Regenstein, J. M. (2006). Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. *Journal of Food Science, 71,* C313-C321.

III. CHAPTER ONE:

EVALUATION OF TEXTURAL PROPERTIES OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) FILLET WITH THE NATURAL CONTOUR METHOD

Abstract

Textural properties played an important role in the quality control and acceptability of both raw and processed fish products. Yet there is limited information on this topic as well as methods for evaluation of textural properties in catfish fillets. Textural properties of raw and smoked channel catfish (*Ictalurus punctatus*) fillets were measured by the "finger" and "tooth" methods with a Texture Analyzer. A novel sampling technique was used to sample thickness contours on the fillets. Indentation force (g) of the "finger" method and shear force (g) of the "tooth" method were measured at different contour levels of four myomere cone bands on the fillets.

Shear force and indentation force of the fresh catfish fillets increased with the increasing thickness as measured by the "tooth" and "finger" methods. Both methods could be used to measure the textural properties of catfish fillet. The "finger" method was recommended because of its non-destructive nature and applicability to both raw and smoked catfish samples. The novel sampling technique used in this study was rapid and applicable to irregular fillet shapes, including catfish and other fish species. Smoked

catfish had decreased indentation force with increased thickness. The dehydration effects and denaturization of fish muscle during the smoking process were the main reasons for the decrease in the indentation force.

Keywords: Textural properties, smoked channel catfish, contours, sampling technique

1. Introduction

Global fish production from capture and aquaculture reached about 155 million tons in 2004 (FAO, 2004[a]). In the past ten years, the proportion of fish protein as a fraction of total animal protein consumed has increased from 14.9 % (1992) to 15.9% (2001) (FAO, 2004[b]). Fish is very important in the human diet because it supplies a high nutritional value protein along with a variety of vitamins and minerals (Sikorski, 1990). Channel catfish (*Ictalurus punctatus*) is the most important aquaculture species in the southern U.S. Farm-raised catfish production in the U.S. reached an estimated 289 million kg in 2005 with a present market value of over USD 480 million (USDA, 2006).

Freshness monitoring is a critical control point in the processing of fish to ensure good quality of the final products. Raw materials freshness influences odor and appearance of smoked catfishes (*Pseudoplatystoma fasciatum* and *P. pirinampu*) (Tome, Kodaira, & Matsunaga, 1999). Loss of freshness will negatively affect the overall acceptance of smoked catfish. Freshness of channel catfish (*I. punctatus*) is affected by preharvest and postharvest handling and transportation (Silva, Nunez, Figueroa-Garcia, Chamul & Kim, 1998; Nunez, Silva, & Chamul, 1999). Evaluation of the catfish freshness can be achieved either by chemical (Beuchat, 1973; Nunez et al., 1999) or physical measurement techniques (Heia et al., 1998; Olafsdottir et al., 2004).

Texture as an indicator of the food quality (Lawless & Heymann, 1998) can also be used to predict the fillet freshness. When whole, ungutted and gutted sea bass (*Dicentrarchus labrax*) were cooked and evaluated by panelists, all scores for texture, odor, taste, and overall acceptance decreased with increased storage time. (Papadopoulos, Chouliara, Badeka, Savvaidis, & Kontominas, 2003). The longer the storage time, the

softer the texture of the fish. Studies on sea bream (*Sparus aurata*) and channel catfish fillets confirmed that indentation force of the fillets decreased with increased storage time after harvest (Silva et al., 1998; Alasalvar, Taylor, Oksuz, Garthwaite, Alexis, & Grigorakis, 2001).

The texture of a food product is often difficult to define and as many as ten different definitions of "texture" are listed by Bourne (1982). Hence, the recommended term now is "textural properties" to indicate a group of related properties. Many approaches have been used to measure the textural properties of fish products. Most of them can be classified as either sensory or instrumental approaches but there is no accepted standard method. Sensory tests of the fish texture are common (Gill, Keith, & Lall, 1979; Cardello et al., 1982; Cardello, Sawyer, Maller, & Digman, 1982; Raksakulthai, Lee, & Haard, 1986; Slattery, 1998), but these are time consuming, complex, and expensive to conduct (Coppes, Pavlisko, & Vecchi, 2002). Sensory evaluation also has a subjective element that is affected not only by the physical property of the sample, but also by the psychological perspectives of the panels involved in the testing (Stone & Sidel, 2004).

Compared to sensory evaluations, instrumental methods are more objective but not necessarily more reliable. Several studies on the texture measurement were reported in different fish species such as haddock, hake (Gill et al. 1979), cod (Botta 1989; 1991), and salmon (Sigurgisladottir et al. 1999; Veland & Torrissen, 1999). Because of the irregular shapes of fish fillets, reformations of samples into regular shapes are typically involved, which is time consuming and labor intensive. Gill et al. (1979) used a modified four-blade Kramer shear compression cell attached to an Instron Universal testing

machine to measure the textural properties of both raw and cooked haddock (*Melanogrammus aeglefinus* L.) and hake (*Urophycis chuss* Mitchill) fillets. A correlation coefficient (r_s=0.71) was observed between the subjective and instrumental measurement using the maximum peak force. However, reformation and destruction of the samples were needed to complete the tests. A patented, portable, nondestructive texture tester was designed by Botta (1989, 1991) for raw Atlantic cod (*Gadus morhua* L.) fillets. The firmness and resilience of the raw cod fillets were measured using the texture index (*dr/di, dr: springiness; di: hardness*). When results of the texture tester were compared to the results of individual fish inspection officers, a 75% to 86% match was achieved using this device. Chamberlain, Kow, and Balasubramaniam (1993) developed a fish shearing device attached to an Instron Texture Analyzer and used reformed sample pieces to test shear force of raw flathead (*Platycephalus richardsoni* Castelnau), white trevally (*Pseudocaranx dentex* Bloch & Schneider), and blue morwong (*Nemadactylus douglasii* Hector). The shear force measured by the circular gadget, as the authors called it, had a correlation of 0.92 with sensory analysis, but the drawback was the required strict and time-consuming sample preparation steps. Sigurgisladottir et al. (1999) used the Texture Analyzer to study the textural properties of raw Atlantic salmon (*Salmo salar*) fillets at different locations above the lateral line on the fillets with both destructive (cutting blade) and non-destructive methods (flat-ended cylinder and spherical probe). The destructive shear force (cutting blade) method was found to be more sensitive than the other two methods for the salmon fillets. The most reliable results were obtained from a location below the dorsal fin.

A new value-added smoked catfish product is under development to explore potential markets and provide job opportunities. Acceptance of the final products is of great concern which is influenced by the characteristics of the products such as texture, flavor, appearance, etc. Few reports on properties of channel catfish texture are available. Likewise, little work has been done on the textural properties of fillets or different locations on the single fillet. Rapid and accurate measurement methods along with efficient sampling techniques are needed to determine the texture properties of catfish. The goal of this research was to explore and develop reliable instrumental texture measurement methods with an easy and novel sampling technique for both raw and cooked irregular shaped catfish fillets.

2. Materials and methods

2.1. Sample preparation

2.1.1. Raw catfish fillet

Twenty-four pieces of farm-raised, fresh, whole channel catfish, approximately 15-18 months of age, were purchased from a local grocery store and filleted by hand. All fillets (skin-off, with ribs and nugget) were weighed on an electronic balance (Rite-weight A-200D, Denver Instrument Company, Denver, CO, U.S.A.). The average weight of each fillet was 86±5 g.

2.1.2. Smoked catfish fillet

Smoked fillets were tested as a representative of cooked catfish fillets. Thirteen pieces of channel catfish fillets were cured (4 °C, v: w = 1.4:1) in a brine solution (60° salinity) for one hour. Fillets were then dried at 30 °C for one hour, smoked at 60 °C for one hour, and cooked at 71 °C for 45 min using an electronic smokehouse (KOCH

323345, KOCH Equipment LLC. Kansas City, MO.). Hickory sawdust was used to generate the smoke. All the fillets were cooled to room temperature (25 °C) before texture evaluation.

2.2. Sampling technique

When a whole piece of catfish fillet was placed on a flat surface with skin side down, thickness of the fillet changes gradually from tail to head and from side to middle forming a contoured slope. Fortunately, natural thickness contours existing on the catfish fillet are just like the elevation contours on a hill (Fig. 1). A combination of fillet contours and the height calibration function of a Texture Analyzer (5 kg load cell, Model TA-XT plus, Texture Technologies Corp., Scarsdale, N.Y./ StableMicro Systems, Godalming, Surrey, U.K.) were used to develop a new instrumental sampling method for texture measurement. The novel sampling technique for fillet texture testing was developed by sampling certain thickness points on contour plots without reforming the fillet into regular shapes. To sample a certain thickness level, height of the probe was first set at specific height. Then, the equivalent thickness level on the fillet can be located easily by sliding the fillet under the probe until a touch was made between the probe tip and the fillet surface.

Only the shank fillet part (fillet part excluding the nugget and ribs, Fig. 1) was used for the test. Channel catfish fillet has a typical teleostean segmented "W"- shaped trunk muscles which are separated into epaxial myomeres and hypaxial myomeres by the horizontal septum (Arratia, Kapoor, Chardon, & Diogo, 2003). When seen from the internal surface, four cone bands of the myomeres can be observed (Fig. 2). For this study, the four myomere cone bands were numbered as band 1 to 4 from the back to the

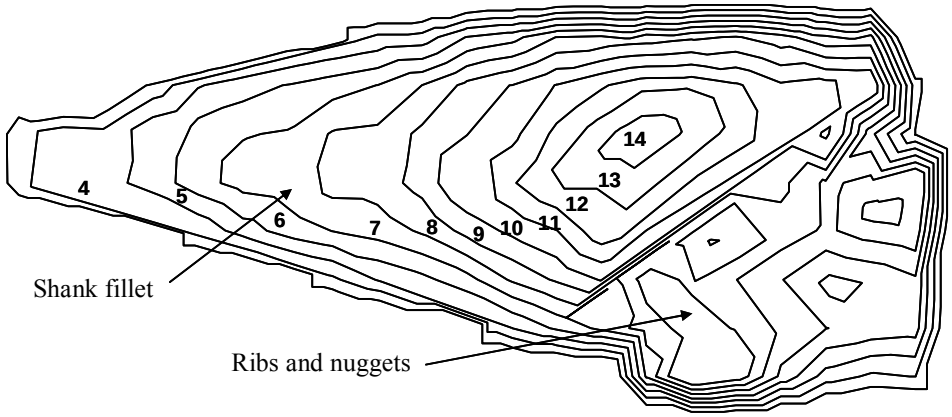


Fig. 1. Thickness contour plot of channel catfish fillet existing on the shank fillet part. Numbers on the contour denote different thickness (mm) levels.

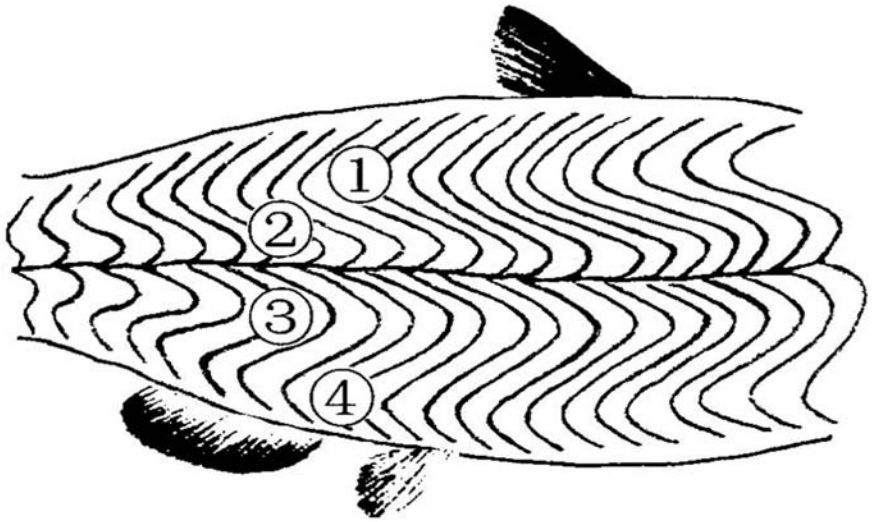


Fig. 2. Schematic catfish myomere cone bands (internal surface). Numbers stand for myomere cone bands 1 to 4 from back to the belly on a piece of channel catfish fillet.

belly side. Tests were made on different contour levels (6 mm - 13 mm) on each of the cone bands, i.e., each cone band contributed one point on every contour level tested.

2.3. Texture measurement

Two methods with two distinct probes were applied to different locations (cone band×thickness combinations) of raw and hot smoked catfish fillets. Height (distance between the probe tip and the platform) and weight (applied force: 5 kg) of the probes were calibrated each time the instrument was reconfigured.

2.3.1. "Tooth" method

The destructive "tooth" method used an incisor blade (length=34.04 mm, width=9.90 mm, thickness=1.50 mm) to imitate a human tooth. The probe was attached to the Texture Analyzer with its longitude side perpendicular to the test platform and blade side (9.90×1.50 mm) facing the fillet surface. Each cut was made at directions perpendicular to the middle horizontal septum on the fillet (Fig. 2). After a touch was achieved between the blade tip and fillet surface, the "tooth" probe was allowed to cut the muscle fiber perpendicularly at a speed of 2 mm/sec with the trigger type of "button" and then returned to the starting position. Fillet was compressed first and then cut through by the blade. The shear force (g) was measured at the maximum force required to cut through the samples (failure point). Tests were made at 7, 8, 9, 10, and 11 mm thickness for each of the four cone bands on a fillet.

2.3.2. "Finger" method

The non-destructive "finger" method used an aluminum cylinder probe (length=123.0 mm, diameter=7.9 mm) with a ball tip (diameter=6.6 mm) to imitate compression with a human finger. The probe was attached to the Texture Analyzer with

its longitude side perpendicular to the test platform. After a touch was made between the ball tip and fillet surface, a test was performed at a speed of 1 mm/sec with the trigger type "button". The probe was allowed to press the muscle fiber to certain depth and then returned to the starting position. A one-time indentation was made at 6, 7, 8, 9, 10, 11, and 12 mm thickness on each of the four cone bands of a fillet. According to preliminary tests, different press depths (2 - 5 mm) based on different contour levels (6 - 12mm) were selected. The press depths were determined by the formula

$$\mathrm{Pr}\,ess \quad depth(mm) = \frac{Thickness(mm)}{2} - 1(mm)$$

such that indentation force of the fillet was measured without breaking the muscle fiber. The indentation force (g) at the deepest press depth was recorded. A positive linear relationship between the fillet thickness and indentation force was observed in salmon (*Salmo salar*) fillets (Veland et al., 1999). The same positive linear relationship was assumed here on the catfish fillet. Prior to statistical analysis, all results collected at different thicknesses were converted to a 10 mm thickness using the following formula:

$$Converted \quad Indentation \quad Force(g) = \frac{Raw \quad Indentation \quad Force(g)}{\mathrm{Pr}\,ess \quad Depth(mm)} \times 10(mm)$$

2.3.3. Limitations

Because the fillets were dried for one hour before smoking, a thin dried layer or pellicle was formed on the fillet surface, which made it difficult for the "tooth" probe to break through the surface. Furthermore, the cooked muscle fiber under the surface was very fragile and tended to fall apart under the pressure of the "tooth" probe. No reliable results could be obtained with the "tooth" method on smoked fillets and thus only results from the "finger" method are presented for smoked fillets.

2.4. Statistical analysis

The experimental design was a randomized complete block design (RCBD), where fillets (r = 12 for raw fillet and r = 13 for smoked fillet) were considered the random blocking factor. 'Treatment' factors were myomere cone band and thickness along the longitudinal axis. The 'treatment' structure thus was a factorial combination of cone band and thickness. Data were analyzed using SAS® procedure GLIMMIX (http://support.sas.com/rnd/app/papers/glimmix.pdf). This new procedure enables the user to check underlying assumptions by creating plots of studentized residuals against linear predictors, histograms, Q-Q plots, and box plots in a single panel. Because an individual fillet was considered a block and levels of 'treatment' factors (cone band and thickness) were not randomized, observations (measurements) for a given method on a particular fillet were not truly independent and a correlated error structure must be assumed. The residual error structure was modeled assuming either a correlation among cone bands or among thickness levels within cone bands. The Akaike Information Criterion with Correction (AICC) was used to decide among competing models (Burnham & Anderson, 2002). Treatment effects were assessed as a standard two-factor model with interactions and in a second step by direct regression approach in a mixed model setting as outlined by Little, Milliken, Stroup, Wolfinger, & Schabenberger (2006).

3. Results and discussion

3.1. Basic statistical analysis

The data met the underlying assumptions although histogram and Q-Q plots indicated a slight skewness to the right for the "Finger" method used on raw fillets. Based

on the improvement in the AICC, the best fitting structure for all three data sets (raw fillet analyzed by the "Tooth" method, raw fillet analyzed by the "Finger" method, and smoked fillet analyzed by the "Finger" method) was a first order autoregressive correlation [Type = AR(1)]. AICC values for correlations among measurements within a myomere band versus measurements among cone bands were very close. Both resulted in a substantially lower residual variance and, hence, standard errors compared to the standard model with residual error.

Cone band×thickness interaction was significant for fresh fillets ($P \leq 0.002$), but not for smoked ones ($P \geq 0.51$, Table 1). Based on variance components estimated from a model where all effects were treated as random, it was shown that for the "finger" method fillet thickness is of utmost importance, followed by cone band and the interaction between these two factors (data not shown). As indicated by the non-significant interaction for smoked fillets evaluated by the "finger" method, the interaction variance component estimate was essentially zero. For the "tooth" method, the variance component for cone band was largest, followed by the slightly smaller estimate for thickness and a much smaller cone band×thickness variance component. The relationship between cone band and thickness was modeled using regression techniques within the framework of mixed models analysis. The quadratic relationship was never significant ($P > 0.30$, data not shown).

3.2. Raw catfish fillet

3.2.1. "Tooth" method

Shear force obtained by the "tooth" method increased with increasing thickness, regardless of cone band (Fig. 3A). Similar patterns of shear force change along the

Table 1. Interaction analysis of the test results using “finger” and “tooth” methods on textural properties of fresh and smoked channel catfish fillets.

Type	Method	Source	Degrees of Freedom		F-Value	Prob>F
			Numerator	Denominator[a]		
Fresh	Finger	Band (B)	3	160	23.2	<0.001
Fresh	Finger	Thickness (T)	6	55	22.8	<0.001
Fresh	Finger	B*T	17	153	2.5	0.002
Fresh	Tooth	Band (B)	3	130	31.8	<0.001
Fresh	Tooth	Thickness (T)	6	64	11.9	<0.001
Fresh	Tooth	B*T	16	137	2.9	<0.001
Smoked	Finger	Band (B)	3	127	23.5	<0.001
Smoked	Finger	Thickness (T)	4	59	5.6	0.001
Smoked	Finger	B*T	11	127	0.9	0.513

[a] Denominator degrees of freedom adjusted with the Kenward-Rogers option.

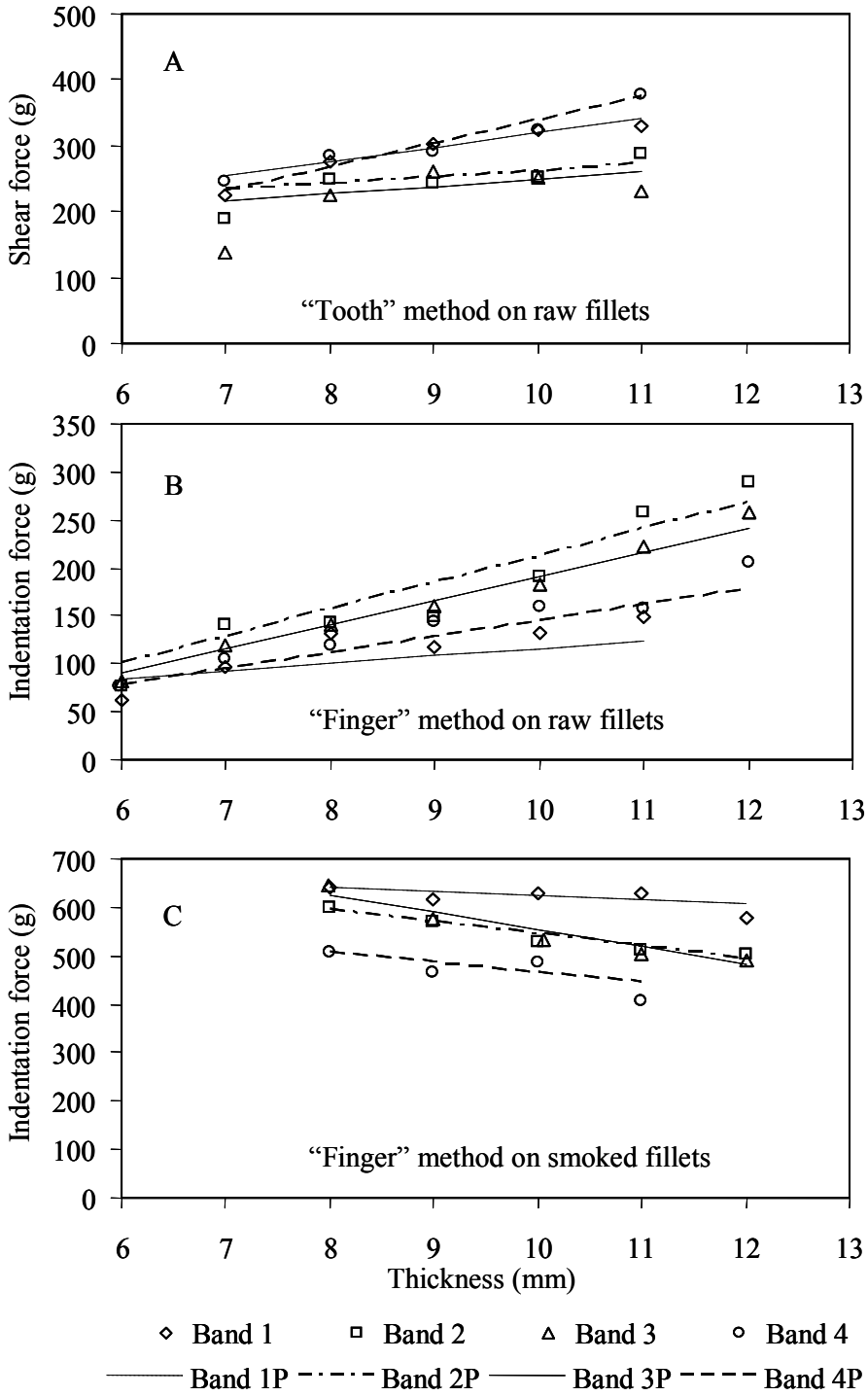


Fig. 3. Effects of cone band and fillet thickness on textural properties of fresh and smoked channel catfish fillets evaluated by the "tooth" (A) and "finger" (B, C) methods. Regression parameter estimates are given in Table 2.

thickness were observed on band 2 and 3. The slopes for the inner bands (2, 3) did not differ significantly from one another ($P = 0.776$, Table 2), whereas the outer bands (1, 4) did ($P = 0.066$, Table 2).

Shape of the fillet negatively influenced on the tests performed on bands 1 and 4. The region where bands 1 and 4 were located was the marginal sides of the fillet (Fig. 2). It was difficult for the blade tip to achieve full contact with the fillet surface on these marginal bands, which was not a problem for tests made on middle bands (bands 2 and 3). When the tests were executed on marginal bands (bands 1 and 4), the contact area between the blade tip and fillet muscle was always changing until a full touch was made at some time after the start of each test. No reliable results could be obtained on these two bands (1 and 4) with the "tooth" method. Results also showed that outer bands had significantly greater shear force slope estimates than inner bands ($P = 0.049$, Table 2). This was not truly reflecting the nature of the fillet due to the reason explained above. Only results from the middle part (bands 2 and 3) of the fillet reflect its textural properties objectively.

There is limited information on textural properties of different locations of a fillet. Shear force change along different locations on the fillet was also measured by Sigurgisladottir et al. (1999) with a cutting blade (70 mm width) on salmon (*Salmo salar*) fillets. Tests were taken above the fillet lateral line from head to tail. Shear force from the tail part was significantly greater than that of the head part and middle part of the fillet. But the head part shear force (test location 1) was greater than that of the middle part (test location 2, 3, and 4) which was not different from each others. In our shear force study, the starting point of measurement was usually taken near the middle part of the fillet and

Table 2. Estimates for intercepts and slopes from regression of texture response on thickness of fresh and smoked channel catfish fillets.

Type	Method	Band	Intercept		Slope			Contrast among Slopes	
			Est.[a]	SE[b]	Est.	SE	Prob>t	Label	Prob>t
Fresh	Tooth	1	102	62.5	21.8	6.55	0.001	Comparing outer	0.066
Fresh	Tooth	2	164	38.1	9.8	3.51	0.006	Comparing inner	0.776
Fresh	Tooth	3	137	38.3	11.2	3.54	0.002		
Fresh	Tooth	4	-25	45.4	36.3	4.38	<0.001	Outer vs. inner	0.049
Fresh	Finger	1	33	33.7	8.3	4.04	0.040	Comparing outer	0.105
Fresh	Finger	2	-67	26.3	27.9	2.79	<0.001	Comparing inner	0.484
Fresh	Finger	3	-61	26.8	25.1	2.84	<0.001		
Fresh	Finger	4	-22	28.6	16.6	3.16	<0.001	Outer vs. inner	<0.001
Smoked	Finger	1	714	114.0	-8.9	11.77	0.452	Comparing outer	0.465
Smoked	Finger	2	795	88.8	-25.0	8.25	0.003	Comparing inner	0.366
Smoked	Finger	3	911	89.6	-35.6	8.37	<0.001		
Smoked	Finger	4	677	118.4	-21.3	12.27	0.084	Outer vs. inner	0.021

[a] Estimated; [b] Standard error.

went up towards the head part. The salmon samples were also cut into equal shapes before the shear force tests, which was not the case in this catfish shear force test. A food-size catfish fillet is much thinner than that of a salmon. A test method with uniform test shapes will not be applicable to fresh catfish fillets. Considerations of thickness on catfish fillet textural properties should be taken no matter what method is used. In this case, due to unavailability of equal sample shapes, fillet thickness might contribute to the increasing shear force trend from tail to head.

3.2.2. "Finger" method

Indentation force on the raw catfish fillet increased gradually along the thickness on all four myomere cone bands (Fig. 3B). Neither the outer bands ($P = 0.105$) nor the inner bands ($P = 0.484$, Table 2) showed significant difference in slope. The change per unit increase in thickness was significantly lower ($P < 0.001$) for the outer bands than for the inner bands, which was a difference from the "tooth" method.

Negative influence of the fillet margin to the test was not so evident in the "finger" method as in the "tooth" method because of the relatively small contact area between the ball tip and muscle. A full contact between the ball tip and fillet could be achieved easily when the probe approached the fillet surface no matter which cone band was tested. Indentation force obtained from the "finger" method could be regarded as a better indication of textural properties of the whole fillet. Again, similar patterns of indentation force change along the thickness were observed on bands 2 and 3.

Fillet thickness must be taken into consideration when a compression test is applied to the fillet (Veland et al., 1999). In a study of Sigurgisladottir et al. (1999), no difference in indentation force was observed on the head and middle part of salmon

(*Salmo salar*) fillets when a 5 mm indention was made by a ball probe (Diameter=25.4 mm) on the fillet samples of natural thickness. Only the tail part had greater indentation force than that of the head and middle part. The average fish weight was 4 kg in the salmon study. The 5 mm press depth might be a possible reason for not obtaining enough response from thicker fillet part, such as the head and middle area, when a large-size fish (e.g. salmon) is studied. In contrast, Veland et al. (1999) measured the indentation force of salmon (*Salmo salar*) fillets at different thicknesses with two press depths set at 6 and 10 mm. Change of indentation force along thickness was significant for both press depths. The average weight of the salmon used was below 3 kg. The catfish fillet is much smaller than the salmon fillet. It is even more difficult to set a fixed press depth when the textural properties of the whole fillet were studied. Besides this, the non-linear relationship between the indentation force and thickness (Veland et al., 1999), if measured by fixed press depth method, makes the interpretation of results complicated. This was why a different press depth was chosen for different thicknesses in this study.

3.3. Smoked catfish fillet

The measured indentation force values from smoked catfish fillets were also converted to a 10 mm thickness values using the same equation as the raw fillets before the data were analyzed. The same assumption was made on the smoked catfish fillets that a linear relationship exists between the indentation force and thickness.

Unlike fresh catfish fillets, the smoked fillets' indentation force decreased with increasing thickness for all cone bands (Fig. 3C). Outer bands differed significantly from the inner bands ($P = 0.021$, Table 2), whereas the outer bands did not differ significantly

from one another ($P = 0.465$). Indentation force slopes of the inner two cone bands (2, 3) did not differ from one another ($P = 0.366$, Table 2).

Measurements of textural properties of cooked fish meat are necessary during the development of a food product. Changes must be monitored due to modifications of processing parameters while the quality optimization is the goal (Coppes et al., 2002). Unlike the collagen of most mammalian muscle, which makes the meat tougher after cooking, collagen in fish muscle does not contribute significantly to the firmness of cooked fish meat and fish muscle becomes very fragile after cooking (Dunajski, 1979). A dried layer was formed on the fillet surface during the drying and cooking process. The thicker the fillet was, the more fragile the muscle, it had under the dried layer and the softer it became on the texture. This was why the indentation force value decreased with increased thickness on the smoked catfish fillets.

4. Conclusions

Both the "finger" method and the "tooth" method can be used to evaluate the textural properties of the raw channel catfish fillets. The "finger" method is recommended in practical situations not only because it is a non-destructive method but also because it is applicable to both raw and smoked fillets. The height calibration function of the Texture Analyzer and the natural contours existing on the fillet was combined together to generate a novel sampling technique to be used in the textural measurement of irregularly shaped fish fillets like catfish fillets. This method is rapid and easy to execute without reforming the samples. Measurement of textural properties of other fish fillets, such as tilapia and salmon was recommended using this novel sampling technique.

References:

Alasalvar, C., Taylor, K. D. A., Oksuz, A., Garthwaite, T., Alexis, M. N., & Grigorakis, K. (2001). Freshness assessment of cultured sea bream (*Sparus aurata*) by chemical, physical, and sensory methods. *Food Chemistry*, *72*, 33-44.

Arratia, G., Kapoor, B. G., Chardon, M., & Diogo, R. (2003). *Catfishes*, *volume 1* (pp. 166 - 169). Enfield: Science Publishers. Inc.

Beuchat, L. R. (1973). Hypoxanthine measurement on assessing freshness of chilled channel catfish (*Ictalurus punctatus*). *Journal of Agriculture and Food Chemistry*, *21*(3), 453-455.

Botta, J. R. (1989). *Method of measuring the firmness of meat*. Canadian Intellectual Property Office. No. 2004560.

Botta, J. R. (1991). Instrument for nondestructive texture measurement of raw Atlantic cod (*Gadus morhua*) fillets. *Journal of Food Science*, *56*(4), 962-964, 968.

Bourne, M. C. (1982). *Food texture and viscosity: concept and measurement*. (pp8-12). New York: Academic Press.

Burnham, K. P. & Anderson D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretical Approach* (2nd ed.). New York: Springer-Verlag.

Cardello, A. V., Maller, O., Kapsalis, J. G.., Segars, R. A., Sawyer, M. F., Murphy, C., & Moskowitz, H. R. (1982). Perception of texture by trained and consumer panelists. *Journal of Food Science*, *47*, 1186-1197.

Cardello, A. V., Sawyer, M. F., Maller, O., & Digman, L. (1982). Sensory evaluation of the texture and appearance of 17 species of North Atlantic fish. *Journal of Food Science*, *47*, 1818-1823.

Chamberlain, A. I., Kow, F., & Balasubramaniam, E. (1993). Instrumental method for measuring texture of fish. *Food Australia*, *45*, 439-433.

Coppes, Z., Pavlisko, A., & Vecchi, S. D. (2002). Texture measurements in fish and fish products. *Journal of Aquatic Food Product Technology*, *11*(1), 89 - 105.

Dunajski E. (1979). Texture of fish muscle. *Journal of Texture Studies*, *10*, 301-318.

FAO. (2004) a. *Total production 1950-2004*. (Release date: March 2006).

FAO. (2004) b. *The State of World Fisheries and Aquaculture*.

Gill, T. A., Keith, R. A., & Lall, B. S. (1979). Textural deterioration of red hake and haddock muscle in frozen storage as related to chemical parameters and changes in the myofibrillar proteins. *Journal of Food Science*, *44*, 661-667.

Heia, K., Sigernes, F., Nilsen, H., Oehlenschlager, J., Schubring, K., Borderias, J., Nilsson, K., Jorgensen, B. M., & Nesvadba, P. (1998). Evaluation of fish freshness by physical measurement techniques. *Proceedings of the Final Meeting of the Concerted Action "Evaluation of Fish Freshness",* AIR3CT94 2283.

Lawless, H. T. & Heymann, H. (1998). *Sensory evaluation of food: principles and practices*. Chapman and Hall, New York, NY, USA.

Little, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D., & Schabenberger, O. (2006). *SAS® for Mixed Models.* 2nd ed. SAS Institute Inc. Cary, NC. USA.

Nunez, A. L., Silva, J. L., & Chamul, R. S. (1999). Onset of rigor and quality of channel catfish (*Ictalurus punctatus*) fillets as affected by preprocessing. *IFT Annual Meeting Abstract*, 79E-32.

Olafsdottir, G., Nesvadba, P., Natale, C. D., Careche, M., Oehlenschlager, J., Tryggvadottir, S. V., Schubring, R., Kroeger, M., Heia, K., Esaiassen, M., Macagnano, A., & Jorgensen, B. M. (2004). Multisensor for fish quality determination. *Trends in Food Science & Technology*, *15*, 86-93.

Papadopoulos, V., Chouliara, I., Badeka, A., Savvaidis, I. N., & Kontominas, M. G. (2003). Effect of gutting on microbiological, chemical, and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*) stored in ice. *Food Microbiology*, *20*, 411-420.

Raksakulthai, N., Lee, Y. Z., & Haard, N. F. (1986). Effect of enzyme supplements on the production of fish sauce frommale capelin (*Mallotus villosus*). *Canadian Institute of Food Science and Technology Journal*, *19*(1), 28-33.

Sigurgisladottir, S., Hafsteinsson, H., Jonsson, A., Lie, O., Nortvedt, R., Thomassen, M., & Torrissen, O. (1999). Textural properties of raw salmon fillets as related to sampling method. *Journal of Food Science*, *64*(1), 99-104.

Sikorski, Z. E. (1990). *Seafood resources, nutritional composition, and preservation*. Boca Raton: CRC Press, Inc.

Silva, J. L., Nunez, A., Figueroa-Garcia, J. E., Chamul, R. S., & Kim, T. (1998). Influence of preharvest and postharvest handling on quality of channel catfish fillets. In *Proceedings: 1998 Catfish Processors' Workshop. Mississippi Agricultural and Forestry Experiment Station* (pp4-6).

Slattery, S. L. (1998). Shelf-life of Spanish mackerel (*Scomberomorus commerson*) from Northern Australian waters. *Journal of Aquatic Food Product Technology*, *7*(4), 63-79.

Stone, H. & Sidel, J. L. (2004). *Sensory Evaluation Practices*. San Diego: Elsevier Academic Press.

Tome, E., Kodaira, M., & Matsunaga, Y. (1999). Effect of processing conditions, lipids content and freshness on acceptability of smoked catfish. *Food Science and Technology International*, *5*(2), 167-176.

USDA. (2006). *Catfish Production*. NASS.

Veland, J. O. & Torrissen O. J. (1999). The texture of Atlnatic salmon (*Salmo salar*) muscle as measured instrumentally using TPA and Warner-Brazler shear test. *Journal of the Science of Food and Agricuture*, *79*, 1737-1746.

IV. CHAPTER TWO:

STUDIES ON THE BRINING BEHAVIOR AND SALT CONTENT OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) FILLETS

Abstract

Hot-smoked channel catfish (*Ictalurus punctatus*) products have been developed to improve the potential market value of catfish. FDA guidelines on the hot-smoked fish products require at least 3.5% water phase salt (WPS) in the loin muscle to inhibit the formation of toxins due to the growth of *Clostridium botulinum* in reduced oxygen packaging. Manipulations of the salt and moisture content of the fish are critical to the control of the WPS level. Fresh catfish fillets were brined in a saline solution with different concentrations (5, 10, 15, and 20%, w/v) for 7 pre-determined time intervals (10, 20, 30, 45, 60, 90, and 120 min) at 4 °C. Salt content of smoked catfish was determined by both titration and near infrared spectroscopy (NIR) methods. Fresh catfish brining behavior was explained by both nonlinear and multiple linear regression (MLR) analyses. The possibility of using NIR to determine the salt content in hot-smoked catfish fillet was investigated.

Keywords: Channel catfish, *Ictalurus punctatus*, brining, salting, hot smoked, NIR, WPS

1. Introduction

Smoking is a traditional way to preserve perishable food items, such as fish. It can be traced back several thousand years (Eyo, 2001). Smoking, together with salt curing, was originally used to improve food preservation, but today it is mainly used for producing desired flavors in meats and other foods (Lawrie, 1985). The combined effects of drying and deposition of chemical compounds from incompletely burned woods is believed to mostly attribute to the prolonged shelf-life of smoked fish products. Cold smoking and hot smoking are two traditional ways of smoking. The temperatures of cold smoking typically do not exceed 30 °C, while hot smoking usually occurs at temperatures above 70 °C. Therefore, hot-smoked fish are already cooked and are ready for consumption after smoking (Wheaton & Lawson, 1985).

Before fish is smoked, it needs to be brined in saline solutions to achieve certain levels of salt content in the muscle. Water phase salt (WPS) in the fish muscle is an important criterion used to monitor and evaluate this brining process and it is also a critical control point during the production of smoked fish products. As the salt content in the fish muscle increases water activity (a_w) decreases so that the growth of spoilage and pathogenic microorganisms is inhibited (Horner, 1997). FDA guidelines for hot-smoked fish products in reduced oxygen packaging require a minimum of 3.5% WPS in fish muscle without other preservatives. This prevents the production of toxins by *Clostridium botulinum* (FDA, 2001). These types of neurotoxins will cause vomiting, constipation, respiratory difficulties, or heart failure in humans (Adam & Moss, 2000).

Brining operations play a key role in the control of salt content of final fish products. Many studies on the brining behaviors have been carried out on various fish

species. For example, yellowtail (*Trachurus mccullochi* Nichols) fish was brined in 5, 10, 15, 21%, and saturated brine solutions at temperatures of 25 °C and 35 °C. Results showed that fish brined in higher brine concentrations absorbed salt more rapidly and reached a higher equilibrium salt content than those brined in lower concentrations (Berhimpon et al., 1990; Berhimpon et al., 1991). Temperature also has a significant effect on the salt absorption rate. Fish brined at 35 °C always reached the equilibrium salt content higher and faster than those brined at 25 °C. Water activity and moisture content in the fish muscle decrease at a rate positively related to the rate of the salt absorption (Berhimpon et al., 1991). When previously frozen rainbow trout (*Oncorhynchus mykiss*) fillets (340 ± 50g) were brined in 8.7 and 17.4% brine solutions at various intervals (30, 60, 90, and 120 min) at 3 °C and then smoked, the salt content of smoked fillets increased linearly with elongated brine times. Brining fish in higher brine concentrations resulted in higher salt content and WPS, and lower water activity of the smoked fillets. The combination of 8.7% brine concentration and a 90 min brining period was optimal based on the FDA requirements of 3.5% WPS as mentioned above (Jittinandana et al., 2002). Overall quality and yield increased in fresh filleted cod (*Gadus morhua*) salted at low salinity (17.5%) compared to two other salinity levels (22.5% and 25.6% respectively,Thorarinsdottir et al., 2004).

Mathematical models have also been developed to interpret the brining behavior of various fish species. Deng (1977) used a first order kinetics equation to explain the salt penetration characteristics of frozen mullet fillets (25% brine solution). Zugarramurdi & Lupin (1980) developed a nonlinear model depicting the characteristics of fish brining for wet or dry salting processes of fish species including anchovy (*Engraulis anchoita*),

patagonian haddock (*Egleginops maclovinus*), and fueguine sardine (*Clupea fueguensis*). This brining behavior was also verified using several other fish species.

Channel catfish (*I. punctatus*) is a typical warm-water fish species and also a widely accepted food item around the world. Smoking of catfish generally involves a marinating or a brining process to provide more tastes and flavors to the fish. Little work has been done concerning the effects of brining on the quality of channel catfish fillet. In the early 1970's, two size groups of frozen skinned whole channel catfish (248 and 347 g group) were put in a brine solution of about 12.5% salinity (w: v) for four different brining periods (4, 8, 16, and 24 hr) at 4.4 °C. Fish were then smoked and evaluated for sensory attributes by trained panels. Based on the results, the longer brining time was recommended for further use because it produced better texture and flavor than those made with shorter brining times (Boggess et al., 1973). However, how the brine solution affects the salt content of brined fish during different brine periods is not clear.

The salt and moisture contents in the fish play important roles in controlling the WPS levels. WPS is the concentration of salt (wet basis) in the water portion of the fish flesh (FDA, 2001). It is calculated as:

$$WPS = \frac{\%Salt}{\%Salt + \%Moisture} \times 100$$

Assuming zero level of salt content in raw, unprocessed fish, a required WPS content in the smoked fish can be expressed as:

$$\frac{S_2}{S_2 + M_2} \geq \frac{3.5}{100} \text{ or } \frac{M_2}{S_2} \leq 27.57 \qquad (1)$$

where S_2 and M_2 are salt and moisture content of smoked fish, respectively. Equation (1) indicates that ratio of moisture to the salt content must be less than or equal to 27.57 to provide a WPS larger than or equal to 3.5% in the smoked fish. Since the salt content of the smoked fish (S_2) can be described as:

$$S_2 = \frac{S_1 \times (1 - M_2)}{1 - M_1} \tag{2}$$

where S_1 and M_1 are salt and moisture content of brined fish, respectively. Replacing S_2 in equation (1) with equation (2) we will have:

$$\frac{M_2 \times (1 - M_1)}{S_1 \times (1 - M_2)} \leq 27.57 \tag{3}$$

By rearranging equation (3) we can obtain:

$$M_2 \leq \frac{27.57 \times S_1}{27.57 \times S_1 - M_1 + 1} \tag{4}$$

and

$$S_1 \geq \frac{M_2 \times (1 - M_1)}{27.57 \times (1 - M_2)} \tag{5}$$

Equation (4) means that if the salt (S_1) and moisture (M_1) content of brined fish is known, the 3.5% WPS level can be achieved by controlling the moisture (M_2) content in the smoked fish product below certain levels (Hilderbrand, 2000). Equation (5) means that the 3.5% level can also be controlled by adjusting the salt content in brined fish (S_1) given the moisture content of brined fish (M_1) and smoked fish (M_2). Both brine concentration and brine time are believed to have major impacts on the salt contents of brined fish (S_1). The goal of this study was to find the effects of different brine

concentrations and brine times on salt contents of brined catfish fillets. Results of this study will help develop fish brining practices for fish processors.

To study brining behavior properly, it is important to determine the salt content accurately. There are several methods available for measuring salt content, including titration (AOAC, 1995), Quantab titrator (Hilderbrand, 2000), and conductivity method (Hilderbrand, 1992). The titration method is used primarily as a reference method. This method produces accurate results but the sample preparation and testing are time consuming (Huang et al., 2002; Lin et al., 2003). The Quantab titrator works well with clear sample solutions but clogging problems inside the titrators are commonly encountered when working with ground fish samples, such as smoked catfish samples in this study. Based on our experiences, the conductivity method does not produce reliable and reproducible results. In addition, all the aforementioned methods require samples to be totally destroyed before the testing. Therefore, it is important to develop a rapid, accurate, and non-destructive analytical technique for measuring salt in fish.

NIR is a rapid and non-invasive analytical technique for food quality analysis and control (Huang et al., 2001, 2002; Lin et al., 2003). In the NIR region (700 - 2500 nm), the O-H stretch of water has absorption bands at approximately 960, 1200, 1450, and 1940 nm. Sodium chloride molecules do not have absorption bands in NIR region per se. However, salt is measurable indirectly because a sample with higher salt content gives rise to water bands shifting to a longer wavelength through salt-water interactions (Lin & Brown, 1992). NIR has been successfully used to determine salt content in commercial hot smoked King and Chum salmon fillets (Lin et al., 2003), cold smoked Atlantic salmon (Huang et al., 2002), and cured salmon roe (Huang et al., 2001).

The objectives of this study were to investigate brine behaviors of fresh catfish fillets submerged in varied saline solutions. The possibility of using NIR as a replacement of salt determination in smoked catfish products was also investigated.

2. Materials and methods

2.1. Brining

Sixty pieces of farm-raised, fresh channel catfish fillets were purchased directly from a catfish processing plant (Harvest Select, Uniontown, Alabama). All the fillets were stored on ice until experimentation began.

Fillet weights ranged from 62 to 123 g with an average value of 92 g. Thicknesses of the fillets on the sampling locations were measured with a caliper (Model C-09923-18, Newton, MA). Thickness ranged between12.6 to 17.4 mm with an average of 14.9 mm.

Plain table salt (Morton® Salt) was used to prepare brine solutions at 4 different salinity levels: 5%, 10%, 15%, and 20% (w / v). The salinity levels were set from relatively low (5%) to high (20%) levels; so that brining behaviors of the catfish fillets could be observed over a wide range. The whole fillets were submerged into brine solutions (v / w = 1.5 / 1) at 4 °C, then taken out at pre-determined intervals (10, 20, 30, 45, 60, 90, and 120 min). The superficial brine solution was removed by dipping the fillets into deionized water (v / w = 1.5 / 1) for 2 seconds. Excess water on the surface of fillets was removed using absorbent tissues.

The thickest part of the fillet, usually between the head and the dorsal muscle (Part 4 on Fig. 1), was sampled for the determination of salt content because it was most likely to have the lowest salt content.

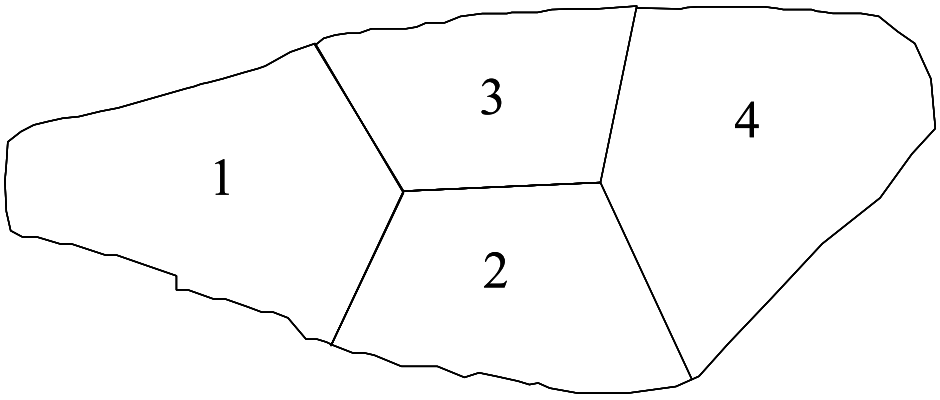


Fig. 1. Sampling locations on a piece of channel catfish (*I. punctatus*) fillet. Number 1, 2, 3, and 4 indicate tail, middle belly, middle back, and head part, respectively.

2.2. Determination of salt content with titration method

The salt content of brined and smoked fish samples was determined by using the AOAC Official Method 937.09-Silver Nitrate Titration (AOAC, 1995) as a reference method. Each fish was blended into small particles and a 5 g sample was used in the titration method.

2.3. Determination of salt content with NIR method

An NIR Composition Analyzer KJT270 (Kett Inc., Villa Park, CA, USA) was used to determine salt content in smoked catfish samples. During the determination, sample was placed into a sample container and exposed to both NIR absorption light and reference light. Fifteen samples were used to construct a calibration curve, while 12 samples were selected as unknown samples for salt testing.

For the NIR method, a proper calibration curve was established prior to determination operations. Correlations between NIR spectral features and chemical

reference values were used to predict salt concentrations in test samples. The predicted values were then compared with the reference values. The standard error of prediction (*SEP*) was used to indicate the predictive performance of the calibration models:

$$SEP = \sqrt{\frac{\sum_{i=1}^{n}(X-Y)^2}{n-1}}$$

where X is the NIR method value, Y is the reference method value, and n is the number of samples.

2.4. Model verification

Relationships between the brine concentration and brine time with the salt content of brined fish were verified in smoked catfish products. Four different levels of salt content of brined fish (S_1) were calculated according to equation (1) and (5) to give the final product a WPS of 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%. With a 75% moisture in the raw fish and 60% smoking yield, the salt content of brined fish (S_1) could be calculated backward from the required WPS (Table 1). The brine concentration and brine time needed for specific salt content (S_1) of brined fish was then predicted by the equation obtained from the brine experiment.

Brined fillets were laid on racks and transferred into a smoke house (KOCH 323345, KOCH Equipment LLC. Kansas City, MO, USA). Smoke was generated by burning hickory sawdust. Fillets were first dried using hot air for one hour and then smoked to a final internal temperature of 100 °C for 30 min. The internal temperature of the fish was monitored by inserting thermocouples into representative fillets. After the smoking process, fillets were allowed to cool to room temperature (25 °C) before samples were taken for salt determinations.

Table 1. Salt content needed in brined catfish fillet as calculated according to predicted WPS levels assuming 75% moisture content in raw fillet and 60% smoking yield in the final products.

Predicted WPS	M_1 ($\approx M_0$)	Yield	M_2	S_2	$\frac{1-M_1}{1-M_2}$	S_1
1.0%	75.0%	60.0%	58.3%	0.59%	0.6	0.35%
2.0%	75.0%	60.0%	58.3%	1.19%	0.6	0.71%
2.5%	75.0%	60.0%	58.3%	1.50%	0.6	0.90%
3.0%	75.0%	60.0%	58.3%	1.80%	0.6	1.08%
3.5%	75.0%	60.0%	58.3%	2.12%	0.6	1.27%
4.0%	75.0%	60.0%	58.3%	2.43%	0.6	1.46%
4.5%	75.0%	60.0%	58.3%	2.75%	0.6	1.65%
5.0%	75.0%	60.0%	58.3%	3.07%	0.6	1.84%

M_0: Moisture content of raw catfish fillet.

M_1: Moisture content of brined catfish fillet. S_1: Salt content of brined catfish fillet.

M_2: Moisture content of smoked catfish fillet. S_2: Salt content of smoked catfish fillet.

M_2=1-[(1- M_0) / yield]. S_2=WPS ×M_2 × 100 / (100-WPS × 100).

S_1= S_2 × (1- M_1) / (1- M_2).

2.5. Statistical analysis

Data analysis was performed with SAS 9.1.3 software package (SAS Institute Inc. NC, USA). Data from the brine experiment were fitted with both nonlinear (Seber & Wild, 2003) and multiple linear regression (Montgomery et al., 2001) approaches. A first order kinetics equation, $Y = C \cdot (1 - e^{-k \cdot t})$, was fitted through PROC NLIN procedure with Gauss-Newton algorithm in SAS, where Y is the salt content at time t (g salt / 100 g sample), C is the equilibrium salt content (g salt / 100 g sample), and k is the initial salt absorption rate (/ min) (Deng, 1997). Parameters obtained from this model fitting (C and k) were used to study the brine characteristics including equilibrium salt content of each of the 4 brine concentration levels. A multiple linear regression (MLR) model was used to include both brine concentration and brine time as predictors for a single equation. Hence, predictions on other salt concentrations could be easily made. Paired t-test was used to check the similarity between the salt, moisture, and WPS from Titration and NIR in the verification experiment. A p-value ≤ 0.05 indicates a significant difference in an analyzed dataset.

3. Results and discussion

3.1. Brining characteristics of catfish fillet

An equilibrium salt content will be reached between fish and brine solution if the fish is immersed in the brine solution for a long period of time (Wheaton et al., 1985). This was observed in this study too, although fish were treated for limited and predetermined periods of time. Based on the fitting of the first order kinetics equation on the 4 brine concentrations in this study, different equilibrium salt content (C) would be reached at the final stage of brining treatment (0.82, 1.72, 2.27, and 3.41 g salt / 100g

sample, respectively, Table 2). Similar results were reported on yellowtail fish when fish were brined in 5, 10, 15, 21%, and saturated salt solutions at 23, 25, and 35 °C (Berhimpon et al., 1990; Berhimpon et al., 1991). Higher final equilibrium salt levels were observed with increased brine concentrations.

Different brining concentrations result in different initial brining levels for the fish, but a higher brining level does not necessarily result in a higher initial salt absorption rate. When fresh yellowtail fillets were brined in 5 salt concentration levels (5, 10, 15, 21, and 26.5%) at 25 and 35 °C for 50 hr the initial salt absorption rate (k) of the 25 °C treatments in some higher brine solutions (15 and 21%) were smaller than those of the lower brine solutions (5 and 10%). No explanation was attempted for those results by the authors (Berhimpon et al., 1991). Similar results were observed in our study, in which the initial salt absorption rates (k) did not show any clear patterns with increased brine concentrations (Table 2). The interactions between the different brine solutions and the fish fillets submerged into them might provide some explanations for the observed irregular salt absorption rates. In the fish salting theory, there is a "critical" salt level, above which protein denaturation occurs. The water holding capacity of denatured protein is decreased, thus influences the brine uptake of the fillets (Jittinandana et al., 2002). This "critical" salt level is believed to be about 8% (Wheaton et al., 1985; Duerr & Dyer, 1952). In our experiment, three out of the four brine concentrations were above this critical line and it was most likely that protein denaturation caused by high brine solution was the reason for the unclear initial salt absorption rate (k) pattern.

Four brine concentrations (5, 10, 15, and 20%, w / v) were selected for the brine treatment. Brining behavior of different brine concentrations can be well described by the

Table 2. Model fitting results for each brine concentration (5, 10, 15, 20%, w / v) with first order kinetics equation $Y = C \cdot (1 - e^{-k \cdot t})$.

Brine concentration	C	k
5%	0.82	0.06
10%	1.72	0.04
15%	2.27	0.09
20%	3.41	0.05

C : equilibrium salt content (g salt / 100 g sample)

k : initial salt absorption rate (/ min)

first order kinetics equation (Table 2). Other combinations between varied brine concentration and time may be of interest in real situations. Incorporation of both brine concentration and brine time factors was made possible by fitting a MLR model with the brine treatment data. Relationships between the brine concentration and brine time with the salt content of fish were explained with the following equation ($R^2 = 0.88$):

$$Salt\ content\ (\%) = 0.32 Ln(Time) + 13.65 Concentration - 1.20 \quad (6)$$

Prediction curves generated from this equation were plotted on the raw data in the Fig. 2.

3.2. Verification of the MLR model

According to equation (6) brine concentration and time combinations were drawn to produce salt contents (S_1) of 0.35, 0.71, 0.90, 1.08, 1.27, 1.46, 1.65, and 1.84% in brined fish, so that WPS levels of 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0% could be expected in the smoked fish (Table 1). The eight combinations were calculated as 0.06%×9 min, 0.06%×29 min, 0.06%×52 min, 0.06%×91 min, 0.12%×13 min, 0.12%×24

min, 0.12%×44 min, and 0.12%×80 min, respectively. After the fillets were brined and smoked, both salt and moisture contents were determined. Actual WPS contents were calculated, and a plot with predicted WPS level and 95% confidence prediction bands is shown in Fig. 3. The actual WPS at low WPS prediction level (WPS=1%) was slightly higher than the predicted value. This was because the raw catfish fillet itself possesses a very low level (~ 0.16%) of salt, which may have a relatively greater influence to the prediction at lower levels than that at higher levels. Predictions made on other higher WPS levels followed closely with the prediction line and in the middle part of the prediction band. Therefore, this MLR model (equation 6) can be regarded as effective in predicting certain salt contents in brine catfish fillets.

3.3. Determination of salt content with NIR method

The efficacy of using NIR spectroscopy as a non-destructive and quick salt measurement method in smoked catfish was evaluated in this experiment. Salt determination results from both titration and NIR methods were compared at four salt levels in smoked catfish samples (Table 3). According to paired t-tests on the four different salt levels, no significant differences were observed between the results from "Titration" and "NIR" methods ($p = 0.529$, $p = 0.068$, $p = 0.243$, and $p = 0.868$, respectively). However, for samples at 1.8% predicted salt level, NIR data (3.2%) appeared to over estimated the salt content which was determined at 1.9% by the reference titration method. NIR light can only penetrate into fish muscle tissue for a few millimeters (Fahlstrom et al., 2001; Nord, et al., 2002), making NIR determinations only reflects information on the surface or skin layer of muscle tissue which generally contains slightly higher salt contents than deeper layers of fish muscle. Systemic error may also

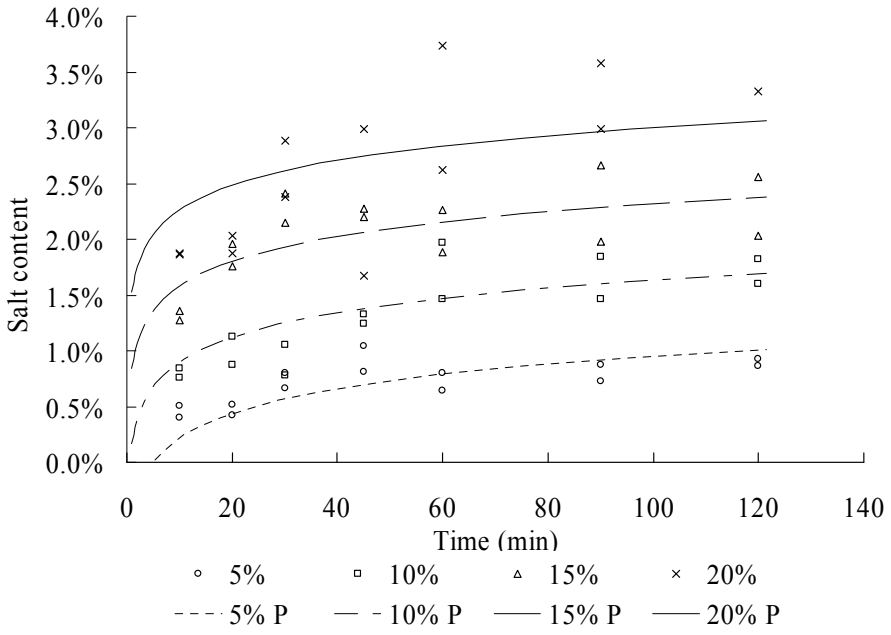


Fig. 2. Salt content (%) in brined catfish fillet as affected by brine concentration and brine time. Prediction curves (P) were produced by equation (6).

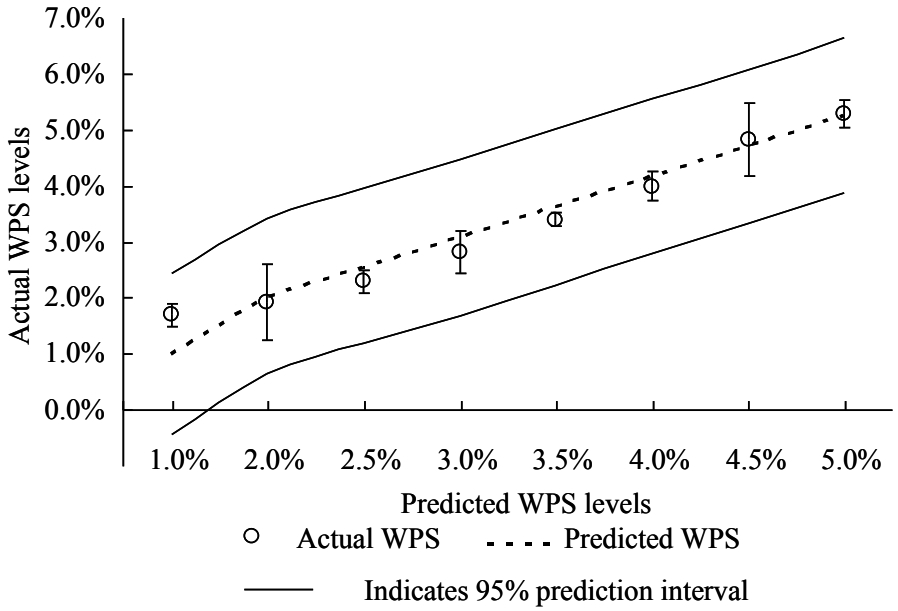


Fig. 3. Plot of actual WPS levels of smoked catfish with predicted WPS levels and 95% confidence prediction bands.

Table 3. Comparison of salt content of smoked catfish fillet determined with titration and NIR method.

Predicted	1.2%	1.8%	2.4%	3.1%
Titration (± SD[a])	1.7 ± 0.1%	1.9 ± 0.1%	2.8 ± 0.3%	3.9 ± 0.6%
NIR (± SD)	1.5 ± 0.5%	3.2 ± 0.7%	3.3 ± 0.7%	4.0 ± 0.6%

a: SD = Standard deviation

contribute to the observed differences because NIR methods typically require large data sets to calibrate the readings for each food type. In this study fifteen samples were used to construct a calibration curve with three replicates for each calibration level. Increasing replicate number at calibration level may enhance the performance of the NIR method on the smoked catfish samples.

4. Conclusions

As value-added products, smoked catfish has considerable potential to bring increased profits to the catfish industry. Brining operations are critical to meet the FDA requirements on hot-smoked and ready-to-eat seafood products. According to this study, higher equilibrium salt contents in fish would result from higher brine concentrations. But initial salt absorption rates in the fresh catfish fillets were not positively related to the brine concentrations due to the protein denaturation introduced by salt. A multiple linear regression model was developed to predict salt content in catfish fillet as influenced by the brine concentration and brine time. The NIR method has the potential to be used as a rapid, accurate, and non-invasive method to determine salt content in hot-smoked catfish.

References:

AOAC. (1995). Method 937.09-Salt (Chlorine as Sodium Chloride) in Seafood Volumetric Method. *Official Method of Analysis of AOAC International*, *16th edition*. Washington, D.C: Association of Official Analytical Chemists.

Adams, M. R. & Moss, M. O. (2000). Bacterial agents of foodborne illness. In *Food Microbiology, 2nd editon*. Pp. 200-211. London, UK: Royal Society of Chemistry.

Berhimpon, S., Souness, R. A., Driscoll, R. H., Buckle, K. A., & Edwards, R. A. (1991). Salting behavior of yellowtail (*Trachurus mccullochi* Nichols). *Journal of Food Processing and Preservation,* 15, 101-114.

Berhimpon, S., Souness, R., & Edwards, R. A. (1990). The effect of brine concentration on the salting and drying of yellowtail (*Trachurus mccullochi*). *FAO Fisheries Report*, Pp. 153-159.

Boggess, T. S., Jr., Heaton, E. K., & Beuchat, L. R. (1973). A procedure for processing smoked channel catfish. *Journal of Milk and Food Technology,* 36, 469-473.

Deng, J. C. (1977). Effect of freezing and frozen storage on salt penetration into fish muscle immersed in brine. *Journal of Food Science,* 42, 348-351.

Duerr J. D. & Dyer, W. J. (1952). Proteins in fish muscle IV. Denaturation by salt. *Journal of Fisheries Research Board of Canada,* 5, 325-331.

Eyo, A. A. (2001). Fish smoking. In *Fish Processing Technology in the Tropics*, Pp. 153-192. New Bussa, Nigeria: NIFFR.

Fahlstrom, C. A., Cavinato, A. G., Mayes, D. M. 2001. Penetration Depth of Short-Wavelength Near Infrared Light in Fish Tissue. Eastern Oregon Science Journal, Volume XVI, 9-12.

FDA. (2001). Clostridium botulinum toxin formation. Ch. 13. In *Fish and Fishery Products Hazards and Controls Guidance, 3rd edition*, Pp. 167-190. Washington, D.C: Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood.

Hilderbrand, Jr. K. S. (1992). Fish smoking procedures for forced convection smokehouse. *Special report 887*. Newport, OR. U.S.A: Oregon State Extension Service.

Hilderbrand, Jr. K. S. (2000). Quick determination of water phase salt content of smoked fish. *Oregon State University Extension Sea Grant*. Revised November 21, 2000.

Horner, W. F. A. (1997). Preservation of fish by curing (drying, salting and smoking). In *Fish Processiong Technology by Hall, G. M. 2nd Edition*, Pp. 32-73. London, UK: Chapman & Hall.

Huang, Y., Cavinato, A. G., Mayes, D. M., Bledsoe, G. E., & Rasco, B. A. (2002). Non-destructive prediction of moisture and sodium chloride in cold smoked Atlantic salmon (*Salmo salar*). *Journal of Food Science,* 67, 2543-2547.

Huang, Y., Rogers, T. M., Wenz, M. A., Cavinato, A. G., Mayes, D. M., Bledsoe, G. E., Rasco, B. A. (2001). Detection of sodium chloride in cured salmon roe by SW-NIR spectroscopy. *Journal of Agriculture and Food Chemistry,* 49, 4161-4167.

Jittinandana, S. Kenney, P. B., Slider, S. D., & Kiser, R. A. (2002). Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. J*ournal of Food Science,* 67, 2095-2099.

Lawrie, R. A. (1985). The storage and preservation of meat. II. Moisture control. In: *Lawrie's Meat Science, 4th edition*, Pp. 149. Cambridge, UK: Woodhead Publishing Limited.

Lin, J. & Brown, C. W. (1992). Near-IR spectroscopic determination of NaCl in aqueous solutions. *Applied Spectroscopy,* 46, 1809-1815.

Lin, M., Cavinato, A. G., Huang, Y., Rasco, B. A. (2003). Predicting sodium chloride content in commercial King (*Oncorhynchus tshawytscha*) and Chum (*O. keta*) hot smoked salmon fillet portions by short-wavelength near-infrared (SW-NIR) spectroscopy. *Food Research International,* 36, 761-766.

Montgomery, D. C., Peck, E. A., & Vining, G. G. (2001). *Introduction to Linear Regression Analysis, 3rd Edition*. New York: Wiley.

Nord, S.P., DuBreuil, R., Cavinato, A.G., Mayes, D.M., Lin, M. and Rasco, B.A. 2002. Penetration depth studies in cod tissue using short-wave near infrared spectroscopy. Eastern Oregon Science J., Volume XVII, 37-41.

Seber, G. A. F. & Wild, C. J. (2003). *Nonlinear Regression*. New York: Wiley.

Thorarinsdottir, K. A., Arason, S., Bogason, S. G., & Kristbergsson, K. (2004). The effects of various salt concentrations during brine curing of cod (*Gadus morhua*). *International Journal of Food Science and Technology,* 39, 79-89.

Wheaton, F. W. & Lawson, T. B. (1985). Chapter 11: Other preservation methods. in *Processing Aquatic Food Products*, Pp. 273-327. New York: Wiley.

Zugarramurdi, A. & Lupin, H. M. (1980). A model to explain observed behavior on fish salting. *Journal of Food Science,* 45, 1305-1311.

V. CHAPTER THREE:

STUDIES ON SHELF-LIFE OF HOT-SMOKED CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) FILLETS PACKED WITH DIFFERENT OXYGEN TRANSMISSION RATE MATERIALS AND STORED AT DIFFERENT TEMPERATURES

Abstract

Hot-smoked channel catfish (*Ictalurus punctatus*) product is being developed as an alternative high value product. The final products would be vacuum-packed to extend the storage life. Some bacteria spores may still survive the thermal treatments and grow inside the package. This includes spores from the anaerobic *Clostridium botulinum* bacterium whose neurotoxins can cause high mortality rate in human beings. Thus, shelf-life of the products must be evaluated to make sure they are safe by the time they are consumed. For this project, fresh catfish fillets were first brined and then hot-smoked inside an electrical smokehouse. Fillets were individually vacuum-packaged with three types of commercial food packaging films (oxygen transmission rate (OTR) 0, 4,000, and 10,000 $cm^3/m^2/24h/atm$ at 20 °C and 0%RH). Packaged fillets were stored at either 4 or 25 °C. Samples were taken at 1-7 days intervals depending on the growth of bacteria populations. No *C. botulinum* was detected in the samples with current processing procedures. Samples stored at 25 °C had a shelf-life of 3-5 days. Shelf-life of 53-, 35-,

and 33-day was observed for samples stored at 4 °C and packaged with 0, 4,000, and 10,000 OTR films, respectively. A 38-day microbiological growth lag-time was observed in 0 OTR×4 °C treatments. Knowledge of the smoked catfish shelf-life will direct the future storage and distribution of products. Results of this study will help us understand the interactions between the packaging material and the storage temperature on the shelf-life of packaged products. This information will also be useful for further optimization of the product characteristics and storage stability.

Key word: hot-smoked, catfish, shelf-life, vacuum packaging, oxygen transmission rate.

1. Introduction

Catfish is a major fresh-water aquaculture species in the southern United States. In 2008, total sales from catfish were about 410 million U.S. dollars (NASS, 2009). Most of the catfish (about 95%) were directly sold to processing plants and sold as raw fresh or frozen products. Currently, diversity of products from catfish is low.

Smoking is a traditional food processing method which uses smoke to preserve fish and other food items (Eyo, 2001). The drying effects during smoking and the antioxidant and bacteriostatic effects of the smoke preserve the smoked products resulting in extended shelf-life (Efiuvwevwere & Ajiboye, 1996; da Silva, Prinyawiwatkul, King, No, Bankston, & Ge, 2008). The color, taste, and other sensory perceptions produced by smoking are at least as important as its preservative effects. Smoked fish products are also regarded as delicatessen food items which are served on special occasions. Hot and cold smokings are two major smoke techniques used widely. During hot smoking, fish is first brined and then dried. Smoking is then implemented in a smokehouse where fish are separated on different layer of racks. The difference between hot and cold smoking is mainly the temperature applied at the time of smoking. Hot smoking temperature is normally carried out at above 70 °C, while cold smoking rarely exceeds 30 °C (Wheaton & Lawson, 1985). For smoked fish and fisheries products, a minimum thermal process of 30 min at or above 145 °F (62.8 °C) is required by FDA (2001). Thus, the hot-smoked fish is fully cooked and ready for consumption after smoking. Cold-smoked fish is either eaten raw or further cooked before consumption. Smoked fish products have been reported in varied fish species including salmon (Lakshmanan, Parkinson, & Piggott, 2007), rainbow trout (Cakli, Kilinc, Dincer, &

Tolasa, 2006), tilapia (Yanar, Çelik, & Akamca, 2006), and catfish (Benjakul & Aroonrueng, 1999; Yanar, 2007).

Smoking does improve shelf-life, however, unpackaged hot-smoked catfish are still perishable due to the relatively high level of moisture inside the smoked products. Vacuum packaging provides an effective way to deal with this problem. By blocking most oxygen from the outside, bacterial activities can be dramatically decreased so that the shelf-life of food is extended. However, even with vacuum packaging, some facultative bacteria (bacteria that can exist under either aerobic or anaerobic conditions) can still survive and grow. The biggest danger is *C. botulinum*, whose neurotoxins can cause high mortality rate to human beings (Adam & Moss, 2000; Sharma & Whiting, 2005). *C. botulinum* is a gram negative, rod-shaped anaerobic bacterium that is widely distributed in land and water environment. The vacuum condition inside the package will provide favorable living conditions for their growth if they survive the processing procedures. Hence, a systematic study needs to be conducted to make sure the processing procedures, packaging materials and storage conditions will provide safe and high quality products to consumers.

In this study, shelf-life of hot-smoked catfish was evaluated based on the total plate count and detection of *C. botulinum*. Other physical properties (pH and texture) changes were also measured over time.

2. Materials and methods

2.1. Fish and smoking procedure

Farm-raised fresh channel catfish fillets were purchased from a commercial catfish processing plant (Harvest Select, Uniontown, Alabama) immediately after being

processed. Fillets were immediately stored on ice. Before smoking, fillets were submerged into a brine solution (6% salinity, w/v) for 60 min at 4 °C. The fillet to brine solution ratio was 1: 1.5 (w: v). After brining, all the fillets were rinsed with tap water at a ratio of 1: 1 (w: v) for 30 seconds to remove the surface salt solution. Fillets were then laid on racks and transferred into a smoke house (KOCH 323345, KOCH Equipment LLC. Kansas City, MO). Smoke was generated using hickory sawdust. Fillets were first dried in hot air (30 °C, 50% RH) for 1 hour and then smoked to a final internal temperature of 100 °C for 30 min. The internal temperature of fish was monitored by inserting thermocouples into the thickest part on representative fillets. Smoked fillets were allowed to cool down to room temperature (25 °C) and then packaged at the same location of smoking.

2.2. Packaging and storage

Each piece of whole fillet was individually vacuum-packaged using a Multivac Model A316 Vacuum packaging Machine (Multivac Inc., Kansas City, MO). Three types of commercial food packaging films with an oxygen transmission rate (ORT) of 0, 4,000, and 10,000 $cm^3/m^2/24h/atm$, respectively, at 20 °C and 0%RH, were used. All the packaging films were provided by Cryovac® Food Packaging (Duncan, SC). Packaged fillets were divided into two groups and stored at either 4 °C or 25 °C conditions. For each treatment combination (OTR×Temperature), three bags of fish were prepared for each sampling day.

2.3. Microbiological analysis

Total aerobic plate count was performed with AOAC official method 966.23. Anaerobic *C.botulinum* detection was performed with AOAC official method 977.26.

2.4. Physical and chemical analysis

2.4.1. Textural property change during the storage

Hardness is chosen as the indicator of textural property of the fillets and measured with a Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y. / Stable Micro Systems, Godalming, Surrey, U.K.) using the method by Jiang, Wang, van Santen, & Chappell (2008). An aluminum cylinder probe (length=123.0 mm, diameter=7.9 mm) with a ball tip (diameter=6.6 mm) was attached to the Texture Analyzer with its longitude side perpendicular to the test platform. Fillet was placed on the platform with its skin side down. A one-time indentation (4 mm) was made at 10 mm thickness on the fillet at a speed of 1 mm/sec. The maximum force obtained after the probe reached the fillet was recorded as hardness of the fillet.

2.4.2. pH

Sample pH was measured by method provide by Price & Tom (2006). Sample was homogenized with a food chopper (WARING blender 7011, Model 31BL92, New Hartford, CT) for 60 sec. Homogenized samples of 20 g were mixed with 40 mL deionized water. The mixture was then blended using a hand-held food blender for 60 sec. An Orion 370 pH meter (Thermo Fisher Scientific Inc. MA. U.S.A) was used to measure the pH of the mixture.

2.4.3. Moisture and salt content of the fillet

Salt content of brined and smoked fish samples were determined using the AOAC Official Method 937.09. 5 g homogenized sample was taken for each measurement.

Moisture content of the fillet was measured using drying oven procedure (Price & Tom, 2006). Homogenized samples of 5 g were put into a drying pan and dried at 103 °C overnight.

2.5. Statistical analysis

For TPC results from the sample packaged with 0 OTR film and stored at 4 °C, the Gompertz model was used to study the growth characteristics of the bacteria population. The Gompertz model is expressed as:

$$L(t) = A + \frac{C}{\exp(\exp(-B(t-M)))}$$

where *L (t)*: LogCFU/g in time *t*, *A*: Asymptotic amount of growth as time decreases infinitely, *C*: Asymptotic amount of growth as time increases indefinitely, *M*: the time at which the absolute growth rate is at a maximum (days), *B*: the relative growth rate at time *M* (Log CFU/g/day).

3. Results and discussions

3.1. Characteristics of the products

After the brining and smoking process, the final products possessed an average salt content of 2.56% and 65.77% moisture content, which resulted in a 3.75% WPS content. The overall product yield was about 61.79% (Table 1).

3.2. Detection of *C. botulinum*

No *C. botulinum* was detected in the samples by the time that shelf-life endpoint was reached based on TPC standard. From the processing of catfish in the plant to the preparation of smoking (brining, drying, smoking, cooling), good sanitation standards were maintained to ensure no contamination of *C. botulinum*. The brining procedure

Table 1. Production characteristics of raw and smoked channel catfish (*I. punctatus*) fillets (Mean ± SD).

	Raw fillet	Smoked fillet
Salt content	0.16 ± 0.01%	2.56 ± 0.06%
Moisture	72.68 ± 4.27%	65.77 ± 1.93%
WPS	0.22 ± 0.00%	3.75 ± 0.09%
pH	-	6.31 ± 0.08
Yield	-	61.79 ± 5.09%

was also carefully controlled to make sure the final products had a WPS level of 3.75% (Table 1). Therefore, the final WPS level in the product will also pose an inhibitive factor to the germination of *C. botulinum* spores if they did exist in the low oxygen transmission treatment (0 OTR). However, this WPS barrier effect could not be tested in the current experiment settings. Further inoculation tests may provide an answer to that. For the other treatments with certain oxygen permission between the package film, germination of *C. botulinum* spores were unlikely to happen due to the existence of oxygen and growth competition from other aerobic bacteria.

3.3. TPC curve and shelf-life

When the TPC was plotted with the storage time, varied growth patterns were observed on different treatment combinations (Fig. 1). For samples stored at 25 °C, the storage temperature played a dominate role on the bacteria growth. No matter what kind of packaging materials were used, low (0 OTR), medium (4,000 OTR), or high (10,000 OTR), growth of bacteria followed an upward straight line over the time.

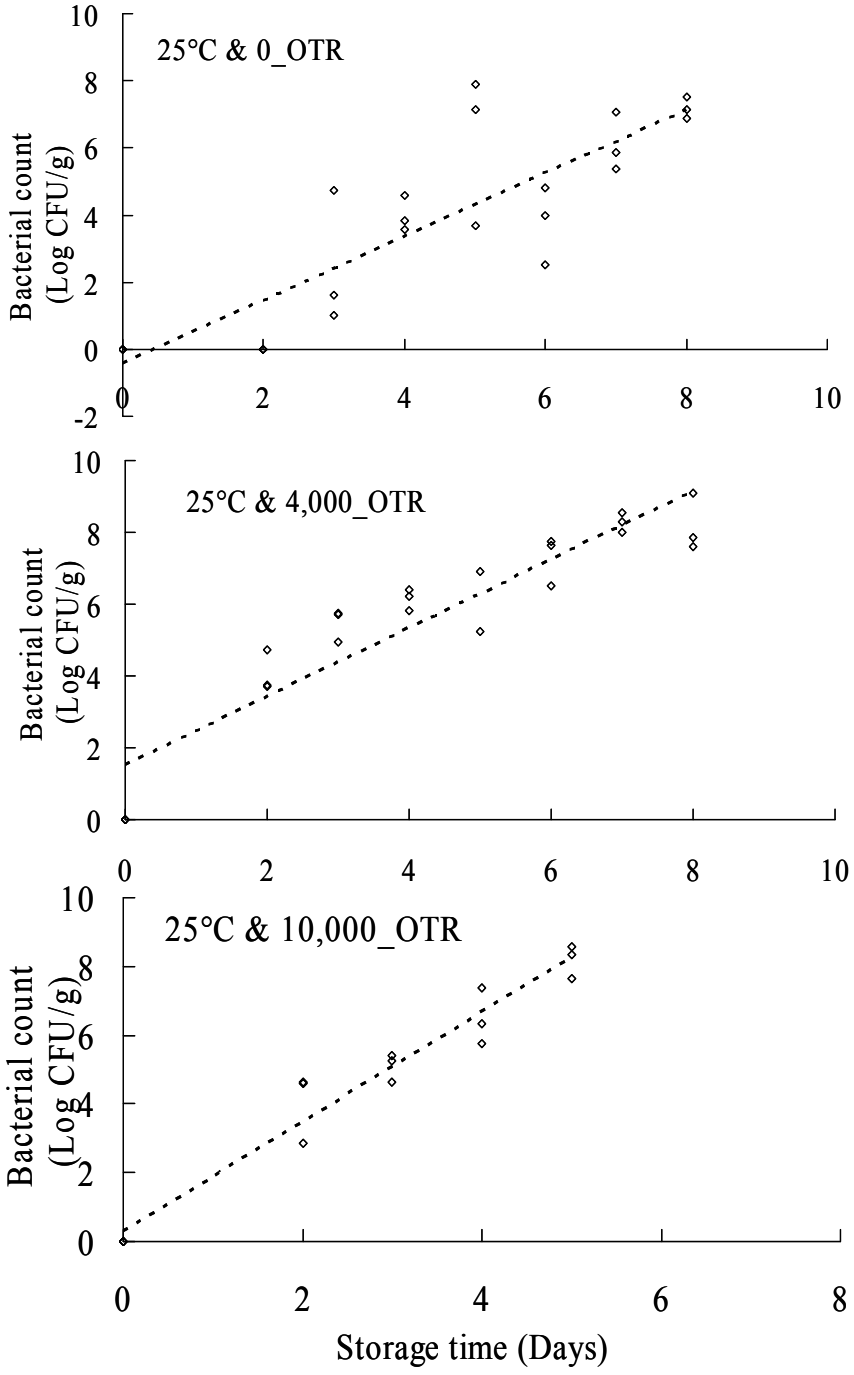


Fig. 1. Plot of bacteria growth (Log CFU/g) in the vacuum-packed (0, 4,000, and 10,000 OTR respectively) hot-smoked channel catfish (*I. punctatus*) stored at 25 °C.

In our study, the endpoint of shelf-life based on TPC was regarded as 6 LogCFU/g. According to this standard, all the 25 °C treatments reached their shelf-life by the 5th day after they were produced and put into storage.

Efiuvwevwere & Ajiboye (1996) also reported that although smoking significantly reduced the bacterial load in all samples, sharp and significant microbial growth occurred during the first 4 days of storage of hot-smoked catfish packaged in perforated bags and stored at ambient tropical temperature (26-33 °C) no matter what chemical preservatives (potassium sorbate and sodium benzoate at two different levels each) were applied.

A long shelf-life at ambient temperature and open air could be expected only if the a_w of the product was decreased so much that most bacteria could not survive. Studies presented by da Silva, Prinyawiwatkul, King, No, Bankston, & Ge (2008) showed that hot-smoked catfish steak can be safely stored for up to 6 weeks if the a_w was dropped under 0.85 through a combined cooking and smoking procedure for over 30 hr.

For samples stored at 4 °C, no clear pattern could be observed on samples packaged with medium or high OTR films (Fig. 2). They did not have a clear lag time as compared to the low OTR×4 °C treatment. Growth of bacteria was slow at the beginning stage of storage. Both of the oxygen barrier effects of the film together with the low temperature might contribute to these irregular growth patterns. A 33-and 35-day shelf-life could be determined based on TPC results. The treatment with the highest OTR (10,000 OTR) reached shelf-life endpoint earlier than that of the 4,000 OTR treatment. The higher access to oxygen was considered as the major factor for this result which helped aerobic microorganism grow inside the package. Growth of bacteria in 4,000 OTR

might have been slightly restricted by the oxygen amount through the package, thus resulting in a 2-day delay in the endpoint.

For TPC results from the sample packaged with 0 OTR film and stored at 4 °C, a 53-day of shelf-life was obtained. In addition, Gompertz model was used to study the growth characteristics of the bacteria population. The fitted growth curve was drawn on the plot (Fig. 2). A 38-day lag time of bacteria growth was also calculated (Table 2).

Smoking significantly decreased the bacteria load of hot-smoked catfish. Benjakul & Aroonrueng (1999) reported that aerobic plate count increased significantly after 8 weeks of storage at 4 °C on control unsmoked sample. The aerobic plate count was 10^5-10^7 CFU/g for the control compared to 10^3-10^5/g for the hot-smoked samples at that time. Yanar (2007) reported that a 24-day shelf-life of hot smoked catfish (*Clarisa gariepinus*) could be obtained based on microbiological and sensory analyses when the fish were packed with 10,000 OTR film and stored under refrigerated conditions (4 °C). Cakli, Kilinc, Dincer, & Tolasa (2006) compared the effects of modified atmosphere packaging (MAP) and vacuum packaging (VP) on the shelf-life of hot-smoked rainbow trout (*Onchoryncus mykiss*) fillet stored under refrigeration condition (<4 °C). A 33-day shelf-life was obtained for VP samples according to microbiological and sensory analysis. The MAP samples had a longer shelf-life of 40-day (50% CO_2, 50% N_2) and 47-day (60% CO_2, 40% N_2), respectively. A 35-day shelf-life was reported on hot-smoked tilapia stored open air at 4 °C (Yanar, 2007).

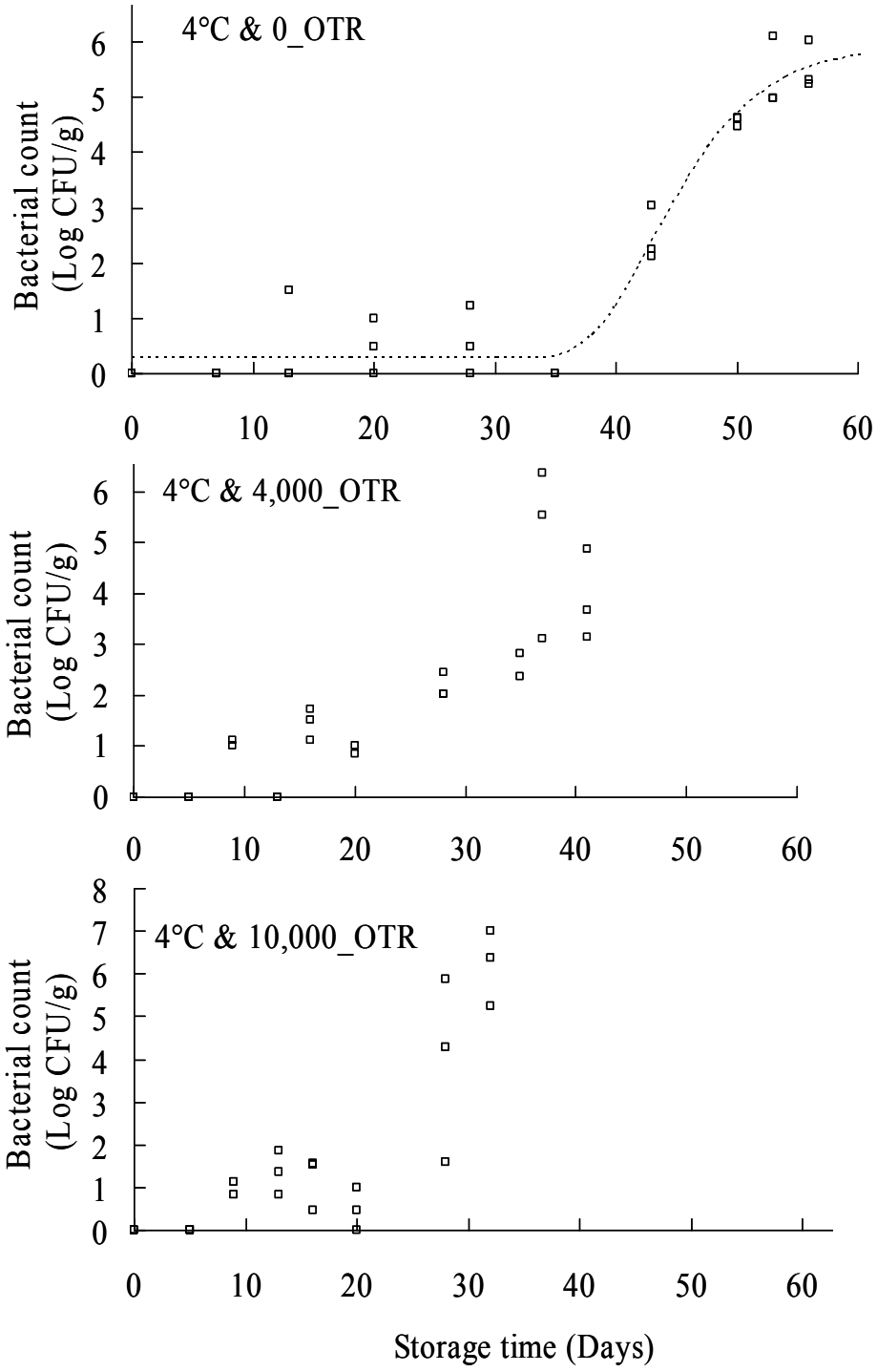


Fig. 2. Plot of bacteria growth (Log CFU/g) in the vacuum-packed (0, 4,000, and 10,000 OTR respectively) hot-smoked channel catfish (*I. punctatus*) stored at 4 °C.

Table 2. Parameter fitting results of Gompertz model for hot-smoked catfish packaged with 0 OTR film and stored at 4 °C.

Parameters				Lag time
A	C	B	M	
0.28	5.68	0.20	42.9	38 days

3.4. pH change

Changes of smoked catfish pH for varied storage conditions were graphed in Fig. 3. Mean changes of pH was not significant at either 25 or 4 °C treatments. Similar results were reported by da Silva, Prinyawiwatkul, King, No, Bankston, & Ge (2008) when antimicrobial agents and antioxidants were used to extend shelf-life of hot-smoked blue catfish at ambient temperature (26-33 °C) and no significant pH change was observed. Yanar (2007) also reported only slight pH changes during the refrigerated storage (4 °C) of hot-smoked catfish (*Clarisa gariepinus*). The degradation of protein caused by bacterial activity may influence the pH of the vacuum packaged products but the effects may not be that significant to change the pH greatly. In another side-by-side study of modified atmosphere packaging (MAP) and vacuum packaging (VP) on the shelf-life of hot-smoked rainbow trout (*Onchoryncus mykiss*) fillet stored under refrigeration condition (<4 °C) by Cakli, Kilinc, Dincer, & Tolasa (2006), decrease of pH was only found in MAP samples due to the CO_2 absorption into the tissue. Sample pH of the VP remained constant over the storage time.

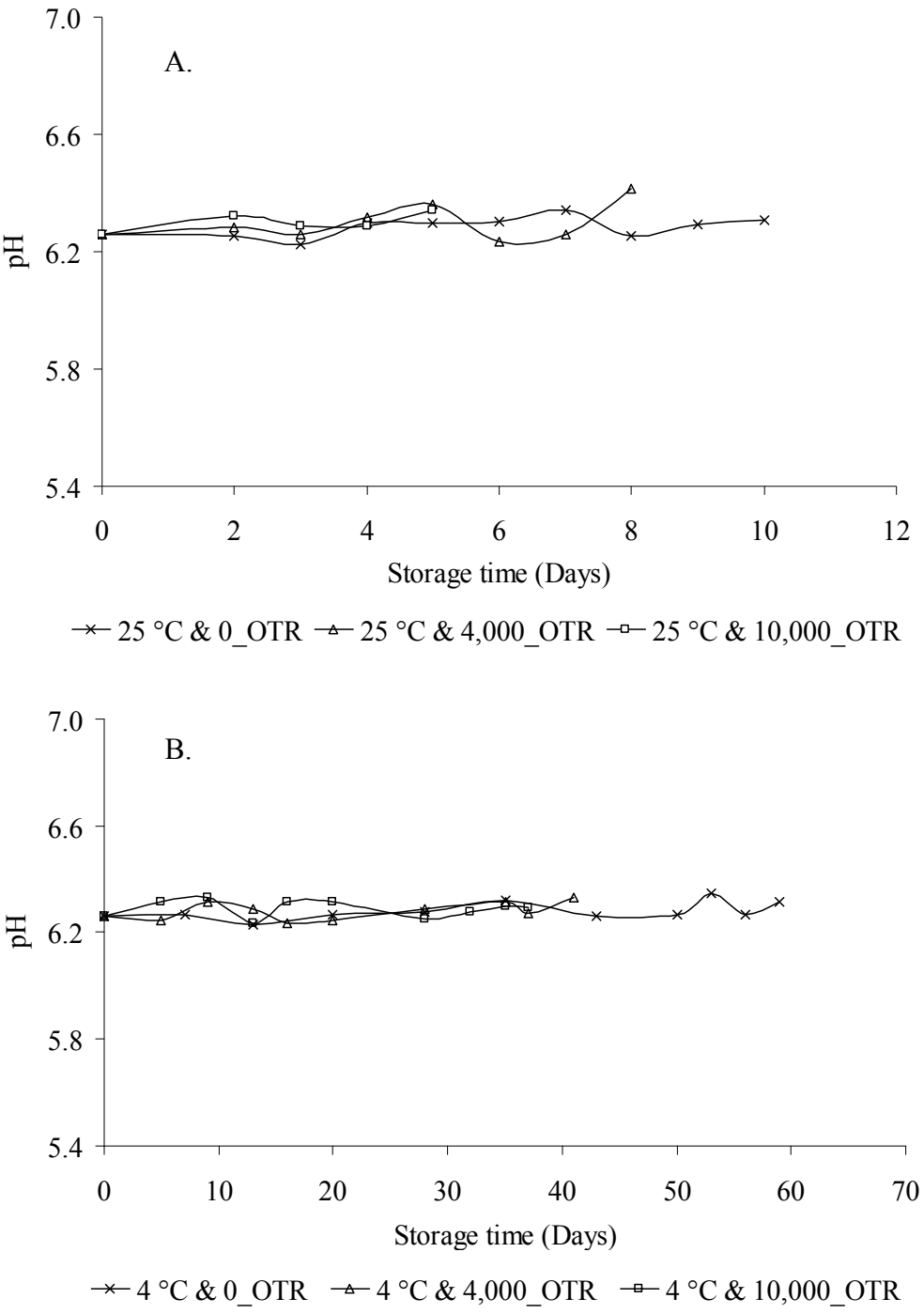


Fig. 3. Plot of pH change in hot-smoked channel catfish (*I. punctatus*) vacuum-packed in films with different OTRs (0, 4,000, and 10,000) and stored at 25 °C (A) and 4 °C (B).

3.5. Texture change

Change of textural properties of hot-smoked catfish fillets during the storage was also studied (Fig. 4). The method used was sensitive enough to detect hardness change of the fillets. A constantly deceasing hardness was observed in 10,000 OTR×25 °C treatment. However, no clear trend could be observed on the hardness of the other treatments over the storage time. Most hardness values fluctuated between certain values. The moisture and fat content of individual fillet might play a dominate role on the textural properties instead of storage temperature and time.

4. Conclusions

Vacuum-packed hot-smoked catfish fillets showed different bacterial growth curves when stored under different temperatures. Temperature played a dominate role affecting the microbial growth at 25 °C. Shelf-life of the 25 °C treatments ended by 5 days after storage. Shelf-life of 53-, 35-, and 33-day was observed for samples stored at 4 °C and packaged with 0, 4,000, and 10,000 OTR films, respectively. A 38-day microbiological growth lag-time was observed in 0 OTR×4 °C treatments.

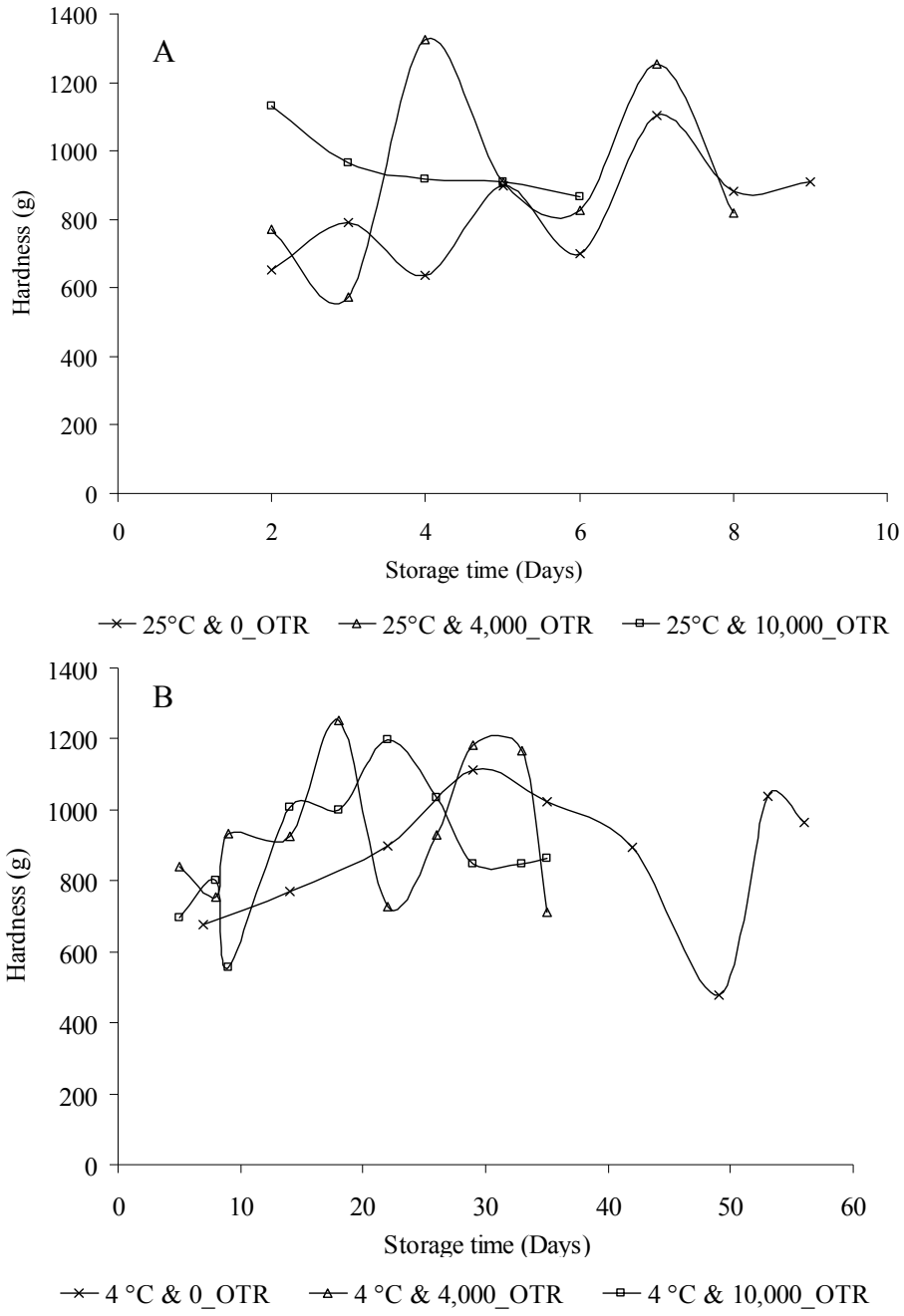


Fig. 4. Plot of hardness change in hot-smoked channel catfish (*I. punctatus*) vacuum-packed in films with different OTRs (0, 4,000, and 10,000) and stored at 25 °C (A) and 4 °C (B).

References:

Adams, M.R. and Moss, M.O. (2000). Bacterial agents of foodborne illness. In *Food Microbiology. Second editon.* 200-211.

Benjakul, S. & Aroonrueng, N. (1999). Effect of smoke sources on quality and storage stability of catfish fillet (*Clarias macrocephatus* Gunther). *Journal of Food Quality, 22,* 213-224.

Cakli, S., Kilinc, B., Dincer, T. & Tolasa, S. (2006). Comparison of the shelf lifes of map and vacuum packaged hot smoked rainbow trout (*Onchoryncus mykiss*). *European Food Research and Technology, 224,* 19-26.

da Silva, L. V. A., Prinyawiwatkul, W., King, J. M., No, H. Y., Bankston, J. D. Jr., & Ge, B. (2008). Effect of preservatives on microbial safety and quality of smoked blue catfish (*Ictalurus furcatus*) steaks during room-temperature storage. *Food Microbiology, 25,* 958-963.

Efiuvwevwere, B. J. O. & Ajiboye, M. O. (1996). Control of microbiological quality and shelf-life of catfish (*Clarias gariepinus*) by chemical preservatives and smoking. *Journal of Applied Bacteriology, 80,* 465-470.

Eyo, A. A. (2001). Fish smoking. In *Fish Processing Technology in the Tropics*, Pp. 153-192. New Bussa, Nigeria: NIFFR.

FDA. (2001). Food and Drug Administration, Chapter 13: *Clostridium botulinum* toxin formation. In *Fish and fisheries products hazards and control guide*, *3rd edition.* Office of Seafood, Food and Drug Administration, Department of Health and Human Services, US Government Printing Office, Washington, DC.

Jiang, M., Wang, Y., van Santen, E., & Chappell, J. A. (2008). Evaluation of textural properties of channel catfish (*Ictalurus punctatus* Rafinesque) fillet with the natural contour method. *LWT-Food Science and Technology, 41,* 1548-1554.

Lakshmanan, R., Parkinson, J. A., & Piggott, J. R. (2007). High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (*Salmo salar*). *LWT-Food Science and Technology, 40,* 544-551.

NASS. (2009). *Catfish Processing*. USDA Economics, Statistics and Market Information System. Publication date: 01.30.2009. Washington D.C.

Price, R. J. & Tom, P. D. (2006) (updated). Chapter 1: Acidified, Fermented, and Salted Fish and Fisheries Products. In *Compendium of Fish and Fishery Product Processing Methods, Hazards and Controls*. Sea Grant Extension Program. Davis, CA. U.S.A. (http://seafood.ucdavis.edu/haccp/compendium/compend.htm)

Sharma, S. K. and Whiting, R. C. 2005. Methods for detection of *Clostridium botulinum* toxin in foods. *Journal of Food Protection, 68,* 1256-1263.

Wheaton, F. W. & Lawson, T. B. (1985). Chapter 11: Other preservation methods. in *Processing Aquatic Food Products*, Pp. 273-327. New York: Wiley.

Yanar, Y., Çelik, M. & Akamca, E. (2006). Effects of brine concentration on shelf-life of hot-smoked tilapia (*Oreochromis niloticus*) stored at 4 °C. *Food Chemistry, 97,* 244-247.

Yanar, Y. (2007). Quality changes of hot smoked catfish (*Clarias gariepinus*) during refrigerated storage. *Journal of Muscle Foods, 18,*391-400.

VI. CHAPTER FOUR:

STUDIES ON THE PHYSICAL PROPERTIES OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) SKIN GELATIN FILM USING TRANSMISSION X-RAY MICROSCOPE

Abstract

Gelatin from catfish skin was obtained by thermal extraction. Triacetin was added to the gelatin in proportions of 0, 50, 100, and 150% of gelatin content to improve the hydrophobic properties of the resulting films. Tween 80 was also added as an emulsifier. Distribution of the introduced triacetin in the film was examined using transmission X-ray microscope (TXM). Other film properties, such as thickness, mechanical properties, water vapor permeability, water solubility, light transmission, and transparency were also evaluated. Possible relationships between the distribution characteristics and the film properties were studied. Triacetin distribution changed from homogeneous to heterogeneous with increased content in the film. The addition of triacetin resulted in decreased tensile strength (TS) and increased percent elongation (E%), water solubility, UV and visible light barrier properties of the film. Water vapor permeability of the film increased in some treatments (100 and 150% triacetin) possibly

due to the heterogeneous distribution of the triacetin and also the increased emulsifier amount in the film.

Keywords: catfish skin gelatin, edible film, triacetin, water vapor permeability, transmission X-ray microscope

1. Introduction

Gelatin is an important biopolymer widely used in food, pharmaceuticals, and photographic industries. Gelatins are traditionally obtained from bones and hides of mammals (mainly beef and pork). Unfortunately gelatins from mammalian sources may not be acceptable for kosher (Jewish) and halal (Muslim) products or for people concerned about Bovine Spongiform Encephalopathy (BSE) and Foot-and-Mouth Disease (FMD). As an alternative source and solution, gelatins extracted from fish products have attracted increased attention in recent years. Extractions of gelatin from many fish species have been studied. These species include cod (Gudmundsson & Hafsteinsson, 1997), hake (Montero, Gómez-Guillén, & Borderias, 1999), blue shark (Yoshimura, Terashima, Hozan, & Shirai, 2000), tilapia (Choi & Regenstein, 2000; Jamilah & Harvinder, 2002), yellowfin tuna (Cho, Gu, & Kim, 2005), Alaska pollock (Zhou, Mulvaney, & Regenstein, 2006), horse mackerel (Badii & Howell, 2006), skate (Cho, Jahncke, Chin, & Eun, 2006), and catfish (Yang, Wang, Jiang, Oh, Herring, & Zhou, 2007a).

Channel catfish (*Ictalurus punctatus*) is a major fresh-water aquaculture species in the southern part of the United States. Processing of catfish in 2007 was about 2.25×0^5 tons (NASS, 2008), and catfish skin accounted for about 6% of processed fish weight (Lovell, 1980). As a rich source of collagen, catfish skin is also an alternative source for gelatin. Thermal extraction of gelatin from catfish skin has been reported (Yang et al., 2007a; Yang, Wang, Zhou, & Regenstein, 2008) as well as studies on its physical properties (Yang, Wang, Regenstein, & Rouse, 2007; Wang, Yang, & Regenstein, 2008; Yang & Wang, 2009).

One possible usage of the catfish skin gelatin is edible film or package. Edible films are biodegradable natural polymers obtained from agricultural products. They are safe for the environment and also reduce the packaging waste associated with the packaged products (Debeaufort, Quezada-Gallo, & Voilley, 1998). Protein based edible film, such as gelatin film, is typically fabricated by casting a certain amount of film forming solutions onto a leveled surface followed by drying under a controlled temperature and relative humidity for a certain time. Gelatin films generally have good barrier properties to oxygen, carbon dioxide, and lipid (McHugh & Krochta, 1994). Other ingredients like sugar (Veiga-Santos, Oliveira, Cereda, & Scamparini, 2007), inorganic salts (Park, Rhee, Bae, & Hettiarachchy, 2001; Rhim & Lee, 2004), and lipid (Bertan, Tanada-Palmu, Siani, & Grosso, 2005; Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006; Prodpran, Benjakul, & Artharn, 2007) can also be added to further improve film properties.

Quality of film is determined by the nature of gelatin and components added into the casting solution (de Carvalho & Grosso, 2004). Distributions of the added components in the gelatin film also significantly affect their characteristics. However, researches on film internal structure are still sparse. There are several high-resolution imaging techniques. Scanning electron microscopy (SEM) and atomic force microscope (AFM) can only acquire the information on sample surface, while the penetration ability of transmission electron microscopy (TEM) is quite limited (typically tens of nanometers). A recently developed technique, transmission X-ray microscopy (TXM), provides a powerful tool for imaging the internal structure of matter (Sayre & Chapman, 1995; Jacobsen, 1999). With the merit of high penetration ability of an X-ray, TXM can

investigate the inner part of a sample with a thickness of tens of microns (Attwood, 2006), and it has been successfully used to investigate samples from inorganic materials to living cells (Sayre et al., 1995; Jacobsen, 1999; Attwood, 2006; Zbik, Martens, Frost, Song, Chen, & Chen, 2008).

In this study, catfish skin gelatin was extracted and used to make edible films with ingredients such as triacetin and Tween 80. Triacetin is recognized as a safe (GRAS) human food ingredient by the FDA (Fiume, 2003), and it was incorporated into the film forming solution to improve the water vapor barrier properties of the film. Tween 80 was added as an emulsifier to improve the homogeneity of the solution. First, TXM was used to examine the distribution of introduced triacetin into the film. Secondly, some common film properties, such as thickness, mechanical properties, water vapor permeability, water solubility, light transparency, and thermal properties were also investigated. The goals of this study were a). to investigate the structure characteristics of the added triacetin in the film using TXM; b). to quantify the influence of the triacetin on film properties, and c). to develop possible relationships between the distribution characteristics and the film properties.

2. Materials and methods

2.1. Catfish skin gelatin preparation

Fresh channel catfish skin was provided by Harvest Select Inc. (Uniontown, AL. USA). The skin was frozen and stored at -18 °C for no more than two months before use. Catfish skin gelatin was prepared using the method described by Yang et al. (2007b). Cleaned catfish skins were cut into small pieces (about 2 to 3 cm squares) and then treated with 0.20 M NaOH solution (1:6, w/v) for 84 min (4 °C) followed by a treatment

in 0.115 M acetic acid (1:6, w/v) for another 60 min (4 °C). Catfish skin pieces were mixed with deionized water (1:4, w/v) and subjected to a thermal extraction at 55 °C for 180 min. The gelatin solution was then filtered through cheesecloth and pure gelatin was obtained by lyophilizing (Labconco Corp., Kansas City, MO. USA).

2.2. Preparation of gelatin film

The preparation of catfish gelatin film followed the procedures of Zhang, Wang, Herring, & Oh (2007) with modification. The film-forming solution was prepared by dissolving 1.0 g catfish skin gelatin in deionized water (100 mL) at 50 °C with designated glycerol (20% of the gelatin amount), sodium triphosphate (STP) (50% of the gelatin amount), triacetin (0, 50, 100 and 150% of the gelatin amount), and Tween 80 (added at a ratio of 10% triacetin amount). A 100 mL portion of the film-forming solution was cast onto a right square (250×250mm), leveled plate covered by Teflon tape (Bytac®, Saint-Gobain Performance Plastics, Poestenkill, NY. USA) and dried inside an environmental chamber (Model AA-5460A, Espec Corp., Hudsonville, MI. USA) at 25 °C and 50% relative humidity for 48 hours. The film was then gently peeled off the plate. The margins were trimmed off and only the middle part was used in the following tests.

2.3. Characterization of gelatin film

2.3.1. Film thickness

Film thickness was measured at three different locations using a hand-held electronic micrometer (Model IP54, Fowler Electronic Micrometer, Fred V. Fowler Co., Inc. Newton, MA. USA).

2.3.2. Mechanical properties

Tensile strength (TS) and percent elongation (%E) at break were determined at room temperature (25 °C) using ASTM D882-97 (ASTM, 1998). A 10×100 mm film strip was placed onto grip pairs which were attached to a TA.XTPlus Texture Analyzer (5 kg load cell, Texture Technologies Corp., Scarsdale, NY. USA). The film strip was stretched by the grip at a head speed of 50 mm/min until broken.

2.3.3. Water vapor permeability

Water vapor permeability of the film was measured using the ASTM E96-95 standard method (ASTM, 1999). The catfish gelatin film was cut into 90×90 mm piece and put onto a Fisher permeability cup (Fisher Scientific Ltd.). The cup was previously filled with 15 mL of distilled water. The cup was then sealed with a cover and put under 25 °C and 50% RH condition for 7 hours. The weight of the sealed cup was measured at the beginning and at one hour intervals afterward using a digital balance (Model GP5202, Sartorius North America Inc. Edgewood, New York. USA). The WVP of the film was calculated as the following equation:

$$WVP(g/Pa/S/m) = (W \cdot x)/(A \cdot t \cdot \Delta P)$$

where W is the weight change of the cup (g), x is the film thickness (m), A is the area of exposed film (m^2), t is the time (h), and ΔP is the vapor pressure difference across the film (Pa).

2.3.4. Water solubility

Water solubility of the gelatin film was measured by putting 20×20 mm film portion into an aluminum pan with 15 mL of added distilled water. The pan was shaken gently with a shaker (Orbit 1000, Labnet International Inc., Woodbridge, NJ. USA) set at

70 rpm under 22 °C for 15 hr (Model 307A, Thermo Fisher Scientific, Waltham, MA. USA). The solution was then filtered through a filter paper (Whatman No.1) to get the un-dissolved part. Then the filter paper was dried in an oven (Model 30F, Econotemp Laboratory Oven, Thermo Fisher Scientific, Waltham, MA. USA) at 103 °C overnight. The film solubility (*FS* %) was calculated as the following equation:

$$FS(\%) = (W_i - W_f) \times 100 / W_i$$

where W_i *i*s the initial film weight (g), and W_f is the final undissolved film portion weight after drying (g).

2.3.5. Light transmission and transparency

The ultraviolet (UV) and visible light transmission of the films were measured at selected wavelengths between 200 and 800 nm using method described by Fang, Tung, Britt, Yada, & Dalgleish (2002). Film portions of 10×20 mm were placed into the test cell of a UV-Visible spectrophotometer (Helios Omega UV-Vis Spectrophotometer, Thermo Fisher Scientific. Waltham, MA. USA). Film transparency was calculated using the following equation (Han & Floros, 1997):

$$Transparency(A_{600} / x) = -\log T_{600} / x ,$$

where A_{600} is the absorbance at 600 nm, T_{600} is the transmittance (%) at 600 nm, and x is the film thickness (mm).

2.3.6. Transmission X-ray microscopy

The transmission X-ray imaging was carried out at the U7A endstation of National Synchrotron Radiation Laboratory (NSRL), Hefei, China. The schematic diagram of the transmission X-ray microscope was shown in Fig. 1. A 6 T superconducting wiggler was used as the X-ray source and a Si (111) double crystal was

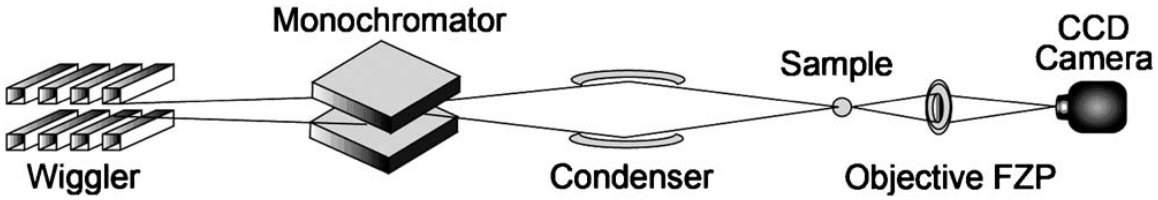


Fig. 1. Schematic diagram of the transmission X-ray microscope at the beamline U7A of NSRL (Chen et al., 2008).

used as the monochromator. An elliptically shaped capillary condenser provided a hollow cone illumination which matches the numerical aperture of the objective Fresnel zone plate with an outermost zone width of 45 nm. A charge coupled device (CCD) camera was employed to record the transmission X-ray images. The whole system had a magnification of 880 folds and a resolution up to 15 nm. More detailed information of the imaging device can be found elsewhere (Chen, Wu, Tian, Li, Yu, & Tian, 2008).

2.4. Statistical analysis

Statistical analysis was performed with the SAS program (V.9.1.3, Statistical Analysis Systems, Cary, NC. USA). Data were subjected to one-way ANOVA (Analysis of Variance) using CONTRAST method for multiple comparisons (Montgomery, 2005). ANCOVA (Analysis of Covariance) was also used to identify the effects of film thickness on the targeted results. A p-value ≤ 0.05 indicates a significant difference in an analyzed dataset.

3. Results and Discussions

3.1. Mechanical properties

Tensile strength (TS) and percent elongation (E%) of four different gelatin films made in this work were investigated. TS of the film decreased with the addition of

triacetin from 17.3 MPa (0% Triacetin) to 6.0 MPa (150% Triacetin), while E% had a reversed trend (from 68.0% to 205.4%) possibly due to the plasticizing effects of the triacetin ($p < 0.001$ for both, Table 1). The biggest drop of TS and also the biggest increase of E% happened between the 50% triacetin treatment and the pure gelatin film treatment, which was -41.0% for TS and 109.6% for E%. Adding more triacetin into the film did not result in larger differences between treatments. A similar effect was observed by Bertan et al. (2005) when stearic acid, palmitic acid, and elemi were added to the gelatin film which caused a decrease in TS and an increase in E% of the film. Yang and Paulson (2000) also reported a significant decrease of TS in their gelatin film incorporated with beeswax or stearic-palmitic acids blend, but the E% decreased in stearic-palmitic acids blend treatment. The weak interaction between the non-polar triacetin molecules and the polar polymer was believed to be the primary reason why their film structure was not softened. In another study on cod skin gelatin incorporated with sunflower oil, a decreased puncture force and percentage puncture deformation were also observed with increased hydrophobic contents (Pérez-Mateos et al., 2009).

3.2. Water vapor permeability

The effect of triacetin on the WVP of the gelatin film is also presented in Table 1. A drop of WVP was observed in 50% triacetin.treatment (from 8.4 to 7.8×10^{-8} g·mm·h^{-1}·cm^{-2}·pa^{-1}). But when the triacetin contents increased to 100% and 150% levels, WVP of the film increased significantly (13.1 and 13.5×10^{-8} g·mm·h^{-1}·cm^{-2}·pa^{-1}, respectively). This is an interesting phenomenon, which means when added at a moderate amount, the triacetin may pose certain barriers to the water vapor but excessive hydrophobic materials do not necessarily bring down the moisture transfer in a composite film.

Table 1. WVP, FS%, and mechanical properties of catfish skin gelatin films incorporated with different levels of triacetin (Mean ± SD).

Film	WVP	FS%	Mechanical properties	
			TS (MPa)	%E
0% Triacetin	8.4 ± 0.5 b*	69.5 ± 4.8 b	17.3 ± 2.0 a	68.0 ± 28.0 c
50%Triacetin	7.8 ± 1.4 b	75.5 ±1.8 ab	10.2 ± 3.0 b	142.5 ± 66.5 b
100%Triacetin	13.1 ± 1.8 a	76.5 ±7.8 ab	9.1 ± 1.8 b	222.1 ± 52.3 a
150%Triacetin	13.5 ± 0.9 a	83.3 ± 2.7 a	6.0 ± 1.7 c	205.4 ± 82.2 a

WVP: 10^{-8}g·mm·h^{-1}·cm^{-2}·pa^{-1}.

* Letters a, b, and c indicate significant differences between the treatments.

3.3. Water solubility

Water solubility of the films incorporated with different levels of triacetin is presented in Table 1. Water solubility increased steadily from 69.5% (0% Triacetin) to 83.3% (150% Triacetin). Films with triacetin possessed better water solubility than pure gelatin film ($p = 0.048$, Table 1). In a gelatin-lipid composite film, the higher the lipid portion, the lower the gelatin fraction in a certain piece of film, thus resulting in higher FS% in films with higher lipid levels if no interaction between the introduced lipid and gelatin happens. Similar results were reported by Bertan et al. (2005) on composite gelatin film incorporated with different lipid (elemi) concentrations (1, 2.5, 5, and 10%), in which the FS% increased steadily from 34.75% to 36.05%. Pérez-Mateos, Montero, & Gómez-Guillén (2009) also reported increased FS% of cod gelatin film when sunflower

oil was added into the film at a level greater than 0.6%, which significantly increased the non-protein soluble matter of the film.

3.4. Light transmission and transparency

The light transmission and transparency of catfish skin gelatin at selected wavelengths are listed in Table 2. Visually, the pure gelatin film was much more transparent than the films with triacetin. Introduction of the triacetin into the film effectively increased the barrier properties of the gelatin film to both the UV and visible light ($p<0.0001$). At the 280 nm wavelength, light transmission dropped dramatically from 20 to 9% (55% drop) with 50% triacetin added in the film. This could mean that the triacetin containing film could be a good barrier to the UV light-induced lipid oxidation. Slight changes of light transmission were also observed among films with different triacetin levels at 280 nm wavelength. Although varied thickness might pose some noise to the observed differences, ANCOVA confirmed that differences in the light transmission came from treatments instead of thickness variability (Table 3). Higher levels of triacetin in the film resulted in decreased light transmission. As a result of the increased light blocking properties, films with triacetin also possessed lower transparency values than pure gelatin film.

The addition of lipid typically produces films with more opacity due to the light scattering effects of the tiny lipid droplets spread in the gelatin film (Yang et al., 2000). A decrease of light transmission and transparency caused by the lipid components was also reported on cod skin gelatin film incorporated with different levels of sunflower oil (0-1%) (Pérez-Mateos et al., 2009) and bigeye and brownstripe red snapper skin gelatin incorporated with palmitic acid and stearic acid (Jongjareonrak et al., 2006).

Table 2. Light transmission (%) and transparency of catfish skin gelatin films incorporated with different levels of triacetin at selected wavelengths (Mean ± SD).

Film	Wavelength (nm)							Transparency
	200	280	350	400	500	600	800	
0% Triacetin	0	20 a*	65 a	74 a	81 a	83 a	86 a	1.8 c
50%Triacetin	0	9 b	24 b	29 b	34 b	38 b	43 b	9.4 b
100%Triacetin	0	8 bc	17 c	19 c	22 c	24 c	27 c	19.9 a
150%Triacetin	0	5 c	13 c	15 c	17 c	18 c	20 d	12.7 b

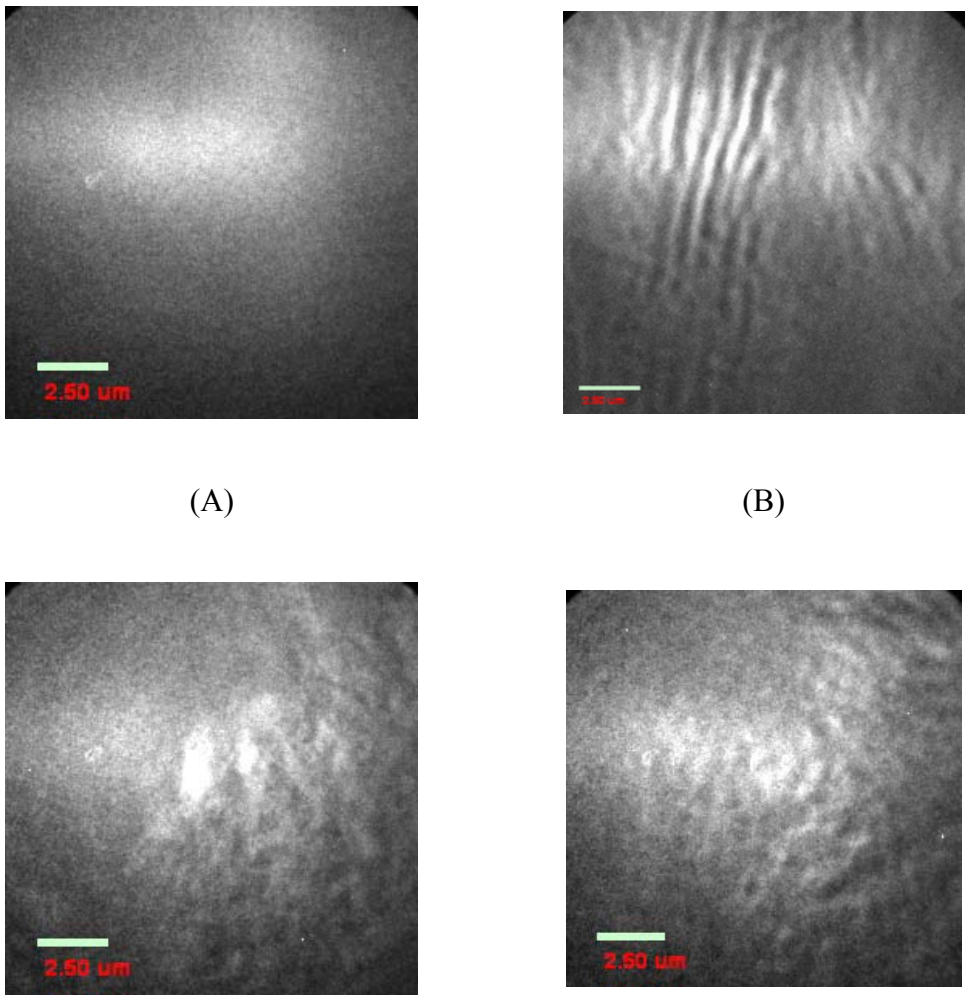
* Letters a, b, and c indicate significant differences between the treatments.

Table 3. ANCOVA results of treatment and thickness effects on the observed difference of channel catfish skin gelatin film incorporated with different levels of triacetin at selected wavelengths.

Variance Sources	Wavelength (nm)						
	200	280	350	400	500	600	800
Treatment	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
Thickness	$p = 0.96$	$p = 0.83$	$p = 0.66$	$p = 0.69$	$p = 0.88$	$p = 0.88$	$p = 0.93$

3.5. Transmission X-ray microscopy

With the increase of triacetin additives, the films structure changed from homogeneous to heterogeneous (Fig. 2). An even distribution of triacetin in the film was clearly shown in Fig. 2-A. Despite the shrinkage part on Fig. 2-B, the distribution of triacetin could still be considered as homogeneous distributed. However, with the increased amount, the homogeneousness of the triacetin decreased in Fig. 2-C and D, in which some congregations could be observed. This could partially explain why the increased triacetin amount did not lower the WVP. The triacetin was initially incorporated into the film to improve the water barrier properties of the films. It was assumed that there was an even distribution of triacetin in the gelatin film. This assumption might not be met which meant the heterogeneous repartition of the triacetin might happen during the forming of the film, resulting in an ineffective moisture blocking role of the lipid component. Similar results were also obtained by Cheng, Abd Karim, & Seow (2008). In their study, the WVP of the konjac glucomannan (KGM) based film increased instead of dropping after palm olein oil (PO) was mixed into the film forming solution. The heterogeneous repartition of the hydrophobic substance and the presence of cracks were considered as the primary reasons for the observed results. In another study of cod gelatin film by Pérez-Mateos et al. (2009), WVP of the gelatin film dropped slightly immediately after the sunflower oil was blended in the film forming solution, but WVP increases in all the oil treated samples were observed after 30 days of storage at 58% RH and 22 °C. A change of film matrix was suspected as the primary reason of increased WVP values.



(A) (B)

(C) (D)

Fig. 2. X-ray images of catfish skin gelatin films incorporated with different levels of triacetin (A. 0% Triacetin; B. 50% Triacetin; C. 100% Triacetin; D. 150% Triacetin).

Due to the hydrophobic nature of the triacetin, Tween 80 was added (10% of triacetin) as an emulsifier to assist the dissolving of triacetin and it might also play a role in the increased WVP at higher triacetin levels (100 and 150% Triacetin). When more triacetin were introduced, the amount of Tween 80 was also increased, which might facilitate the migration of moisture to some degree.

4. Conclusions

Gelatin was extracted from catfish skin and edible film was made from it. Increased triacetin content in the catfish gelatin film resulted in a distribution change of the ingredient from homogeneous to heterogeneous as examined by the transmission X-ray microscope (TXM). The addition of triacetin also caused decreased tensile strength (TS) and increased percent elongation (E%), water solubility, UV and visible light barrier properties. WVP of the film increased in some treatments (100 and 150% triacetin) possibly due to the heterogeneous distribution of the triacetin. The increased emulsifier amount in those films might also play a role to the increased WVP.

References:

ASTM. (1998). Standard test method for tensile properties of thin plastic sheeting. D 882-97. Philadelphia, Pa.

ASTM. (1999). Standard test method for water vapor transmission of materials. E96-95. Philadelphia, Pa.

Attwood, D. (2006). Nanotomography comes of age. *Nature*, *442*, 642-643.

Badii, F. & Howell, N. K. (2006). Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. *Food Hydrocolloids, 20*, 630-640.

Bertan, L. C., Tanada-Palmu, P. S., Siani, A. C., & Grosso, C. R. F. (2005). Effect of fatty acids and 'Brazilian elemi' on composite films based on gelatin. *Food Hydrocolloids, 19*, 73-82.

Chen, J., Wu, C., Tian, J., Li, W., Yu, S., & Tian, Y. (2008). Three-dimensional imaging of a complex concaved cuboctahedron copper sulfide crystal by x-ray nanotomography. *Applied Physics Letters, 92*, id. 233104.

Cheng, L. H., Abd Karim, A., & Seow, C. C. (2008). Characterisation of composite films made of konjac glucomannan (KGM), carboxymethyl cellulose (CMC) and lipid. *Food Chemistry, 107,* 411-418.

Cho, S. M., Gu, Y. S., & Kim, S. B. (2005). Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocolloids, 19,* 221-229.

Cho, S. H., Jahncke, M. L., Chin, K. B., & Eun, J. B. (2006). The effect of processing conditions on the properties of gelatin from skate (*Raja Kenojei*) skins. *Food Hydrocolloids, 20,* 810-816.

Choi, S. S. & Regenstein, J. M. (2000). Physicochemical and sensory characteristics of fish gelatin. *Journal of Food Science, 65*, 194-199.

Debeaufort, F., Quezada-Gallo, J. A., & Voilley, A. (1998). Edible films and coatings: Tomorrow's packagings: A review. *Critical Reviews in Food Science and Nutrition, 38,* 299-313.

de Carvalho, R. A. & Grosso, C. R. F. (2004). Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. *Food Hydrocolloids, 18,* 717-726.

Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey protein. *Journal of Food Science, 67,* 188-193.

Fiume, M. Z. (2003). Final Report on the Safety Assessment of Triacetin. *International Journal of Toxicology*, *22 (Supplement 2),* 1-10.

Gudmundsson, M. & Hafsteinsson, H. (1997). Gelatin from cod skins as affected by chemical treatment. *Journal of Food Science, 62,* 37-47.

Han, J. H. & Floros, J. D. (1997). Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *Journal of Plastic Film and Sheeting, 13*, 287-298.

Jacobsen, C. (1999). Soft x-ray microscopy. *Trends in Cell Biology, 9*, 44-47.

Jamilah, B. & Harvinder, K.G. (2002). Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chemistry, 77,* 81-84.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2006). Fatty acids and their sucrose esters affect the properties of fish skin gelatin-based film. *European Food Research and Technology, 222,* 650-657.

Lovell, R. T. (1980). Utilization of catfish processing waste. *Agriculture Experiment Station. Auburn University. Bulletin 521.*

McHugh, T. H. & Krochta, J. M. (1994). Permeability properties of edible films. In: Krochta, J. M., Baldwin, E. A. and Nisperos-Carriedo, M. O., Editors. *Edible coatings and films to improve food quality*, Technomic, Lancaster pp. 139-187.

Montero, P., Gómez-Guillén, M. C., & Borderías, A. J. (1999). Functional characterization of muscle and skin collagenous material from hake (*Merluccius merluccius L.*). *Food Chemistry, 65*, 55-59.

Montgomery, D. C. (2005). *Design and Analysis of Experiments, 6th Edition*. John Wiley & Sons, Inc. Hoboken, N.J. USA.

NASS. (2008). *Catfish Processing*. USDA Economics, Statistics and Market Information System. Publication date: 02.21.2008. Washington D.C.

Pérez-Mateos, M., Montero, P., & Gómez-Guillén, M.C. 2009. Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends. *Food Hydrocolloids,22,* 53-61.

Park, S. K., Rhee, C. O., Bae, D. H., & Hettiarachchy, N. S. (2001). Mechanical properties and water-vapor permeability of soy-protein films affected by calcium salts and glucono-delta-lactone. *Journal of Agricultural and Food Chemistry, 49,* 2308-2312.

Prodpran, T., Benjakul, S., & Artharn, A. (2007). Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. *International Journal of Biological Macromolecules, 41,* 605-614.

Rhim, J. W. & Lee, J. H. (2004). Effect of $CaCl_2$ treatment on mechanical and moisture barrier properties of sodium alginate and soy protein-based films. *Food Science and Biotechnology, 13,* 728-732.

Sayre, D. & Chapman, H. N. (1995). X-ray microscopy. *Acta crystallographica, A51*, 237-252.

Veiga-Santos, P., Oliveira, L. M., Cereda, M. P., & Scamparini, A. R. P. (2007). Sucrose and inverted sugar as plasticizer. Effect on cassava starch-gelatin film mechanical properties, hydrophilicity and water activity. *Food Chemistry, 103,* 255-262.

Wang, Y., Yang, H., & Regenstein, J. M. (2008). Characterization of fish gelatin at nanoscale using atomic force microscopy. *Food Biophysics, 3,* 269-272.

Yang, H. & Wang, Y. (2009). Effects of concentration on nanostructural images and physical properties of gelatin from channel catfish skins. *Food Hydrocolloids, 23,* 577-584.

Yang, H., Wang, Y., Jiang, M., Oh, J. H., Herring, J., & Zhou, P. (2007a). 2-step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. *Journal of Food Science, 72,* C188-C195.

Yang, H., Wang, Y., Regenstein, J. M., & Rouse, D. B. (2007b). Nanostructural characterization of catfish skin gelatin using atomic force microscopy. *Journal of Food Science, 72,* C430-C440.

Yang, H., Wang, Y., Zhou, P., & Regenstein, J. M. (2008). Effects of alkaline and acid pretreatment on the physical properties and nanostructures of the gelatin from channel catfish skins. *Food Hydrocolloids, 22,* 1541-1550.

Yang, L. & Paulson, A. T. (2000). Effects of lipids on mechanical and moisture barriers properties of edible gellan film. *Food Research International, 33,* 571-578.

Yoshimura, K., Terashima, M., Hozan, D., & Shirai, K. (2000). Preparation and dynamic viscoelasticity characterization of alkali-solubilized collagen from shark skin. *Journal of Agricultural and Food Chemistry, 48,* 685-690.

Zbik, M. S., Martens, W. N., Frost, R. L., Song, Y.-F., Chen, Y.-M., & Chen, J.-H. (2008). Transmission x-ray microscopy (TXM) reveals the nanostructure of a smectite gel. *Langmuir, 24,* 8954-8958.

Zhang, S., Wang, Y., Herring, J. L., & Oh, J.-H. 2007. Characterization of edible film fabricated with channel catfish (*Ictalurus punctatus*) gelatin extract using selected pretreatment methods. *Journal of Food Science*, *72,* C498-C503.

Zhou, P., Mulvaney, S. J., & Regenstein, J. M. (2006). Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. *Journal of Food Science, 71,* C313-C321.

VII. OVERALL CONCLUSIONS

A value-added catfish product, hot-smoked catfish, was developed which had potential to bring more profits to the catfish industry. Quality characteristics during the producing of hot-smoked catfish were studied.

Textural properties of raw and smoked catfish can be evaluated with the "finger" method used in our study. The novel sampling technique could be used in the textural measurement of not only catfish but also other fish fillet with irregular shapes.

Brining operations are critical to meet the FDA requirements on hot-smoked and ready-to-eat seafood products. According to our study, higher equilibrium salt contents in fish would result from higher brine concentrations. But initial salt absorption rates in the fresh catfish fillets were not positively related to the brine concentrations due to the protein denaturation introduced by salt. A multiple linear regression model was obtained to make the control of this procedure possible. Brine concentration and time combinations could be obtained easily for any specific WPS level needed. Accuracy of the model was verified through smoked catfish samples at different WPS levels from 1 to 5%. The NIR method was used to determine salts content of tested fish sample. Results from both NIR method and traditional method were consistent, indicating that the NIR method could be used as a rapid, accurate, and non-invasive method for measuring salt in smoked catfish.

When hot-smoked catfish fillets were individually vacuum-packed and stored at different temperatures different growth curves were observed. Temperature played a dominate role affecting the microbial growth of the 25 °C treatments. Shelf-life of the 25 °C treatments ended by 5 days after storage. Shelf-life of 53-, 35-, and 33-day was observed for samples stored at 4 °C and packaged with 0, 4,000, and 10,000 OTR films respectively. A 38-day microbiological growth lag-time was observed in 0 OTR×4 °C treatments. No *C. botulinum* was detected in any of the samples.

Gelatin was extracted from catfish skin and edible film was made from it. Increased triacetin content in the catfish gelatin film resulted in a distribution change of the ingredient from homogeneous to heterogeneous as examined by the transmission X-ray microscope (TXM). The addition of triacetin also caused decreased tensile strength (TS) and increased percent elongation (E%), water solubility, UV and visible light barrier properties. WVP of the film increased in some treatments (100 and 150% triacetin) possibly due to the heterogeneous distribution of the triacetin. The increased emulsifier amount in those films might also play a role to the increased WVP.

VIII. FUTURE WORK

Smoked catfish is of a product with great potential. Upon completion of the current project several notes can be made in case any further efforts to be put in this product.

1. Current smoking procedure takes about 3 hours to finish in the smokehouse. This procedure produces reliable products with several flavor options. However there is still room to improve the processing efficiency if needed. Combination of temperature set, smoke use, relative humidity set, and other parameter in the smokehouse can be further modified to produce the same product in shorter time and maybe use less power and materials at the same time.
2. Due to the uneven thickness of catfish fillet the thinner part on the fillet may taste salty if the thick part needs to obtain the required salt level. A traditional brine method can not solve this problem. Injection salting may be a possible solution to this by delivering brine solution rapid and evenly over the fillet.
3. There is also room for smoked catfish shelf-life extension. A lot of food grade preservatives can be used on hot-smoked catfish to make it available longer.
4. Value-added catfish processing work is far from over. Utilization of low value catfish products such as nuggets can be the next one to work on. Cold or liquid smoked catfish is also worth trying in the future.