KINETICS OF SALMON (*ONCORHYNCHUS GORBUSCHA*) QUALITY CHANGES DURING THERMAL PROCESSING

By

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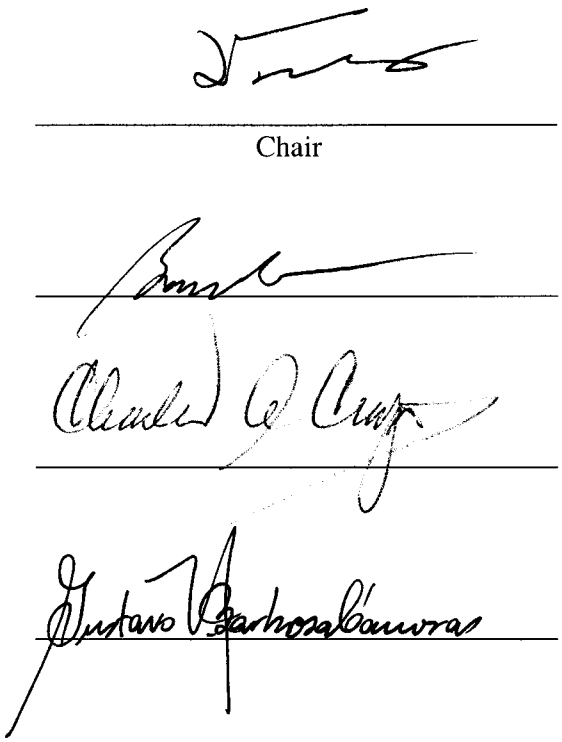
To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of FANBIN KONG find it satisfactory and recommend that it be accepted.



Chair

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ABSTRACT

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The objective of this study was to investigate the reaction kinetics along with changes in salmon quality during high temperature thermal processing. Small samples (D 30 mm × H 6 mm) cut from pink salmon (*Oncorhynchus gorbuscha*) fillets were sealed in aluminum containers and heated in an oil bath to simulate a commercial thermal process. A thorough examination of the quality changes occurring during heating were conducted by investigating quality attributes of raw and cooked salmon, including color, shear force to break muscle fibers, cook loss, area shrinkage, lipid composition and thiamin content. The kinetics of reactions leading to the quality changes were evaluated.

Significant quality deterioration occurred in all of the studied quality attributes as reflected in color changes, increased cook losses, shrinkage, muscle toughening, lipid oxidation and thiamin losses. However, no measurable loss in polyunsaturated fatty acid (PUFA) was observed during thermal treatments at 121.1 ˚C for up to 2 hrs. Addition of salt (1.5% w/w) reduced the cook loss, area shrinkage and shear force of the heated salmon muscle, as well as slightly darkening the color compared to muscle samples to which no salt was added.

Mathematical models were developed relating changes in different quality attributes with heating conditions including processing temperatures from 100 to 131.1 ˚C and heating time from 90 min to 3 hrs with rate constants, activation energy and reaction order determined. Heat-induced shear force changes were compared between salmon and chicken muscle to gain insights into the mechanism(s) underlying the shear force change based on physical, chemical and structural changes in the muscle during heating.

The results of this study increase the understanding of quality changes in salmon during high temperature thermal processing, and provide kinetic models that can be used to design or optimize thermal processes for high quality shelf-stable food products.

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CHAPTER 1. INTRODUCTION

Summary of Related Study and Problem Statement

Pink salmon is one of the most important commercial fish species harvested in the United States. A substantial portion of the pink salmon (*Oncorhynchus gorbuscha*) harvest is processed into canned foods using conventional retorting processes. Most of the remainder is frozen. During retorting, the packaged salmon meat is heated at a high temperature, typically around 120 ˚C, for various periods from 20 to 90 min, depending upon package size and gemometry. The high temperature heating cause quality changes, such as degradations in color, texture, cook loss (weight loss), shrinkage, lipid oxidation and nutrient losses.

Major changes in salmon fillets during heating are associated with protein denaturation. Proteins present in muscle foods include myofibrillar proteins (myosin and actin), connective tissue proteins (mainly collagen) and sarcoplasmic proteins. Heating induces denaturation of heme proteins and oxidation of carotenoid pigments that increase the lightness and reduce both redness and yellowness of the muscle (Bhattacharya and others 1993). Extensive heating causes Mailard reactions that darken the product color (Haard, 1992). Denaturation of meat proteins results in structural changes, such as aggregation and gel formation of sarcoplasmic proteins, solubilization of connective tissue, shrinkage and disintegration of myofibril (Leander and others1980; Hatae and others 1990). The structural changes in both transverse and longitudinal directions of muscle fibre expel the sarcoplasmic fluid from the muscle fibers, resulting in cook losses from meat tissue (Offer and others 1984). Both reduced water holding capacity and shrunken muscle fibers could lead to a harder and more compact tissue texture in the resultant cooked meat (Harris and Shorthose1988). Heating also causes loss of nutrients such as thiamin, which is one of the least thermostable of the water soluble vitamins. Lipid oxidation may occur

generating secondary lipid oxidation products that lead to rancidity and other off flavors in foods.

Studies carried out to investigate the quality changes of salmon during thermal processing have mostly focused on lower temperature (< 100 ^{o}C) and /or short time heating (< 1 hr) (Skrede and Storebakken 1986; Bhattacharya and others 1993). There is a lack of information on the quality changes of salmon at processing temperatures above 100 ^{o}C and heating time longer than 1 hr. Understanding how high temperature processing affects salmon quality could shed light on quality changes that occur in other muscle foods and more generally to shelf-stable low acid food products. Chemical composition and muscle structure vary along the length of a fish fillet and from the dorsal to the ventral side, all affecting the quality properties being measured (Sigurgisladottir and others1999). Sodium chloride is commonly used as an additive in the thermally processed shelf-stable products that may cause significant difference in final product quality (Hutton 2002). However, no reports could be found on how the quality of thermally processed salmon was influenced by salt addition or variations in quality or physical properties of the muscle depending upon the locations of the muscle along the length of a salmon fillet. New thermal processing technologies such as microwave sterilization processes have been investigated as a means of producing commercially sterile food with higher quality (Guan and others 2002). Knowledge of degradation kinetics, including reaction order, rate constant and activation energy, is essential if new thermal processes are to be developed or existing retort processes optimized. Unfortunately, information on kinetics of salmon quality changes during thermal processing is limited.

Meat tenderness, often indicated by shear force, is the most significant factor affecting consumer's satisfaction (Sigurgisladottir and others 1999). During heating, solubilization of

connective tissue improves meat tenderness, while heat-denaturation of myofibrillar proteins generally causes meats toughening (Harris and Shorthose 1988). The change of meat tenderness during heating has been correlated with collagen solubility (Harris and Shorthose 1988), cook loss (Palka and Daun 1999), sarcomere shortening and the shrinkage or expansion of muscle fibres (Hatae and others 1990; Sigurgisladottir and others 2001). Higher number of fibers of smaller diameter was found in cooked salmon muscle than raw muscle resulting in increased toughness (Skrede and Storebakken 1986). However, little information can be found on heat-induced changes in tenderness of salmon fillet as related to physical, chemical and structural changes resulting from thermal process. Also, the mechanism behind the tenderness changes during heating remains unknown.

Objectives

The overall objective of this dissertation aimed at increasing the fundamental understanding of the changes in salmon quality during high temperature thermal processing, and providing kinetic information that could help to design or optimize thermal processes to improve product quality. The specific aims were to:

- to study the changes in quality characteristics of salmon fillet, including physical changes such as color, tenderness, cook loss, area shrinkage, and chemical changes including changes in lipid composition and thiamin content, during high temperature thermal processing; and to determine if these changes were influenced by the sampling position along the length of the salmon fillet or by the addition of salt;

- to investigate the kinetics of salmon quality changes during high temperature thermal processing and develop mathematical models to relate changes of quality attributes with heating conditions; and

- to understand the mechanism underlying tenderness changes of salmon muscle relative to cook loss, area shrinkage, collagen denaturation and microstructure.

Dissertation Outlines

The thesis is comprised of seven chapters:

Chapter 1: Introduction. This chapter summarizes related research, states the current problems, outlines proposed objectives of the study and provides the structure of the dissertation.

Chapter 2: Literature Review. This chapter briefly introduces basic knowledge of muscle structure and composition, explains traditional thermal processing, and presents results and findings of previous research on the heat-induced quality changes of muscle foods.

Chapter 3: Quality Changes of Salmon (*O. gorbuscha*) Muscle during Thermal Processing. In this chapter, a test cell designed at WSU and a multiple thin blade (MTB) texture device were specially developed for sample heating and shear force measurement. A computer vision system (CVS) was used to obtain accurate measurements of color and area shrinkage. Samples were heated in an oil bath at 121.1 ˚C for different time periods from 5 to 120 min. The quality variations along the longitudinal axis of salmon fillets (raw and heated) were examined. Changes in color, shear force, cook loss, and area shrinkage of salmon fillet muscle during thermal sterilization processes were investigated.

Chapter 4: Effect of Salt Addition on the Quality Changes of Salmon Fillet (*O. gorbuscha*) During Thermal Processing. Salmon fillets processed with 1.5% (w/w) added salt were compared with those processed without added salt. Effect on color, cook loss, area shrinkage, shear force, thiamin retention, lipid oxidation and fatty acids profile were examined.

Chapter 5: Kinetics of Salmon Quality Changes during Thermal Processing. Samples were heated at 100, 111.1, 121.1, and 131.1 ˚C for different time periods. Mathematical models

were developed to empirically fit data for color, cook loss, area shrinkage, shear force, and thiamin content. Kinetic parameters such as rate constant, reaction order, and activation energy were predicted.

Chapter 6: Tenderness Changes in Salmon and Chicken Muscle During Thermal Processing: Cook Loss, Area Shrinkage, Collagen Solubility and Microstructure. Washington State grown broiler chicken breast (*Pectoralis Major*) and Pacific pink salmon (*Oncorhynchus gorbuscha*) muscle were heated, and the changes in meat tenderness, cook loss, area shrinkage, collagen solubility and microstructure compared. A correlation relating tenderness with other quality attributes was developed. The mechanism of the tenderness changes of salmon and chicken muscle with heating was explored.

Chapter 7: Conclusions and Recommendations. This chapter summarizes the results of this study and gives recommendations to future work.

Some chapters in the dissertation are being published or submitted to selected journals where styles vary. The format of these chapters follows the style of target journals. Full citations of these chapters included in this dissertation are as follows:

- Chapter 3

Fanbin Kong, Juming Tang, Barbara Rasco, Chuck Crapo and Scott Smiley. Quality Changes of Salmon (*O. gorbuscha*) Muscle during Thermal Processing. *J Food Sci.* In press. Accepted in October 2006.

- Chapter 4

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- Chapter 5

Fanbin Kong, Juming Tang, Barbara Rasco, Chuck Crapo. Kinetics of Salmon Quality Changes during Thermal Processing. Submitted to *J Food Eng*. In second revison.

- Chapter 6

Fanbin Kong, Juming Tang. Changes in the Tenderness of Chicken Breast (you will have to fix this taxonomic designation *P. major*) and Salmon (*O. gorbuscha*) Muscle During Thermal Processing: Cook Loss, Area Shrinkage, Collagen Solubility and Microstructure. Submitted to *Meat Sci.*

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CHAPTER 2. LITERATURE REVIEW

Muscle Composition and Structure

Salmon can be either wild or grown in aquaculture operations. As an important fish resource, salmon contains high contents of protein and polyunsaturated lipids with a high amount of omega-3 fatty acids that are believed to reduce the risk of heart disease and provide benefits for neurological development (Harris and Schacky 2004). Alaska is the world's largest producer of wild salmon, and the pink salmon (*Oncorhynchus gorbuscha*) is often harvestd in the largest volume. About 400 million pounds pink salmon are harvested in US each year, with forty percent processed into canned foods (USDA 2003).

Fish muscle structure is somewhat different than mammalian muscle and this results in differences in how these two types of muscle tissues respond to thermal processing. Although fish muscle fibers are short relative to mammalian muscle fibers (Dunjanski 1979), fish muscle fiber arrangement is similar to mammalians such as poultry and beef, and the muscle cell structure is basically the same as in mammalian tissue. The muscle itself is composed of bundles of muscle fibers (Figure 2-1), with each fiber (10 – 100 μm) consisting of many myofibrils, held together by a collagen network, the perimysium, with a network called the epimysium holding the whole muscle structure together (Flint, 1994). Fish muscle consists of a few long sheets of muscle extending the whole length of the body (Figure 2-1), which are primitive when compared with mammalian muscle. These long sheets are transversely divided by sheets of connective tissue (myocommata) into myotomes (Dunajski 1979). Fish muscle cells join two adjacent myocommata, and are parallel to the long axis of the muscle.

Fish have roughly the same proximate composition as hot-blood animals. The main components of fish muscle tissue after rigor mortis are water (~ 75%), protein (~ 20%), lipids

and to lesser extent carbohydrates, free amino acids, dipeptides, vitamins, minerals and nucleotides. Pink salmon prior to spawning contains ~ 3 - 4% lipids, in which 40 – 50% are polysaturated fatty acids (PUFA) (USDA 2001). Salmon composition is affected by water, season, activity, age, gender, spawning condition and rearing conditions for aquacultured species (Nomura and others, 1999). The chemical composition also varies at different locations in a same fish, particularly the lipid content. In cultivated Atlantic salmon (*Salmo salar*), the dorsal white muscle contains only one tenth of the oil as the belly flap tissue (Ackman and others 1993).

The structural proteins that make up fish muscle function like mammalian proteins, although their physical properties differ slightly. There are three types of muscle proteins: myofibrillar, sarcoplasmatic and stroma proteins. Myofibrillar proteins represents ~ 60% of the muscle proteins and constitute the contractile lattice, whereas sarcoplasmic proteins represents about 30% and consist of myoglobin and enzymes involved in the energy metabolism. About 10% of the muscle proteins are stroma proteins consisting mainly of structural connective tissue proteins collagen and elastin (Lawrie 1991). Fish myosin is more heat labile than mammals. Connective tissue, mainly collagen, is much less in fish (3 - 5%) and more evenly distributed thoughout the muscle tissue than is observed in mammalian muscle, probably due to a lesser need to fight gravity since the water environment supports their bodies (Venugopal and Shahidi 1996). Fish collagen contains a lower proportion of hydroxyproline than is found in red meat, and it is less heat stable and more soluble than collagen from beef or pork (Dunajski 1979; Venugopal and Shahidi 1996). When fish collagen in the myocommata is degraded to gelatin, the myomeres separate readily and give rise to the flakes of cooked fish (Dunajski 1979).

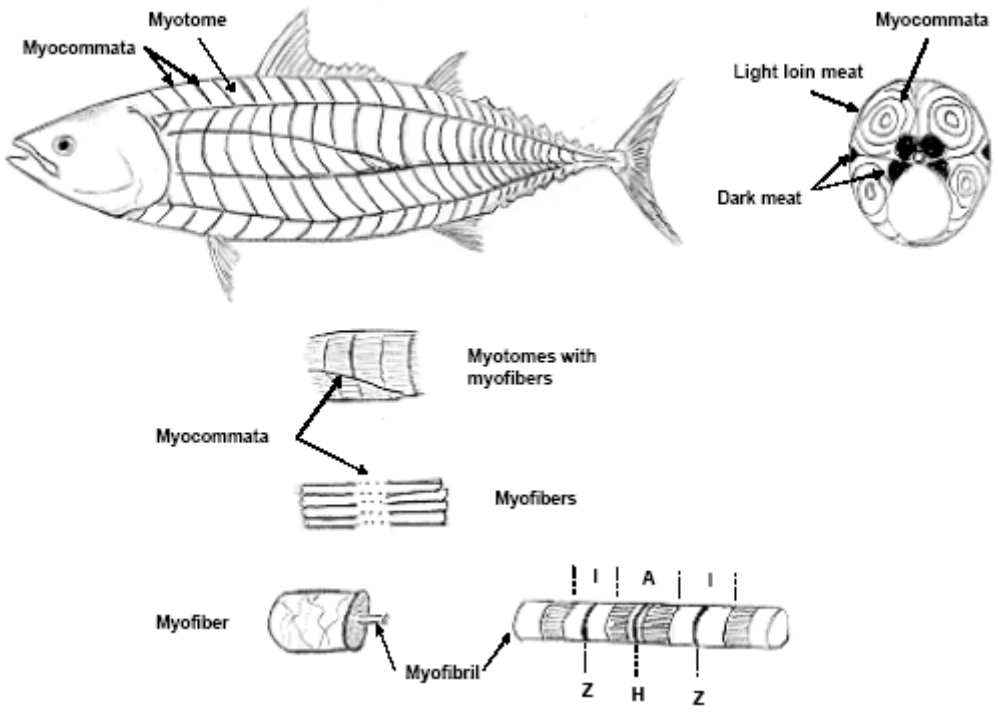

Figure 2-1. Salmon muscle structure (Webb 2003)

Salmon have both red (also referred to as dark) and white muscle. The "red muscle" is that dark, brownish flat strip of muscle that is just under the skin along the lateral line. The "white muscle" of salmon is actually red because of carotenoid pigments. The red muscle is used for continuous swimming, while white muscle is used for short bursts of rapid swimming (Venugopal and Shahidi 1996). Dark muscle contains higher levels of lipids and myoglobin, and is suffused with capillaries to sustain aerobic swimming. White muscle has thicker fibers and is much less vascularized than red muscle (Venugopal and Shahidi 1996).

Thermal Processing of Muscle Foods

Canning is a traditional thermal process to preserve salmon and other fish with a relatively high fat content such as tuna, mackerel and capelin. Canning provides a commercially

sterile product or a product which has received enough heat to kill the spores of the pathogen *Clostridium botulinum* and that will also not support the growth of pathogenic vegetative microbes. Sterile cans properly sealed (hermetically sealed) will not permit the introduction of pathogens following thermal processing. Canned products will not spoil under normal storage conditions, however it is possible that spores of highly heat resistant *Bacillus* sp. can germinate and grow in cans that are contaminated with this spoilage microbe if the containers are held above 110^{o}F for an extended period of time. Since the discovery of canning by the Frenchman Nicholas Appert at the beginning of the 19th century, this method of preserving food using heat has become a fairly well established process. Nowadays, consumers enjoy billions of can of fish along with other canned products worldwide. Over 14 million tons or 12.5% of the world catch destined for human consumption (USDA 2003) is canned.

Canned salmon is characterized by a pH > 4.6 and aw > 0.98. Foods with a pH greater than 4.6 are called "low acid canned foods" for which the microorganism of major concern is *Clostridium botulinum*. Some strains of *C. botulinum* produce spores that are the most heat resistant at all pathogenic microorganisms. Destruction of all spores of *C. botulinum* is the minimum safety requirement when thermally processing low-acid canned foods. Experience has shown that a process equivalent in sterilizing effect to twelve decimal reductions of the population of *C. botulinum* is sufficient to protect consumer safety. Such a process is referred to as a "12 D" process and it is equivalent to holding the contents of the container at 121.1 ˚C for 2.8 min (12 D= 12 x 0.23 = 2.8 min).

The total sterilization effect of a thermal process can be expressed as the sum of all the sterilization effects achieved by all the time-temperature combinations throughout the entire thermal process. By convention, sterilizing effect is expressed in standard units of minutes at

121.1 ˚C, so that an entire processing cycle is expressed as being equivalent to holding the product at 121.l ˚C for a given time. The unit of sterilization is the F_o unit, where a F_o value of one minute is equivalent to holding the product at 121.1 ˚C for one minute and then cooling it instantly. It is calculated from the temperature history inside the product by numerical integration, using a z value of 10 ˚C:

$$F_0 = \int_0^t 10^{\frac{(T-121.1)}{z}} dt \qquad (1)$$

The cook value is a measure of the degree of cooking during thermal treatment, which is calculated from the same equation but with a reference temperature of 100 ˚C and a z value of 33 ˚C.

Salt is often the only added ingredient in canned fish (FAO 1985). It is primarily added as a flavor enhancer. A level of 1.5 - 2.0% is usually the optimal acceptable level from sensory perspective (Ranken 1981). The addition of salt increases water holding capacity (WHC), tenderizes meat texture and increases product yield after cooking (Ranken 1981; Thorarinsdottir and others 2002). This effect is believed to result from the salt increasing protein solubilization or extraction, thereby improving the emulsifying and water binding capacities of proteins in meat products (Hutton 2002). Salt can also promote oxidative and rancidity changes in fats, which are not desirable (Hutton 2002).

While the canning process is likely to be remained popular for the foreseeable future owing to its convenience, new technologies such as microwave (MW) heating has been evaluated as an alternative to traditional canning for salmon processing. Unlike retorting processes in which heat conduction/convection mechanisms are predominant, MW processes

involve volumetric heating in which electromagnetic waves (from 300 MHz to 300 GHz) interacts with food constituents, such as water and NaCl, to generate heat via molecular friction. Therefore, MW sterilization process use shorter heating times (~10 min) compared to traditional retort process (~ 50 - 80 min), and this shorter heating time may potentially improve product quality (Guan and others 2002).

Changes of Salmon Quality During Thermal Process

Thermal processing of meat aims to increase palatability, develop flavor and ensure safety. During thermal processing operations, salmon meat hermetically sealed in cans or pouches is processed in steam retorts at high temperatures for an extended period of time (~ 50 - 90 min) (Guan and others, 2002). The high temperature heating may cause severe quality deterioration, such as degradation in color and texture, nutrient loss, cook loss (weight loss) and area shrinkage, rendering the products less attractive to most consumers. The most drastic changes in salmon fillets during heating result from protein denaturation. In particular, denaturation of heme proteins and oxidation of carotenoid proteins which are the source of muscle pigmentation in salmon lead to a darkened product (Haard 1992). The denaturation of the myofibrillar proteins and collagen leads to reduced water holding capacity and shrunken muscle fibers, and subsequently leading to a harder and more compact tissue texture (Harris and Shorthose 1988). Aroma volatiles are developed in meat during cooking as a result of Maillard reactions, which occur between reducing sugars, amino acids along with other reactions that produce flavor components from degradation of lipids and proteins (Haard 1992; Girard and Durance 2000). Previous reports on the heat induced changes of different quality attributes

including color, texture, water holding capacity (WHC), lipids and thiamin are summarized below.

Color

Color is an important attribute of meat quality that influences consumer acceptance. The pink reddish color of raw salmon flesh results from the deposition of carotenoids (Bjerkeng 2000). Carotenoids, which are lipid soluble pigments, provide yellow to red colors. In the wild, salmonids (salmon and trout) absorb carotenoid from the zooplankton, herbivorous fish and crustaceans they eat. The absorbed carotenoid is then transported in the blood to the muscles and skin where it is deposited. Astaxanthin accounts for more than 90% of the total carotenoid content found in the flesh of wild salmonids (Bjerkeng 2000). A longitudinal variation in carotenoid content and red color is found, with more astaxanthin being deposited in the caudal than in the anterior part (Christiansen and Wallace 1988). Hunter *a* value is significantly correlated with total carotenoid pigments and could be used as indicators of total carotene content of fish (Bjerkeng 2000).

Color measurement

Different methods can be used to determine the extent of color change. The most frequently used instrumental color measures are the CIE [1976] *L*a*b**, in which the primary parameters are lightness (*L**), redness (*a**) and yellowness (*b**). Colorimetric methods have been used for a long time. These involve taking the reflectance spectra of the surface of a sample at one or more wavelengths and comparing this spectra with that of a reference sample. The drawback with this method is that often only a small surface area (normally 2 – 5 cm^2) can

be studied, and thus it does not accurately describe heterogeneity of color commonly found in food products (Louka and others 2004; Briones and Aguilera 2005). Multiple measurements are usually used to overcome this shortcoming. Alternatively, samples can be homogenized to achieve a uniform color, but this method destroys usable information (*e.g.,* color patterns, differences in color distribution) and renders the sample unusable for longitudinal time studies commonly used in product quality evaluation (Briones and Aguilera 2005).

Computer vision system (CVS) for color analysis has been developed and used in many areas to measure the color and color distribution in fruits and vegetables, grains, meats and confectioneries (Yam and Papadakis 2004; Louka and others 2004; Briones and Aguilera 2005). Color image analysis is also used in seafoods such as raw salmon color (Balaban and others 2005) and brown trout (*Salmo trutta*) cutlets (Marty-Mahe and others 2004). Basically, a computer vision system consists of three basic components: the lighting system, a digital camera, computer hardware and software to process the images (Briones and Aguilera 2005). Contrast to colormetric method, CVS measures the color over the entire sample area and can provide useful information for heterogeneous samples like fish fillets.

Changes in color during heating

Heating leads to fading of salmon muscle color causing visual appearance changed from red or reddish-pink to a paler color. Increasing the processing temperature or time increases L^* value, but decreases a^* and b^* values of fish muscle (Bhattacharya and others 1998; Masuda and others 1996). The color change is a result of denaturation of heme proteins (hemoglobin and myoglobin), oxidation of carotenoids, Maillard reactions that occur between reducing sugars, amino acids and degradation of lipids, as well as the protein-lipid browning, *i.e*. the reaction of

lipid peroxide decomposition products with protein (Haard 1992). Different species of fish exhibit different color degradation speed (Masuda and others 1996).

Color changes can be predicted using kinetic models that describe the color changes of meat during thermal process. The rate of cooked meat heme-protein formation (measured as the rate of loss of myoglobin solubility) was found to obey a first order kinetic in beef and lamb (Geileskey and others 1998). When muscle tissue of Pacific chum salmon (*Oncorhynchus keta*) was heated in water (60 – 100 °C) for 0 - 40 min, the L^* and b^* values followed zero order kinetics, a^* value obeyed first order kinetics, and total color difference (ΔE) decreased following zero order kinetics (Bhattacharya and others 1998).

Texture

Texture of muscle foods is a crucial factor of acceptability (Veland & Torrissen 1999). Tenderness is the most important texture parameter of meat for consumer acceptabiltiy and is often closely correlated with the shear force necessary to break muscle fibers. Various measurement methods have been developed to characterize fish texture from different sensory perspectives (Veland & Torrissen 1999).

Texture measurement

The main textural parameters that can be measured experimentally using texture analysis instrumentation for fish may be divided into puncturing, compressing, shearing, cutting and pulling (Bourne 1978). Instrumental methods such as the Kramer shear compression system (multiple blade), Warner–Bratzler shear blade, and Texture Profile Analysis are commonly used

for evaluating meat tenderness (Cavitt and others 2004). Texture profile analysis is a method often most appropriate for food samples as it mimics chewing.

- Texture profile analysis

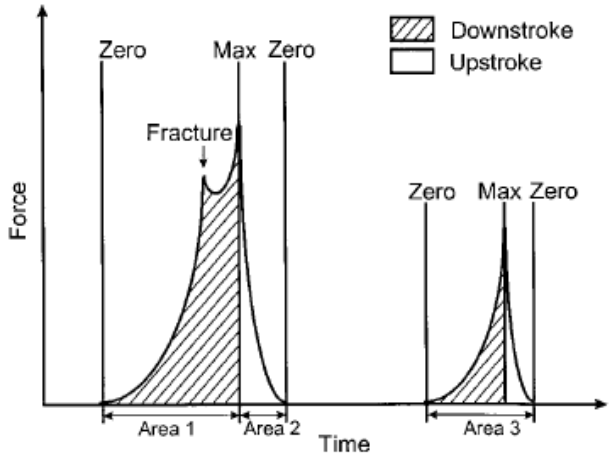

Figure 2-2. Schematic illustration of the force-time plot from a TPA test. The moments of zero or maximum compression are marked with vertical lines (Veland and Torrissen 1999).

Double compression or puncturing methods generate a texture profile analysis (TPA), giving results in terms of force vs. distance (Figure 2-2). TPA parameters are obtained by analyzing the force vs. time curve as shown in Figure 2-2 (Bourne 1978). Hardness is defined as the maximum force (N) that occurs at any time during the first compression cycle. Fracturability, cohesiveness and resilience are calculated using areas and peak heights.

For compression, a flat ended cylinder, or for puncturing, a spherical probe, has been designed to simulate the human finger. These devices are often used for TPA test. Double compression is conducted as follows: the probe approaches the sample and compresses the fillet for a certain depth, retracts from the fillet allowing the muscle to rebound, followed by another compression. For soft muscle tissues, the compression device may penetrate the fillet. The spherical probe is used in TPA to penetrate the surface of the sample, and multiple passes may be

used. The compression test using a cylindrical probe has been found to be the most adequate for differentiating between the textures of fish at the different locations along the length of a fillet (Casas and others 2006), while the spherical probe was found to be more suitable for non-destructive measurement of salmon fillets with the lowest variability in repeated measurements of similar parts on different fillets (Jonsson and others 2001).

- Shear force test

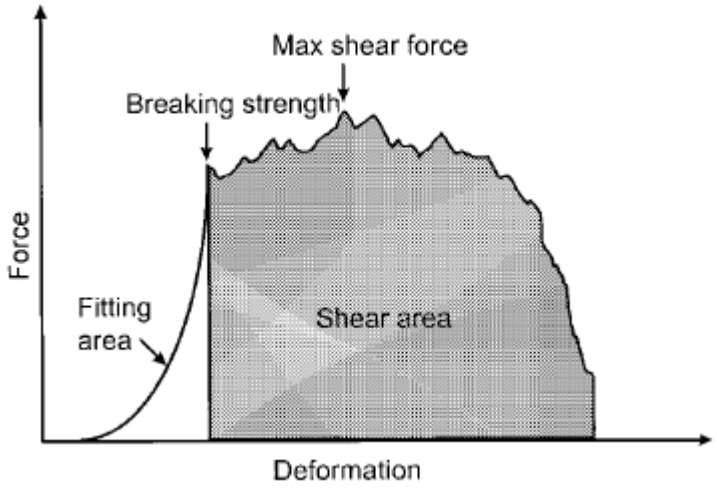

Figure 2-3. Schematic illustration of a force-deformation plot from a shear test (Veland and Torrissen 1999)

Shear force is an indicator of tenderness for muscle foods cited as the most significant factor affecting consumer satisfaction (Sigurgisladottir and others 1999; Jonsson and others 2001). A variety of shearing and cutting devices are commercially available for shear force measurement for muscle, such as Warner-Bratzler shearing device and Kramer shear compression cells. The Kramer shear cell consists of shear bars or blades that pass through the sample and then into a cell holder or box having a corresponding number of slots. The force required to shear through a sample is reported as the maximum peak force (N). The Warner-Bratzler shear blade is a V-shaped blade but is operated in a manner similar to the Kramer cell. The Kramer shear cell is generally preferred for measuring fish muscle (Dunajski 1979;

Sigurgisladottir 1999). This method incorporates compression of fibres beneath the blade, tension in the adjoining fibers and shearing of the fibres (Bouton and others 1975). Maximum shear force and breaking strength are calculated from the force-deformation plot (Figure 3-3).

Factors Influencing Texture

Many factors can influence fish texture, such as the structure and distribution of myofibrillar proteins, collagen, fat, moisture content, fibre density (or fibre diameter) and sarcomere length (Dunajski 1979; Montero and Borderias 1989; Palka and Daun 1999). In red meat, the connective tissue is responsible for the so-called "background toughness", while the myofibrillar proteins largely govern the "myofibrillar toughness" (Dunajski 1979). Both collagen content and collagen solubility seem to influence meat texture. Low collagen solubility indicates high level of cross-linking bonds in the protein molecules, which is related to a tougher meat texture. The firmness and cohesiveness of fresh fish are related with both the density and the arrangement of collagen fibrils in the connective tissue (Bailey and Light 1989). Montero and Borderias (1989) found that the shear strength values of trout were higher near the tail owing to a higher proportion of insoluble collagen. Fish contains less connective tissue with lower cross-links than red meat such as beef muscle, resulting in a generally a more tender texture of the raw muscle compared to red meat (Dunajski 1979).

Muscle texture can be investigated by evaluating the microstructure of muscle tissue. Common methods include SEM (Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy) (Bremner and Hallet 1986; Palka and Daun 1999; Martine and others 2000). The sarcomere length of the muscle fibres and average muscle fibre diameter can be measured using electron microscopy and have been found to be closely related with the

tenderness of cooked meat (Hatae and others 1990; Sigurgisladottir and others 2001). The shear strength tend to be higher in muscle fibres containing a higher number of smaller diameter fibers compared to muscle composed of a lower number of larger diameter fibres (Hatae and others 1990). Kanoh and others (1988) found that the dark muscle of yellowfin tuna had a firmer texture than ordinary muscle as a result of a smaller fiber diameter within the dark muscle.

Fish muscle tissue with higher moisture and fat contents also tends to be softer (Dunajski 1979). In addition, texture varies with biological factors including the size of the fish and stress during slaughter (Taylor and others 2002). Industry practices such as slaughter method, diet, storage time, and storage temperature also have a significant effect on muscle texture (Veland and Torrissen 1999; Taylor and others 2002).

The chemical composition and muscle structure vary along the length of a fish fillet and from the dorsal to the ventral side, all factors affecting the textural properties (Sigurgisladottir 1999). Therefore the location where the sample is taken is of importance and should be considered when measuring the textural properties in the fillet (Sigurgisladottir and others 1999).

Structural and Texture Changes During Heating

Textural changes in fresh or processed fish have been linked to numerous phenomena, such as degradation of myofibrillar proteins or collagen, formation of protein aggregates, and ultrastructural changes (Morzel and others 2000; Murphy and Marks 2000). During heating, the muscle proteins denaturate extensively, causing reduction in WHC and volumetric shrinkage, resulting in a harder and more compact texture. Changes in toughness are generally correlated with structural changes associated with the myofibrils and connective tissue. Heating produced a softening of connective tissue caused by conversion of collagen to gelatin, and a toughening of

meat fibers caused by heat coagulation and fragmentation of myofibrillar proteins (Harris and Shorthose 1988). Denatured and aggregated sarcoplasmic proteins may also contribute to muscle tissue firmness by forming coagulated interstitial material that obstructs or impedes the movements of the fibers (Hatae and others 1990).

In the majority of muscle systems, proteins denature at different temperatures. Ofstad and others (1996) found that the denaturation of myosin, collagen and actin in salmon occurred at 45, 57 and 77 ºC, respectively. Upon heating, myosin and actin denaturation lead to myofibrillar protein shortening thus increasing toughness (Dawson and others 1991). There was further coagulation and aggregation when heating was prolonged. Collagen denatures when heating is started, and then becomes solubilized upon continued heating. The rate and extent of these changes depends upon the cross-linking level and this is related to the maturity of the collagen, as well as exogenous factors such as heating rate, relative humidity, and physical restraints on the muscle fibres during cooking. In solution, isolated collagen molecules are denatured at 36 ºC (Powell and others 2000). However, according to Bailey and Light (1989), the structure of collagen molecules in muscle remains relatively stable due to the crystallization energy of the collagen triple helix which results in a higher denaturation temperature. As denaturation occurs, the molecule shrinks to approximately one-fourth its original length, resulting in toughening of the muscle. However, continued heating above the denaturation temperature causes partial solubilization of collagen, resulting in gelatin formation and eventually a reduction in shear value (Bailey and Light 1989).

There is a lower quantity of connective tissue in fish is much less (3 - 5%) than in red meats, and it appears to be degraded more readily when heated than is that of warm-blooded animals. Therefore, fish collagen plays less of an important role in the texture of fish after

cooking compared to red meat. With fish, the muscle fibres themselves provide the main resistance to mastication (Dunajski 1979) with collagen denaturation being less important.

The review by Offer (1984) summarizes the structural changes of muscle occurring during cooking as follows (Figure 2-4): at 40 – 50 °C, transverse shrinkage occurs to the fibre axis, widening the gap between the fibres and their surrounding endomysium. At 65 – 75 °C, the connective tissue network and the muscle fibres cooperatively shrink longitudinally, with the extent of shrinkage increasing with temperature. This shrinkage causes the greatest amount of water loss that occurs during cooking. It is presumed that water is expelled by the pressure exerted by the shrinking connective tissue forcing aqueous solution into the extracellular void (Offer 1984; Tornberg 2005). Correspondingly, two distinct increases in toughness have been observed when the temperature is increased during cooking. A first increase in toughening is observed at around 40 - 50 °C and a second increase is around 65 - 75 °C (Davey and Gilbert 1974; Harris and Shorthose 1988). The first phase of toughening is suggested to be correlated to the denaturation of the myosin, whereas the second phase is related to heat-induced collagen denaturation, shrinkage and cook loss (Davey and Gilbert 1974).

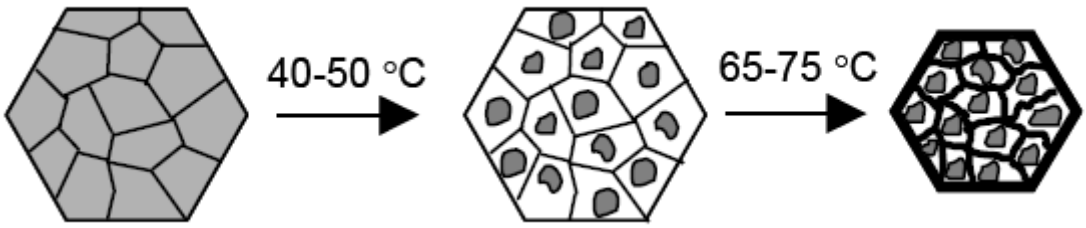

Figure 2-4. Effect of cooking upon shrinkage of muscle. Myofibrillar proteins denature at 45-50 ºC and connective tissue between 60 and 75 ºC. Black lines represent connective tissue and grey areas muscle fibres (Modyfied after Bailey 1988).

Limited information has been found in literature on the change of salmon texture during high temperature long time thermal process. Bhattacharya and others (1993) studied the textural

changes of Pacific chum salmon muscle subjected to hydrothermal treatment (60 – 100 ˚C) for different time intervals (0 - 40 min), and found the hardness of cooked meat was higher than raw muscle. Increases in time or temperature of treatment led to increased hardness. However, softening of muscle occurred with prolonged heating at higher temperatures (90 – 100 ˚C). More study is required to fully understand the changes of fish texture during heating and the mechanisms behind these changes.

Water holding capacity (WHC) and cook loss

The water holding property of the muscle tissue is of major importance to the commercial value and consumer acceptance of fish muscle products. WHC is defined as "the ability of meat to hold its own or added water during application of any force or treatments such as grinding, processing or cooking" (Hamm 1960). Water exist in three forms: bound water, the water (4 - 5%) bound to macromolecules due to their polar properties; immobilized water that is attracted by the bound water; and the free water that is held only by weak surface forces (Hamm 1960). About 90% of the total water is held intracellularly within the myofibrils in the free space between the thick and thin filaments, only a small portion of the water (5 – 12%) present in muscle is located extracellularly outside the fiber wall or between myofibrils (Offer and Kinght 1989). Hence, WHC of muscle is greatly influenced by structural changes including those that result from cooking.

Cooking induces denaturation of proteins and subsequent structural changes in both transverse and longitudinal directions that expel the sarcoplasmic fluid from the muscle fibers, resulting in cook losses from meat tissue (Bertola and others 1994). It has been suggested that collagen is responsible for the majority of juice expulsion from tissue during cooking (Bendall

and Restall 1983). The water is expelled by the pressure exerted by the shrinking connective tissue releasing aqueous solutions into the extracellular void (Ofstad and others 1993). Shortening of sarcomere length during heating also contributes to the liquid loss (Bouton and others 1975).

The extent of meat shrinkage and resultant cook loss varies with different muscles and the meat temperature. Figure 2-5 shows liquid loss in the miscle of cod (*Gadus morhua L.*) and salmon (*Salmo salar*) harvested in Norway as a function of heating temperature from 5 – 70 °C (Ofstad and others 1993). The liquid loss refers to both water and fat loss. The liquid loss was almost constant for both fish species between 5 and 20°C. At higher temperatures, the liquid loss increased rapidly as a function of temperature, attained a maximum when pre-heated to 45/50 ˚C, thereafter the liquid loss was constant or decreased slightly. Most of the liquid lost from salmon was water. Easily separable fat in the liquid fraction was first observed at 40 °C. At 70 °C about 16% of the liquid lost was fat for salmon. It is suggested the water loss at 5 – 50 °C wass due to shrinkage of muscle cell result from denaturation of collagen and myosin and collagen gelatinization. The reduced water loss at higher temperature (50 – 70 °C) is probably caused by aggregates of sarcoplasmic proteins mixed with gelatin forming a gel matrix that is able to hold water and/or plug the intracellular capillaries thus stabilizing the aqueous phase (Ofstad and others 1993; 1996).

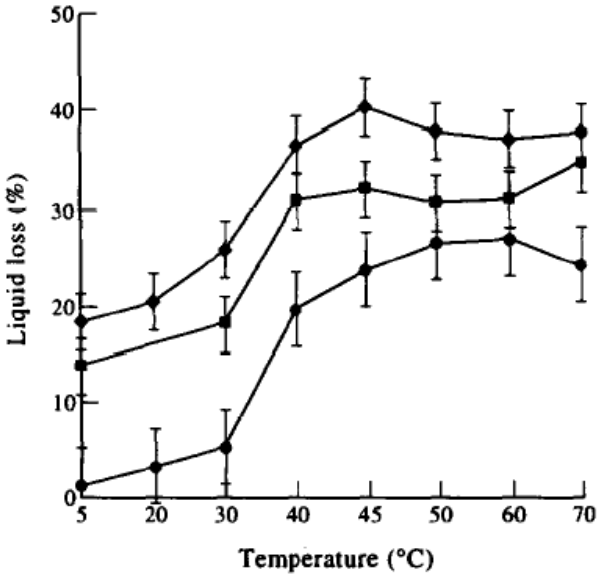

Figure 2-5. Liquid loss (% by weight) as a function of heating temperature of coarsely chopped salmon (-●-), wild cod (-■-) and fed cod (-◆-) muscle (Bars indicate standard deviation of 18 salmon, 15 wild cod and 30 fed cod). The temperature of the fish muscle at slaughter was 47°C (Ofstad and others 1996).

Figure 2-5 indicates the liquid loss is specie dependent. The fed cod muscle had highest liquid loss on heating, as well as a lower pH and the most severe post mortem degradation caused by myofibrillar shrinkage and extensive cell rupture. In wild cod muscle, which had a smaller liquid loss therefore a higher liquid-holding capacity, the myofibrils were closely packed with a lesser sarcomere shortening that favored a higher liquid-holding ability. Salmon muscle possessed better liquid-holding properties than both fed and wild cod muscle. The morphological features of the salmon muscle, the higher fat content and the better stability of the myosin/actomyosin fractions are probably of major importance with regard to the better liquid-holding capacity of the salmon muscle compared to the cod muscle (Ofstad and others 1996).

Lipids

Fish and fish oils are highly susceptible to oxidation due to their high content of polyunsaturated fatty acids (PUFAs). PUFA content of fish in total fatty acids ranges between 30 – 40%, mainly eicosapentaenoic acid (EPA, 20:5n-3) (5 - 18%) and docosahexaenoic acid (DHA, 22:6n-3) (1 - 12%) (USDA 2001). Changes in fish flavor induced by lipid oxidation are one of the major problems in quality deterioration during processing and storage. Cooking and thermal treatment of ω-3 PUFA is known to increase fish tissue susceptibility toward lipid oxidation, and the oxidation enhanced with the levels of ω-3 PUFA in the muscle (Aubourg and others 1997). The level of lipid oxidation of meat during heating can be characterized by measuring both primary products by measuring PV (peroxide value) and secondary oxidation products using a TBARS (2-thiobarbituric acid reactive substances) method (Aubourg and others 1997).

De La Cruz-García and others (2000) found that the canning process, evaluated at 112 °C for 50 min, did not lead to significant changes in fatty acids in sea urchin roe relative to the raw sample. Similar finding was reported in Koøakowska and others (2002) who found that lipid oxidation in the canned cod liver remained a very low level, although the lipid resistance to oxidation decreased during storage. The little effect of canning on lipid oxidation might be partly attributed to the low oxygen availability in the sealed cans, as oxygen availability is critical in propagation stage that converts lipid free radicals to peroxyl radicals (Andreo and others 2003). Addition of salt to meat products greatly enhances lipid peroxidation (Hutton 2002), as sodium chloride can stimulate catalysis of oxidation of phosphatidylcholine liposomes by the soluble fraction of fish muscle (Osinchak and others 1992).

Other thermal processes in addition to canning were also investigated. Al-Saghir and others (2004) measured the degree of lipid oxidation and the formation of cholesterol oxidation products (COP) in salmon fillets subjected to steaming and pan-frying for 6 - 12 minutes. Only very small changes were observed in primary and secondary oxidation products after cooking, indicating an insignificant effect of the heating procedures on lipid oxidation. However, there were significant increases in COP in the fat extracted from the salmon after the various cooking procedures. The study also showed higher degree of unsaturation led to higher lipid oxidation and more COPs formed.

The thermal oxidation of PUFAs could be in part inhibited when oils were used as the filling medium. Among different oils, extra virgin olive oil (EVOO) showed the highest protection, which might be attributed to the natural phenolic compounds in EVOO (Sacchi and others 2002). EDTA has also been found to inhibit lipid oxidation in salmon oil-in-water emulsions, although its effectiveness can be diminished by the presence of calcium (Alamed and others 2002). In addition, gluconic acid, glucono-delta-lactone and glucono-gamma-lactone showed similar effect in keeping seafood texture and flavour during thermal process (Mclntyre 1990).

Thiamin

Water-soluble vitamins, such as thiamin, are known to be sensitive to processing conditions (especially temperature and pH). Salmon contains 2 - 3 μg/g tissue thiamin (Hui and others 2006). Apart from the nutritional significance, thiamin content is commonly used as an index of quality of canned low acid foods. Thiamin can be in free form or in a combined form as a protein-complex or pyrophosphoric acid ester–co-carboxylase (Mulley and others 1975), and

the stability is affected by pH, temperature, heating time, water activity or moisture content, ionic strength, buffer type and processing method (Tannenbaum 1976). Stability of thiamin is also affected by some food components: it is reported that fat, starch and proteins decreases thiamin loss during processing (Tannenbaum 1976; Lešková and others 2006). The thiochrome method (AOAC 942.23, 2000) is commonly used to measure thiamin content, in which thiamin is oxidized to a fluorescent thiochrome which is quantfied.

The thiamin loss in canned foods is mainly due to heat-induced thiamin oxidation, and in addition, to the loss of thiamin in the exudates as thiamin is water-soluble (Janitz and Szymandera, 1998; Lešková and others 2006). The rate of thiamin loss increased with an increase in temperature. Ariahu and others (2000) reported ~ 50% thiamin loss in periwinkle during canning process at 121.1 ºC for 40 min, and the degradation rates were higher in the brine solution. Janitz and Szymandera (1998) reported 50% reduction of thiamin in pork during pasteurization in the presence of iodized salt. Thiamin losses varied significantly with the type and part of meat and the water and fat content. Thiamin retention appeared to be higher in the dark muscles generally (differences in retention of up to 20%) probably because of lower water losses (Lešková and others 2006).

Kinetics of the thermal degradation of thiamin in different food products have been studied by earlier investigators. The thiamin degradation fits an empirical first order model (Mulley and others 1975; Ariahu and others 2000), with *Ea* in the range 107 -123 kJ mol^{-1}. However, second order kinetics have also been reported (Kessler and Fink 1986).

Summary

Previous studies have shown that the high temperature heating cause degradations in color and texture, cook loss (weight loss), muscle shrinkage, lipid oxidation and thiamin losses. Among these quality attributes, meat tenderness received the highest attention in that it is the most significant factor affecting consumer's satisfaction (Sigurgisladottir and others 1999). Studies were carried out to investigate the quality changes of salmon during thermal processing, but these studies have mostly focused on lower temperature (< 100 ^{o}C) and /or short time heating (< 1 hr) (Skrede and Storebakken 1986; Bhattacharya and others 1993). Information is still limited on kinetics of salmon quality changes during thermal processing, which is essential to develop new thermal process and optimize existing retort processes. Furthermore, little information can be found on heat-induced changes in tenderness of salmon fillet and the associated mechanisms. Therefore, this study aimed to investigate the reaction kinetics along with changes in salmon quality during high temperature thermal processing, which involved processing temperatures above 100 ^{o}C and heating time longer than 1 hr. The result could shed light on quality changes that occur in other muscle foods and more generally to shelf-stable low acid food products.

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CHAPTER 3. QUALITY CHANGES OF SALMON (*O. GORBUSCHA*) MUSCLE DURING THERMAL PROCESSING

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Abstract

The objective of this study was to investigate quality changes of salmon fillet muscle during thermal sterilization processes. Small samples (D 30 mm × H 6 mm) from the central dorsal region were heated in an oil bath at 121.1 ˚C for periods varying from 5 to 120 min. The quality variations along the longitudinal axis of salmon fillets (raw and heated) were examined. The quality properties studied included: shear force, color, cook loss and shrinkage. To minimize the influence from the heterogeneity of the salmon muscle, a multiple thin blade (MTB) texture device was developed for shear force measurement and a computer vision system (CVS) used to facilitate accurate measurements of color, and shrinkage. The red muscle was firmer than the white muscle in the raw but not in heated samples. Muscle from the central dorsal region had a lower cook loss and less shrinkage than samples from either the anterior or posterior region following heating. The greatest change in quality occurred within the first 10 min of heating at

121.1 ˚C. Shear force measurements following heating indicated two peaks, one corresponding to 5 min and the second for 60 min processing at 121.1ºC. Possible mechanisms were discussed.

Key words: sterilization; thermal processing, salmon, quality changes, color; shear force; cook loss; area shrinkage

Introduction

About forty percent of salmon harvested in USA are converted into canned or pouched product using conventional retort processes (USDA 2003). A substantial portion of the pink salmon (*Oncorhynchus gorbuscha*) is canned. During retorting, the packaged salmon meat is heated at a high temperature typically around 120 ˚C for various periods from 20 to 90 min, depending upon package size and shape. Knowledge of kinetics for quality changes in salmon muscle during those processes is desired in design of food packages and thermal processes to produce the best possible shelf-stable products.

A wide range of analytical methods has been used to characterize the quality changes that occur to a muscle food during commercial sterilization processes. Color, texture, and cook loss are among the most frequently used quality indicators. Area shrinkage, caused by heat-induced protein denaturation and the resultant shrinkage of the muscle fibers, is also an important cooking quality of muscle foods (Barbera and Tassone 2006).

The instrumental color measurement usually uses the CIE L^*, a^*, b^* scale in which primary parameters are lightness (L^*), redness (a^*) and yellowness (b^*). Conventionally, the color values are measured by a colorimetric or spectrophotometric method, involving reflected light from a sample surface. This method often measures only a small surface area (normally 2–5 cm^2), and thus it does not accurately describe the visual color of a heterogeneous product

(Louka and others 2004; Briones and Aguilera 2005). Computer vision systems (CVS) developed for color analysis have been used in many areas to determine the color of fruits, vegetables, grains, meats, and seafoods (Yam and Papadakis 2004; Louka and others 2004; Briones and Aguilera 2005; Balaban and others 2005) and have a number of advantages. A CVS system can take measurements over an entire sample area, thus significantly decreasing the variation in color prediction from heterogeneity of a sample. The images can be also used for other purposes, such as area measurement (Barbera and Tassone 2006). However, the use of CVS for salmon muscle color is still limited (Lin and others 2003).

As an indicator of tenderness, shear force of muscle foods has been cited as the most significant factor affecting consumer satisfaction (Sigurgisladottir and others 1999; Jonsson and others 2001). A variety of shearing and cutting devices are commercially available for shear force measurement for muscle, such as Warner-Bratzler shearing device, blade probe and Kramer shear compression cells. Among these methods, the Kramer shear cell is generally preferred (Dunajski 1979). The Kramer shear cell is a multi-bladed fixture: the upper part or blade holder holds ten 70 mm wide and 3.0 mm thick blades, and the lower part or cell contains the food specimen and slots to guide the blades. The blades shear the specimen when the slots in the cell become engaged. The maximum force required to move the blades through the specimen is used to characterize the texture quality of the sample. The use of a Kramer cell is, however, problematic for fish muscle due to its unique morphological and compositional features compared to red meat such as beef muscle (Dunajski 1979). Fish muscle has smaller muscle fiber dimensions, the muscle fibers tend to fracture after cooking. The slits in the bottom of a standard Kramer cell are too wide and the blade is too thick, the testing is in a compression mode rather than shearing. In addition, samples of a relatively large dimension are required for a

standard Kramer cell, making it inappropriate for the small sample sizes needed for texture studies with fish fillets in which the muscle morphology and chemical composition changes significantly within a single fillet. Therefore, a fixture with thinner blades would be more suitable (Dunajski 1979) and such a device was developed here. No report on study using a similar thin blade device for fish muscle tenderness measurements was found in the literature.

The chemical composition and muscle structure vary along the length of a fish fillet and from the dorsal to the ventral side, all affecting the quality properties being measured (Sigurgisladottir and others 1999). Therefore the sampling location is important and should be considered when measuring the quality properties of the fillet. Several studies report on the variation of shear force along a salmon from its head to its tail. The muscle is firmer in the tail than in the head, and this might be related to smaller muscle fiber diameter and higher proportion of insoluble collagen in the tail (Love 1970; Montero and Borderias 1989).

Quality changes occurred during cooking (<100 ˚C) have been studied in salmon (Skrede and Storebakken 1986; Bhattacharya and others 1993). Shear force increased as temperature increased, and the higher number of fibers of smaller diameter was found in cooked muscle compared to raw muscle (Skrede and Storebakken 1986). Increasing the processing temperature or time increased the visual lightness, but reduced both the redness and the yellowness of muscle (Bhattacharya and others 1993).

The objectives of this study were to: 1) develop a multiple thin blade texture fixture (MTB) to characterize raw/heated fish muscle tenderness; 2) develop computer vision system (CVS) to characterize raw/heated muscle color as well as changes in sample area attributable to shrinkage; 3) investigate the variation of quality attributes, including color, shear force, cook loss, and shrinkage, in the raw and heated salmon muscle as related to different locations in a

fillet; and 4) investigate the changes in quality characteristics of salmon muscle during thermal processing.

Materials and Methods

Materials

Pacific pink salmon (*Oncorhynchus gorbuscha*) were provided by Ocean Beauty Seafoods, Inc., Seattle. These fish were female, from the same catch harvested in Aug., 2005 near Kodiak, Alaska. The fish weighed 1350 ± 100 g and were of similar size (370 ± 10 mm in length and 120 ± 10 mm in width). The fish were gutted, frozen, stored (-31 ˚C) and shipped to Washington State University, Pullman, WA.

Development of a test cell

To provide uniform heating, a special test cell (WSU Test Cell) was designed to minimize come-up-time (CMT: the time needed for the internal temperature to reach the processing temperature) for meaningful sample sizes for texture and color analyses. Sample dimension, particularly the thickness of sample, is the most important parameter influencing the CMT, the samples were 6 mm in thickness and 30 mm in diameter. The custom-designed cylindrical aluminum test cell is illustrated in Figure 3-1. The cells had a net inner space of 35 mm in diameter and 6 mm in depth. The top and bottom lids with o-rings provided a hermetic seal. The bottom lid allowed easy removal of fragile heated samples for texture and color measurements. A 0.1 mm diameter copper-constantan thermocouple (Type-T) was inserted through the top lid to measure temperature at the geometrical center of the sample during the

heat treatments. The heating was conducted in a 121.1°C oil bath using glycerol as the heating medium. During heating, the signals from the thermocouple junctions were transferred to a computer equipped with a DLZe type data logger (DELTA-T Devices, Cambridge, England). The CMT was ca. 2.5 minutes.

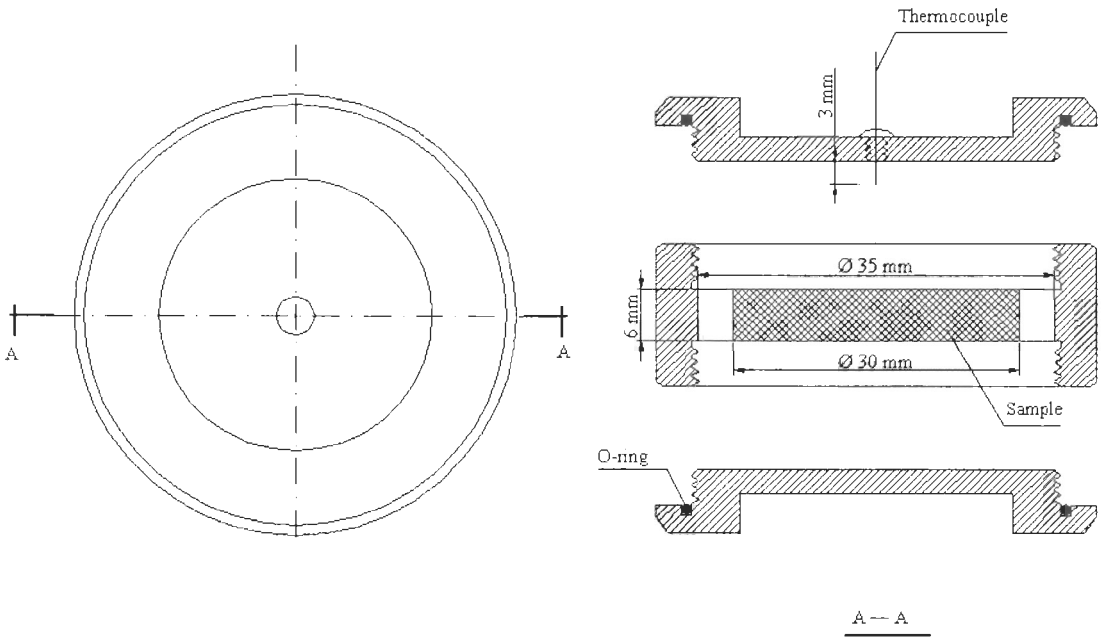

Figure 3-1. Schematic diagram of WSU Test Cell

Sampling position and experimental design

A multi-location sampling technique was employed to examine the influence of sample locations along the length of a salmon fillet, dorsal side only, on the quality attributes measured here. Six fish were used in the sampling. Each fish was cut into nine sections (location 1 - 9) along the length of the fish (Figure 3-2). The belly flaps were removed. Each section was sliced into up to three 6mm-thick layers depending on the thickness of the fillet (Figure 3-2). A 30 mm diameter cutter was used to take disk shaped samples from these thin slices. The samples (size: D 30 mm × H 6 mm) from the three layers were designated as an inner layer, middle layer and outer layer and numbered as shown in the Figure 3-2. The middle and inner layers were primarily composed of white muscle, while the outer layer contained part of the red muscle (Kiessling and others 2006). For the tail sections #7-9, only two (outer and inner) or one (outer) section could be cut because of limited fillet thickness. For each location, a fish provided two replicates, one from each side of the fish. In total, 12 samples were taken from the same location of six fish, of which six replicates were used for raw color, texture and moisture measurements, and the other six were heated in the 121.1 ˚C oil bath for 20 min. After heating, the samples were removed rapidly from the oil bath and cooled in ice to 4 ˚C. The sample temperature dropped to 20 ˚C within a half minute in ice, so that the thermal effect of the cooling step on the product quality could be neglected. After cooling, the samples were dried with a filter paper, weighed in an analytical balance (Ohaus model Analytical Plus, Ohaus, Pine Brook, New Jersey), placed on a sample dish with a cover, and stored in a cooler (4 ˚C) for further analysis.

To investigate the effect of heating time on the quality changes, the muscle (red muscle and tissue along the lateral line removed) near the dorsal fin (inner and middle layer samples of section 3#, 4#, 5#, 6#) of four fish were sampled. In total, sixty samples were obtained, pooled,

and heated at 121.1 ˚C for 0, 2.5, 5, 10, 15, 20, 30, 60, and 120 min. Six replicates were used for each time level. The heated samples were cooled and stored as stated earlier.

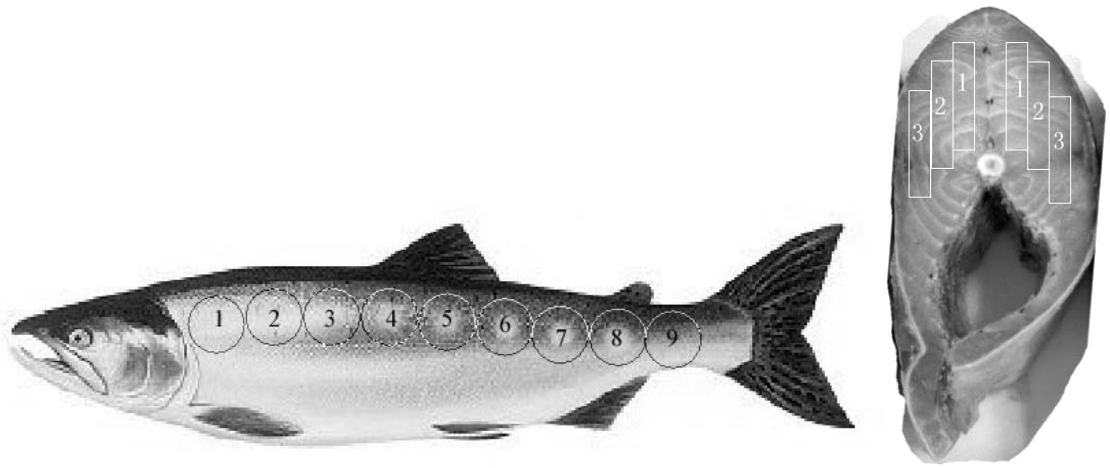

Figure 3-2. Example of sampling locations for the study of quality variation along the salmon length (left: the sampling sections 1-9; right: the sampling layers on the transverse section)

Cook loss and moisture

The weights of raw and heated samples were recorded to calculate cook loss. Percentage of total cooking loss was calculated as:

$$Cook\ loss = \frac{weight\ of\ raw\ sample - weight\ of\ cooked\ sample}{weight\ of\ raw\ sample} \times 100\% \qquad (1)$$

To determine moisture content, 3-5 g raw/cooked samples were dried in a vacuum oven at 65 ˚C to constant weight (Hart and Fisher 1971).

Multiple thin blade (MTB) texture fixture and shear force measurement

A multiple thin blade texture fixture (MTB) similar to a Kramer shear test cell was developed to measure shear force of small salmon samples. Similar to a standard Kramer shear cell (Catalog Number 2830-018, Instron Corporation, 825 University Ave, Norwood, MA), the

MTB consisted of the upper part and lower part: the upper part had thin 10 blades, and the lower part was the support base with slots. Compared to a commercial Kramer shear cell, the new MTB used thinner blades (0.5 mm against 3 mm), shorter blade length (40 mm against 107 mm), and smaller blade width (50 mm against 70 mm) as well as narrower slits (1.2 mm against ~ 3.3 mm) in the base. The spacing between two adjacent blades was 5 mm. The cell was fitted to a Texture Analyser TA-XT2 (Stable Micro Systems Ltd., Surrey, UK) equipped with a load cell of 5 kg. Before shear force measurement, the raw and heated samples were allowed to equilibrate to room temperature (approximately 22˚C), which took approximately half an hour. The samples were placed on the support base in a way so that the blades were perpendicular to muscle fibers. The traveling speed was 1 mm/s. The force-time graphs were recorded by a computer and analyzed using the Texture Expert for Windows (version 1.15, Stable Micro Systems Ltd.). The shear force was measured as the peak height in the force-time profile.

A study was conducted to compare the performance of MTB and a flat, blunted blade in characterizing texture of salmon fillets. The blade probe had a thickness of 3.0 mm and width of 70 mm. This method was commonly used in the literatures for measuring texture of salmon fillets (Sigurgisladottir and others 1999; Casas and others 2006). The blade was fitted to a Texture Analyser TA-XT2 equipped with a load cell of 5 kg. The measurement was made with a loading speed of 1 mm/s. Outer layer samples were used in the comparative study. Paired samples (from each side of the same fish) were divided into two groups. The tests were conducted in four replicates. The texture of one group of the samples was measured with MTB while the other with the blade probe.

Computer vision system (CVS) and color/area determination

A CVS was used to capture color images of fresh and cooked samples for color and area analyses. The CVS included three parts: a lighting system; a Nikon D70 Digital camera, with 6.1 Megapixel solution and 18-70mm DX Zoom Nikkor Lens; and a Pentium IV desktop computer with image processing software to be described later. The lighting systems consisted of a translucent diffusion shooting tent and four fluorescent bulbs (60 HZ 26w) as lighting source. This system is described in details in the paper of Pandit and others (2007). Samples were placed in a black plate on the bottom of the shooting tent. The four fluorescent bulbs were mounted on the two sides of the shooting tent, 25 cm above and at an angle of 45 to the food sample plane. The digital camera was mounted downwards on the top of the tent, 50 cm above the sample plane. The lights were turn on at least 15 min before the pictures were taken. A Roche *Salmo*Fan^{TM} color wheel, comprising of 15 color cards, each with an individual number, ranging from gray pink to deep red, and frequently used for color assessment of Atlantic salmon flesh, was used to verify the experimental settings prior to actual measurements. Color images were downloaded into the computer for analysis.

The color parameters (CIE L^*, a^* and b^*) were derived using Adobe Photoshop CS2 software (Version 8.0) (Adobe Systems Inc., San Jose, CA, USA). The procedures involved using a magic wand tool to select sample area, and a histogram tool to indicate the average lightness, redness and yellowness values for the selected area. In CIE LAB, L^* ranges from 0-100, a^* and b^* from -127 to +128; however, in Photoshop, these color values are encoded between 0-255. The color values from Photoshop (referred as L, a, b) were converted to standard scaling values using formula (Briones and Aguilera 2005):

$$L^* = \frac{L}{2.5} \quad (2)$$

$$a^* = \frac{240a}{255} - 120 \quad (3)$$

$$b^* = \frac{240b}{255} - 120 \quad (4)$$

A comparative study was conducted by using a Minolta Spectrophotometer (Model: CM-2002) and the CVS to measure color cards in the Roche *Salmo*Fan™.

For sample area determination, the Vision IMAQ Builder image processing software Version 6.1 (National Instruments, Austin, Texas) was used. The area of sample image was determined using the "area measurement" menu. The shrinkage ratio was calculated as:

$$Area\ shringkage\ \ ratio = \frac{area\ of\ raw\ \ sample - area\ \ of\ cooked\ \ sample}{area\ \ of\ raw\ \ sample} \times 100\% \quad (5)$$

Statistical analysis

The data of quality attributes of raw and heated samples from different locations of the fillets were compared by using analysis of variance (ANOVA) in the General Linear Models procedure of the SAS System for Windows V8.01 (SAS User's Guide, 1996). Differences between group means were analyzed by Duncan's multiple-range test. Statistical significance was set at a 0.05 probability level. The coefficient of variation (CV) was calculated as a percent of the standard deviation to the mean value (SD/mean×100), and was used to compare the precision of the shear force measurements by different means.

Results and Discussion

Comparison between measuring methods

Color

Figure 3-3 shows the correlations between *L**, *a**, *b** values obtained by the CVS and spectrophotometer measuring the color cards in the Roche *Salmo*Fan™. High correlation coefficients were observed between the two methods for the all three parameters ($R^2 = 0.998$, 0.976 and 0.972 for CIE *L**, *a**, and *b**, respectively). CVS has some special advantages over colorimeter/spectrophotometers. The use of colorimeter/spectrophotometer requires a fairly large flat sample area for accurate measurement; but this is not a limiting factor for a CVS. This is particularly true in our study. The samples used were only about 7 cm^2, which are too small for multiple measurements with a spectrophotometric colorimeter. After heating, the sample surface was oily and sticky, and the muscle fibers tend to fall apart when applying a slight force. All those characteristics in samples make CVS a better choice for color measurement. With CVS, the color values were obtained from the whole sample area under a controlled and reproducible environment. In addition, sample images could be stored electronically for other analyses, for example, subset colors and area. But to use CVS to obtain consistent and reliable results, it is important to use a standardized illumination for each measurement (Briones and Aguilera 2005), along with a reference color with known values to verify the accuracy of the system.

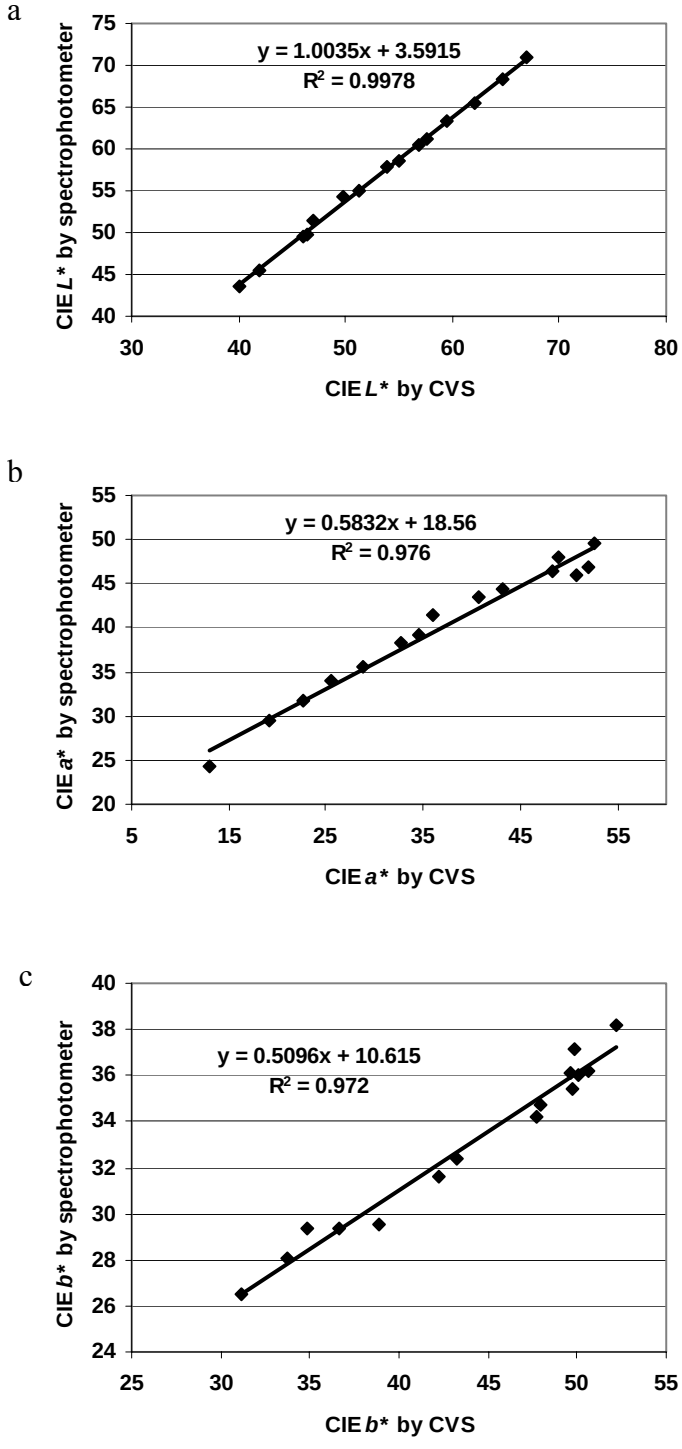

Figure 3-3. Relationships between color values of Roche *SalmoFan*^{TM} color cards measured by the CVS (computer vision system) and a CM-2002 spectrophotometer: (a) CIE L^*, (b) CIE a^*, (c) CIE b^*.

Shear force

Table 3-1. Comparison between the shear force results measured by MTB (multiple thin blade fixture) and blunt blade*

Section	MTB		Blunt blade	
	Mean shear force (N)	CV	Mean shear force (N)	CV
1	39.79	5.67	10.09	20.71
2	36.96	7.47	9.22	7.22
3	37.93	3.90	10.23	4.98
4	46.21	9.10	9.08	10.34
5	45.83	4.93	10.99	23.00
6	62.77	0.71	12.45	14.36
7	75.00	0.05	14.11	36.06
8	82.20	1.12	17.01	18.09
9	103.50	10.74	19.21	5.51

* The samples were outer layer from anterior to posterior (sections 1 to 9).

The shear force value is a mean of four replicatesThe results of a comparative study on the two texture fixtures show a high correlation (R^2=0.932) between MTB and single blade probe methods, indicating that both methods can be used to evaluate shear force of raw and cooked salmon. CV (coefficient of variation) is an important measure of the consistence of the results and reflects the sensitivity of the test method. Table 3-1 compares the CV values for the two methods on the outer layer samples. The single-blade probe method had generally higher CV (4.98 - 36.06%) than MTB (0.05 - 10.74%), indicating the latter is a much more sensitive and consistent method. Compared to standard Kramer shear/compression cell, the denser, thinner blades in the MTB resulted in a higher effective total contact length of the blades on sample over a limited sample area. For small samples, MTB effectively improved the accuracy and reduced the deviation caused by texture heterogeneity. Moreover, the small slit width in the base prevented the cooked fish muscles from falling apart, allowing for measurement of much smaller samples than a standard Kramer cell. The thin blades also reduced compression when cutting

through the samples, leading to results that better correlate with actual shear force measurements (Dunajski 1979).

Quality variation along the longitudinal axis of raw and heated salmon muscle

Color

Significant changes occurred in the CIE L^*, a^* and b^* values when salmon muscle was heated at 121˚C for 20 min. Heating denatures myoglobin and oxidizes carotenoid pigments (Haard 1992), changing the muscle color from red to pale, as reflected by an increase in lightness, and decreases in redness and yellowness. In general, no significant difference was found among samples taken from different locations in either the raw or heated fillet. The average values of L^*, a^* and b^* for the raw samples are 55.19 ± 0.91; 26.27 ± 0.77, and 42.46 ± 1.16, respectively; after 20 min heating, these values became 83.03 ± 1.24, 4.10 ± 0.60, and 24.78 ± 0.58, respectively.

Salmon muscle redness contributes significantly to the overall enjoyment of raw and cooked salmonid flesh (Bjerkeng 2000) and reflects the total carotenoid pigment content (Bjerkeng 2000; Johnston and others 2000). A longitudinal variation in carotenoid content has been reported in literature, with more astaxanthin being deposited in the caudal compared to the anterior part for farm raised Atlantic salmon (Bjerkeng 2000). Although color differences were anticipated from the heat treatments used in this study, little difference in color was expected between different locations of a fish. Pink salmon has a low average carotenoid content. Variations in color that are commonly observed in more highly pigmented Oncorhynhus species such as Chinook (*O. tschawytscha*) or sockeye (*O. nerka*) were not detectable here, and no measurable differences were observed in redness along the length of the fillets. Further, female

salmon muscle loses color during spawning as carotenoid pigment and lipid are mobilized from the flesh and deposited to the eggs.

Shear force

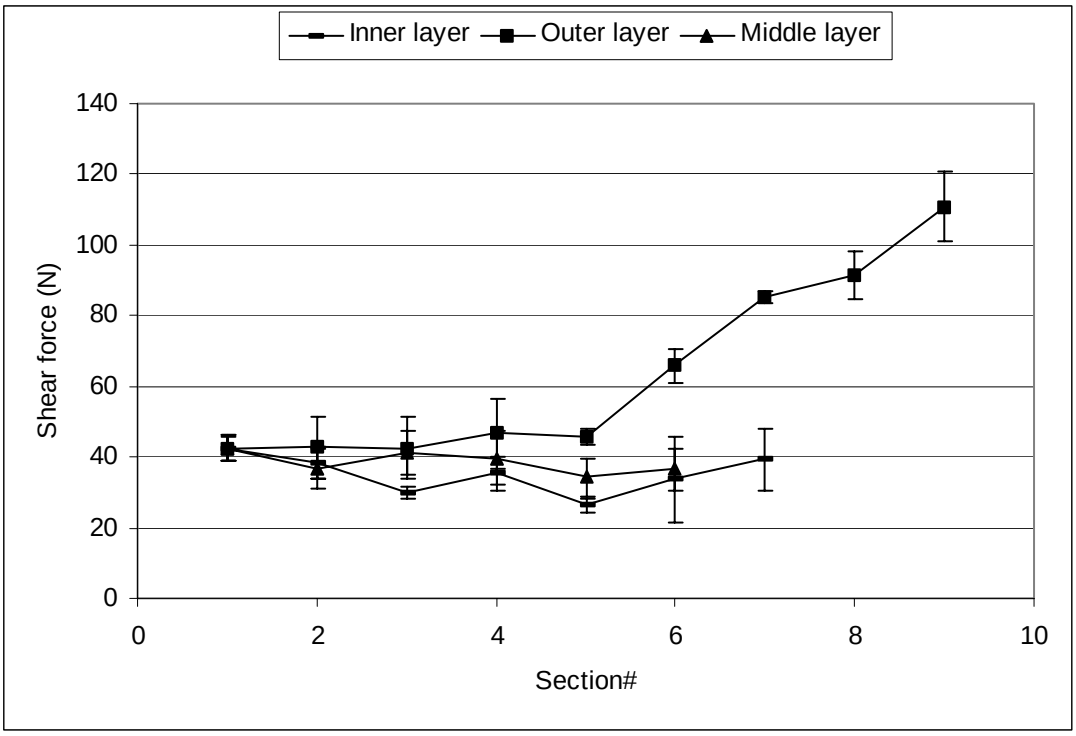

Figure 3-4. Shear force distribution at different locations along raw salmon fillet as shown in Figure 3-2. Bars indicate the standard deviation from 6 determinations.

Figure 3-4 shows the variation of shear force properties with locations in the raw salmon fillet as measured by MTB. The shear force of outer layer samples was significantly ($P < 0.05$) higher than the samples in the inner and middle layer, while the shear force of the samples from inner and middle layers were generally not significantly different from each other. Because the main difference between the outer and inner/middle layer samples is the red muscle being part of the outer layer (Kiessling and others 2006), this result suggests that the red muscle is firmer than white muscle. When shearing outer layer of the samples, it was observed that the red muscle fibers required much higher force to cut than did the white muscle. For the outer layer, the shear

force significantly increased from head (42 N) to the tail (111 N), suggesting that the red muscle in the head area was more tender than in the tail.

The variation of tenderness along the salmon fillet has been related to the varying contents of fat, moisture, and collagen, as well as muscle fiber dimensions and muscle fiber density (Dunajski 1979; Hatae and others 1990; Jonsson and others 2001). The tail area contains the lowest lipid content (Hughes and Robb 2001), and lower lipid content usually leads to a firmer texture (Dunajski 1979). Higher collagen content also contributes to firmer texture. Montero & Borderias (1989) observed higher shear strength values in trout (*Salmo irideus Gibb*) near the tail that has higher proportion of insoluble collagen compared to other parts of the musculature. Muscle cell dimensions and arrangement vary throughout the fillet and this affects texture. Larger number of smaller diameter muscle fibers are present in the tail compared to other part of a same fish (Love 1970). This study confirms previous reports that the tail muscle is firmer than head (Sigurgisladottir and others 1999; Jonsson and others 2001; Casas and others 2006). Our test result suggests that red muscle might play a major role in the firmness of the raw fillet; and its morphological and compositional variation along the fillet is responsible for the difference in shear forces between head and tail. This can be seen clearly in Figure 3-4, from the changes of shear force in different layers of section 6 and 7: the shear force in outer layer increased significantly, while that of inner and middle layer did not show significant difference compared to the anterior sections. The above observations might partly be the result of the smaller diameter of red muscle fibers (25 - 45 μm) compared to white fibers (50 -100 μm) (Kiessling and others 2006). Our results also support the findings of Kanoh and others (1988) that the lower fiber diameter red muscle of yellowfin tuna had a firmer texture than other types of muscle fibers.

Figure 3-5 shows the shear force along salmon fillets after heating at 121.1 ˚C for 20min. The shear force for each section is a mean of samples from different layers. Heating increased the shear force needed to break muscle fibers. The shear force of raw flesh was mostly in the range of 22 - 50 N (Figure 3-4). After heating, the shear force increased to 100 -113 N (Figure 3-5). Heat-induced toughening of fish muscle is a result of heat-denaturation of myofibrillar and sarcoplasmic proteins (Harris and Shorthose 1988; Hatae and others 1990). Protein denaturation leads to a reduced water holding capacity and shrunken muscle fibers, and subsequently a harder and more compact tissue texture. The heated muscle is characterized with higher number of fibers of smaller diameter than raw muscle (Skrede and Storebakken 1986; Hatae and others 1990). In addition, heat-coagulated sarcoplasmic proteins dispersed between muscle fibers might contribute to firmness by impeding the sliding of the muscle fibers over each other (Hatae and others 1990). Our results also indicate that the outer layer in a cooked sample have a similar tenderness as in the middle and inner layers, in contrast to the raw fillet in which the tail and the outer layer are firmer (Figure 3-4). This might have resulted from the liquefaction of collagen and shrinkage of myofibrils. During heating, collagen liquefies readily, with the muscle fibers remaining as the sole element of resistance to shear in cooked fish meat from head to tail (Dunajski 1979). Sigurgisladottir and others (2000) found that freezing- induced protein denaturation in salmon caused the smaller diameter muscle fibers to shrink to a lesser extent than larger diameter fibers. Heat–induced protein denaturation might have a similar effect. That is, the white muscle fibers could shrink to a larger extent than the red muscle fibers. This would result in the difference between the fiber diameter and fiber density of denatured red muscle and that of white muscle fibers diminishing subsequently reducing the differences in the texture between the two types of muscle.

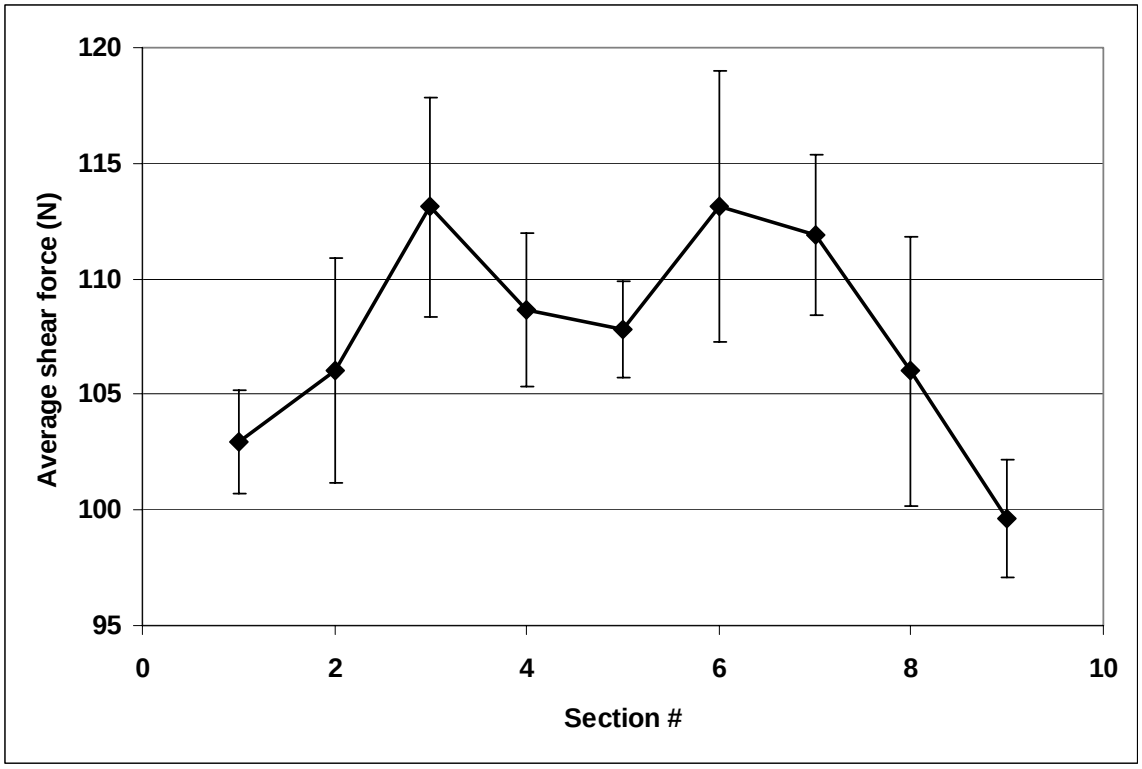

Figure 3-5. Shear force distribution of heated salmon muscle along the length of a pink salmon fillet (121 ˚C, 20 min). The shear force value is a mean of all layers (inner, middle and outer). Bars indicate the standard deviation from 6 to 18 determinations depending upon the muscle thickness.

Cook loss and shrinkage

Figure 3-6 shows variations in cook loss for samples taken over the length of the fillet after heating at 121 ˚C for 20 min. The cook loss ranged from 14% to 22%. The head (section 1, 2) and tail (section 9) had significantly higher cook loss than the middle sections ($P < 0.05$). Our measurements indicate that more than 85% of the cook loss was water, with lipids and solids including collagen or gelatin and muscle fragments and coagulated sarcoplasmic proteins. As a comparison, Ofstad and others (1993) found 84% of liquid was water when salmon muscle was heated at 70 ˚C. Expulsion of water from the myofibrils occurred as they shrank due to denaturation of myosin (Ofstad and others 1993; Palka and Daun 1999). Higher fat content and the higher stability of the myosin/actomyosin fractions are important for better liquid-holding capacity (Ofstad and others 1993).

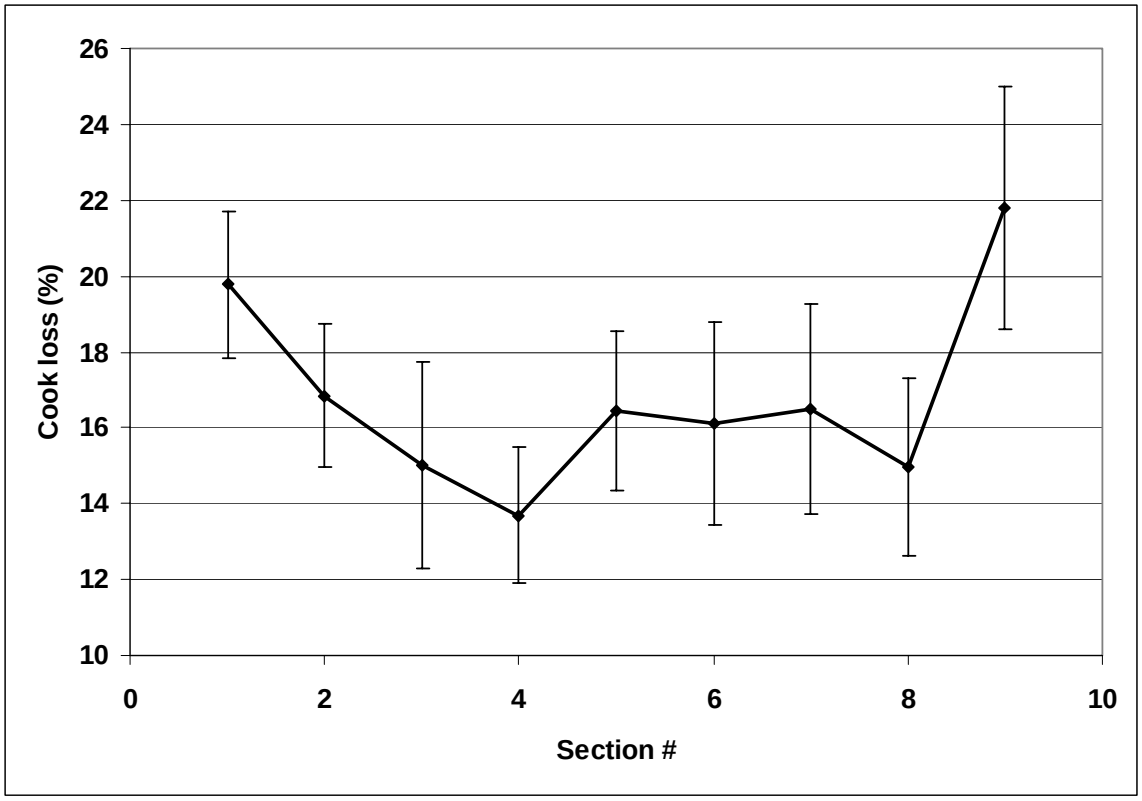

Figure 3-6. Changes of heating-induced cook loss along the length of a pink salmon fillet length (121 ˚C, 20 min). Bars indicate the standard deviation from 6 to 18 determinations depending upon the muscle thickness.

Figure 3-7 shows the moisture distribution along the length of raw salmon fillet. Significantly ($P < 0.05$) higher moisture (> 75.8%, wet basis) exists in the head (section 1, 2) and tail (section 8, 9). The muscle in proximity to the dorsal fin had lower moisture content, which is in agreement with findings of other researchers (Kasai and others 1997; Bell and others 1998). It is well known that the fat and moisture concentrations exhibit an inverse relationship in salmon fillets (Hughes and Robb 2001; Huang and others 2002; Azumaya and others 2003), and a high moisture and lower fat are related to higher cook loss (Ofstad and others 1993).

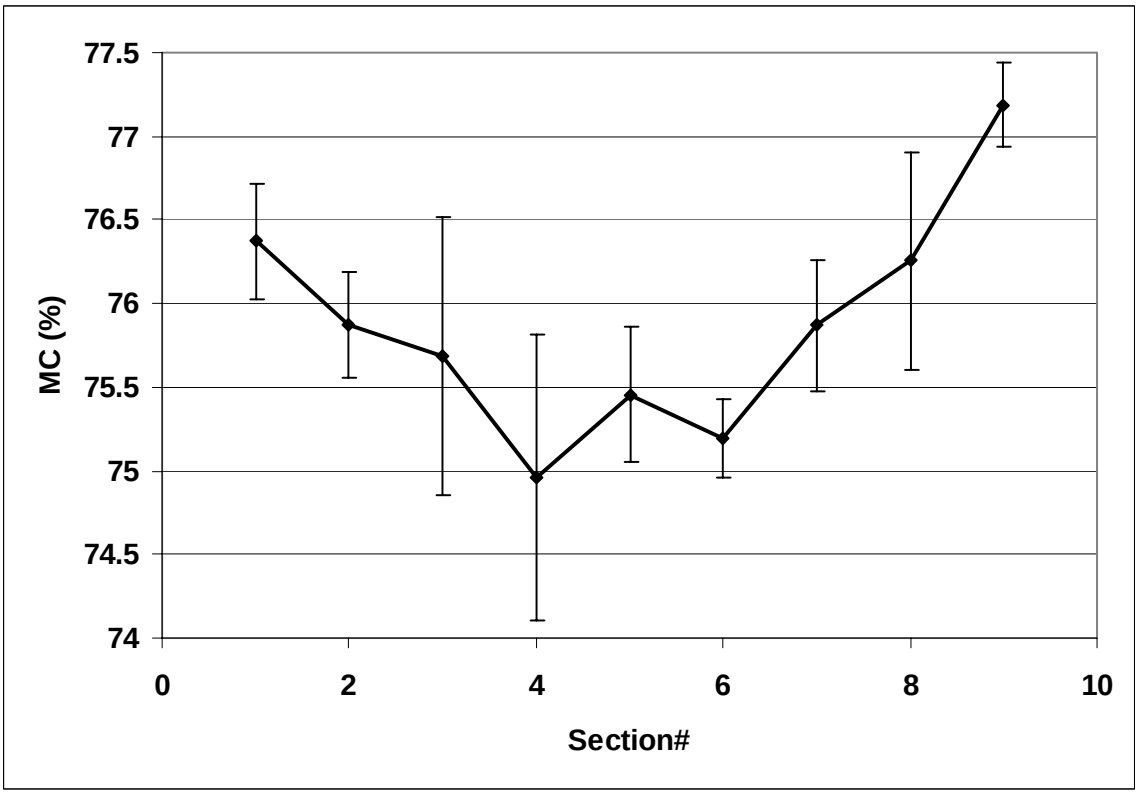

Figure 3-7. Moisture distribution along salmon fillet length. The moisture value is a mean of all layers (inner, middle and outer). Bars indicate the standard deviation from six determinations.

The shrinkage ratio values for samples taken along the length of a fillet after heating at 121.1 ˚C for 20 min are show in Figure 3-8. Overall it ranged from 17% to 25%, with samples from the head showing a significantly higher shrinkage value ($P<0.05$) than the other areas. Cook loss is positively correlated with shrinkage (Palka and Daun 1999; Barbera and Tassone 2006). In addition, the percentage of red muscle increased from head to tail areas, and the front half of the fillet contains mostly white muscle (Love 1970). As stated earlier, the white muscle fibers might shrink to a larger extent than the red muscle fibers. Therefore, the severe area shrinkage in the head region might be also related to greater shrinkage of white muscle.

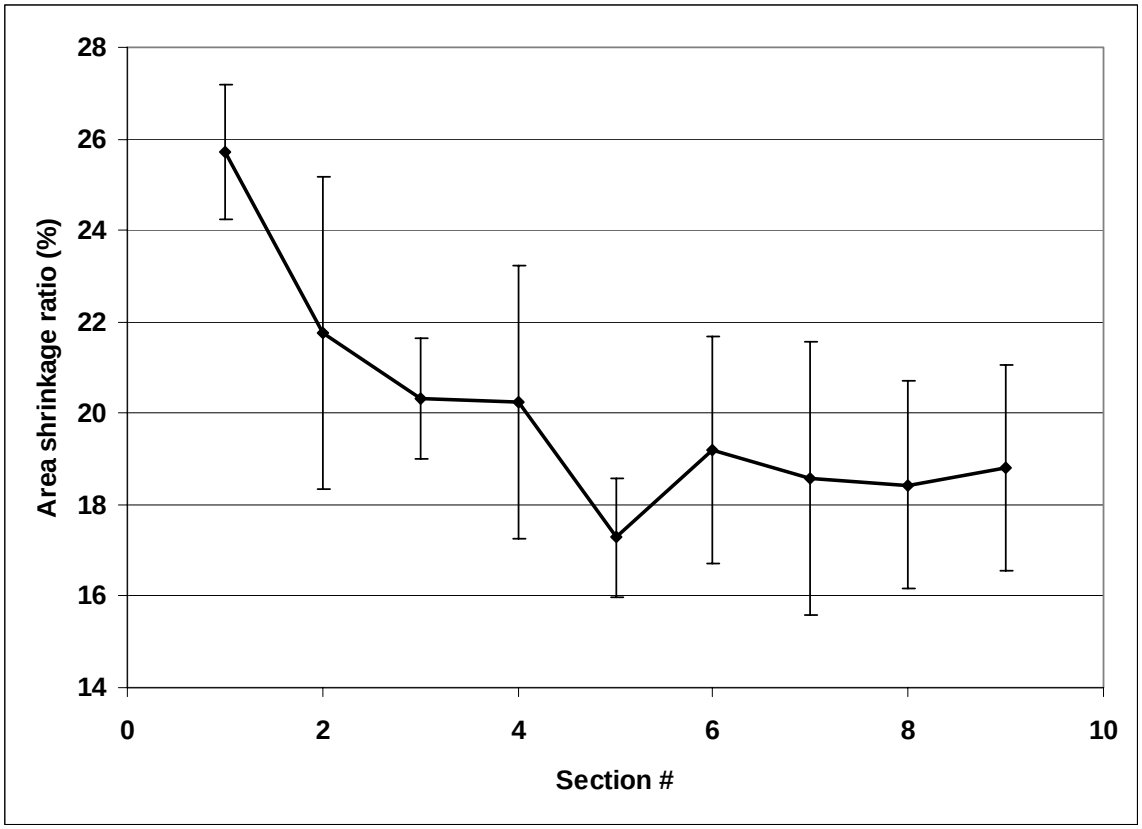

Figure 3-8. Changes of area shrinkage ratio along the length of a pink salmon fillet length after heated at 121 ˚C for 20 min. The ratio value is a mean of all layers (inner, middle and outer). Bars indicate the standard deviation from 6 to 18 determinations depending upon the muscle thickness.

Reproducibility of quality measurements is affected by sampling technique because of the heterogeneity within a salmon fillet. Color, shear force, cook loss and shrinkage changed with the locations from which samples were taken on a salmon fillet, with the sample areas closest to the dorsal fin (inner and middle layers of section 3-6#) having more consistent quality properties. Because results from samples taken in the dorsal region were more reproducible, only the dorsal samples were used for studies on the influence of heating time. These results are summarized in the next section.

Progressive changes of salmon quality properties with heating time at 121.1 ˚C

Color

Figure 3-9 shows the changes of muscle lightness, redness and yellowness with heating time at 121.1 ˚C as measured by CVS. After heating, the color quickly faded from the original pale pink to whitish within 10min: the lightness increased from originally 58 to a maximum 83, yellowness decreased from originally 38 to a minimum 23, and the redness dramatically decreased from originally 17 to 7. The rapid color change resulted from the denaturation of myoglobin and oxidation of carotenoid pigments at 121 ˚C. More than 36% of astaxanthin is destroyed during canning (Haard 1992). As the heating time increased, the color gradually changed to yellow and brown, which was reflected by consistent decrease in lightness and increase in yellowness, and a slight increased redness. The browning is mainly due to Maillard reactions (Haard 1992). Moreover, protein-lipid reactions may contribute to the color change that involves the oxidation of the highly unsaturated fatty acids in the salmon and the reaction of peroxide decomposition products with proteins (Haard 1992). Figure 3-9 shows that browning caused lightness to decrease and yellowness to increase consistently, following zero order kinetics, which is in agreement with previous report (Bhattacharya and others 1994).

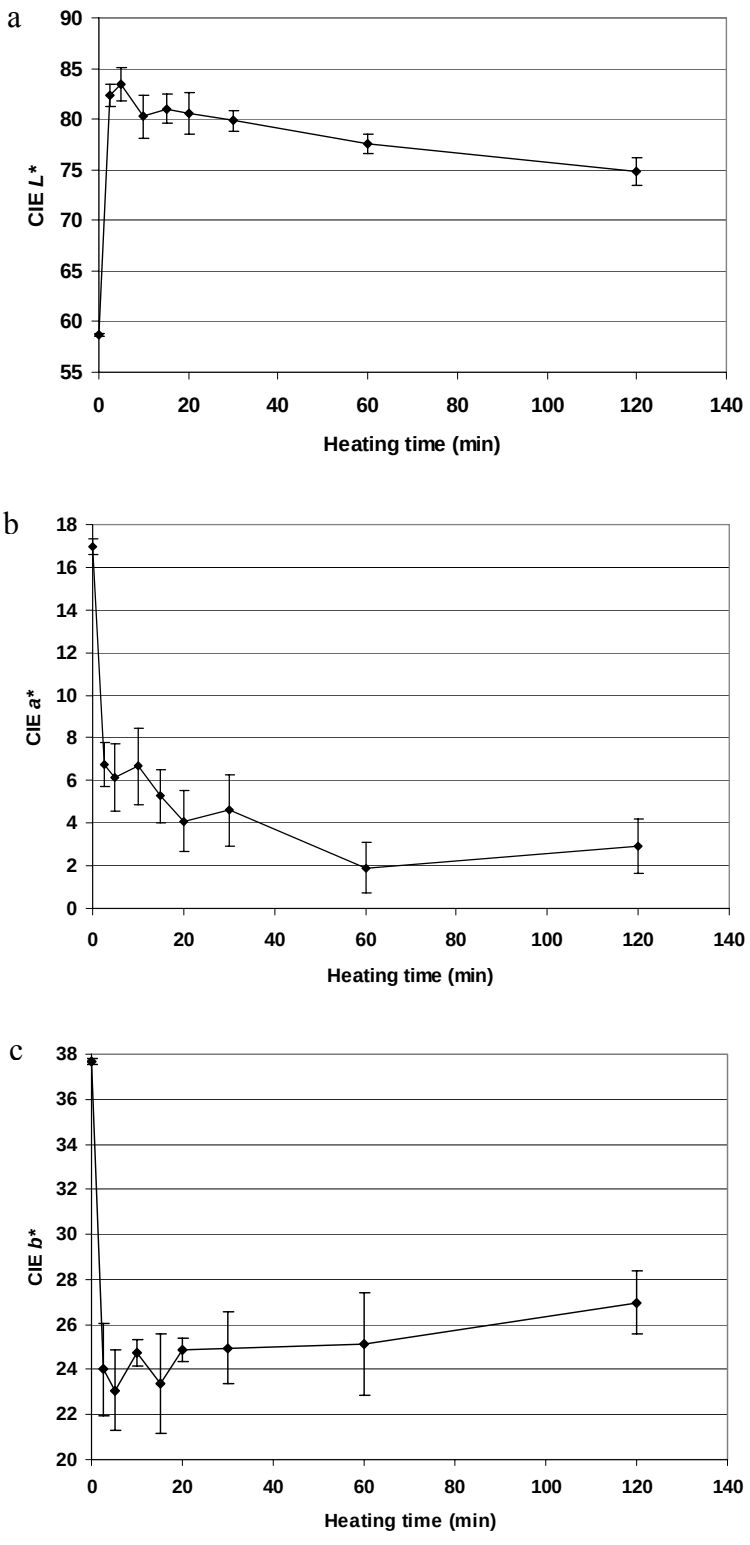

Figure 3-9. Progressive changes of muscle color with time at 121.1°C: (a) CIE *L**, (b) CIE *a**, (c) CIE *b**. Bars indicate the standard deviation from six determinations**.**

Cook loss and area change

Figure 3-10 shows the changes of cook loss and area shrinkage ratio with time at 121.1 ˚C. At the end of 2 hour heating, the area shrinkage ratio and the cook loss increased to 25%, and 20%, respectively. Although both area shrinkage and cook loss consistently increased with heating time, most of the increase occurred within the first 10 min, in which area shrinkage ratio reached 18% and the cook loss reached 14%, both accounting for 70% of the total changes. As a comparison, rabbit meat showed 81% of total cooking losses in 20 min when heated at 80 ˚C (Combes and others 2004). Figure 3-10 shows a strong correlation between cook loss and area shrinkage ratio. The significant shortening in sarcomere length has been positively correlated to cook loss during heating of beef and pork (Palka and Daun 1999; Barbera and Tassone 2006).

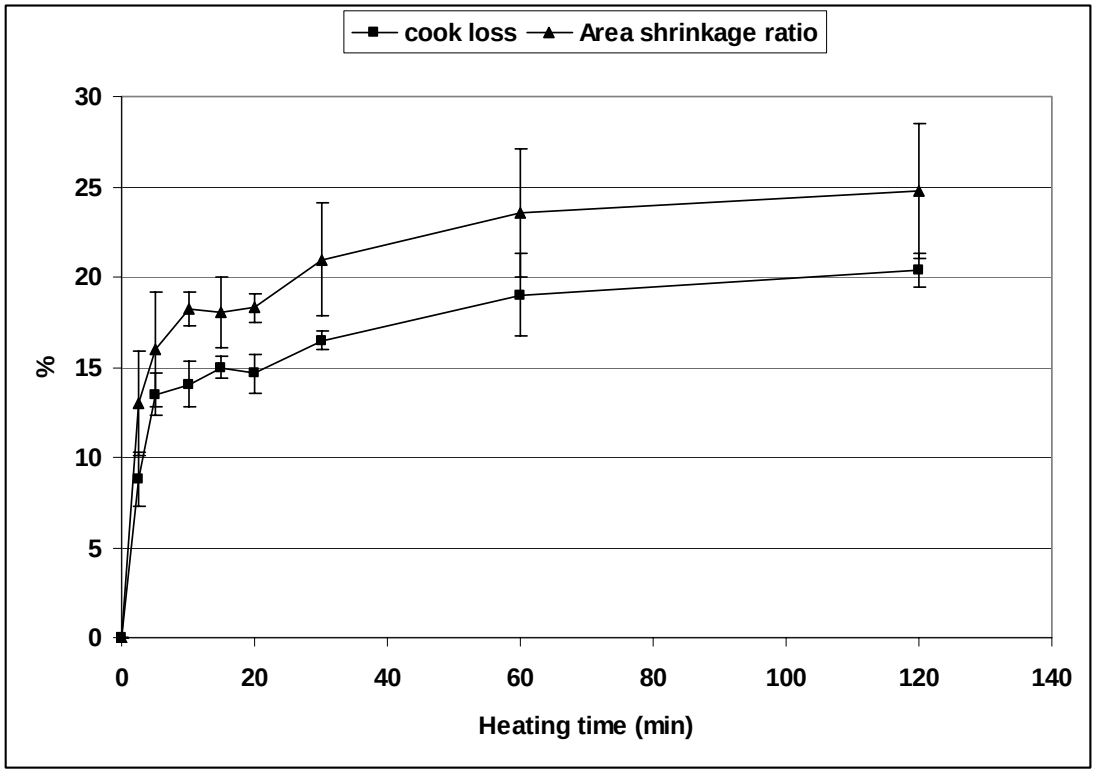

Figure 3-10. Progressive changes of cook loss and area shrinkage ratio of salmon fillet with time at 121.1°C. Bars indicate the standard deviation from six determinations.

Shear Force

Figure 3-11 shows a progressive change of shear force after heating for various time at 121.1 ˚C as measured using the MTB device. Two peaks were observed: the first peak was after about 5 min heating time, when the shear force rapidly increased from 38 N in the raw samples to a maximum of 165 N; after that, the shear force quickly decreased to reach a minimum of 120 N at 20 min, then increased again to a second peak at 130 N about 1 hr heating time. The shear force subsequently decreased consistently and reached 100 N after 2 hr heating.

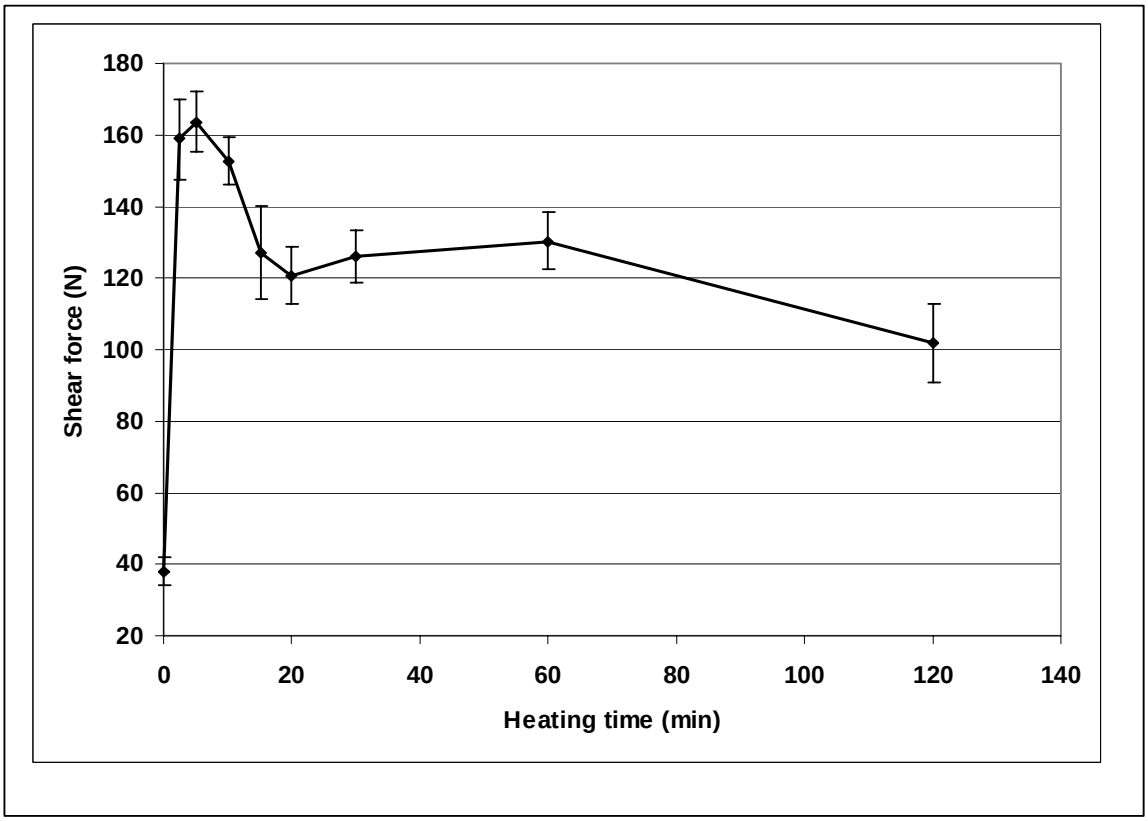

Figure 3-11. Progressive change of shear force with time at 121.1 ˚C for salmon muscle. Bars indicate the standard deviation from six determinations.

Some research has been conducted on the texture changes in fish muscle that occur during cooking (Kanoh and others 1988; Bhattacharya and others 1993); however, to our knowledge, no studies have followed a time course during heat sterilization of fish tissue or have reported a two-peak shear force pattern such as that observed in our study. This may be in part

because the previous research has focused on lower temperature heating. For example, Bhattacharya and others (1993) studied the textural changes of Pacific chum salmon subjected to hydrothermal treatment (60 -100 ˚C) for 40 min, and they found that at higher temperatures (80 - 100 ˚C) the hardness reached a peak within 10 min. In addition, the MTB shear force measurement method used in this study might be more sensitive and reveal minor textural changes. Notably, two peaks have been observed in texture changes involving the heating of beef (Harris and Shorthose 1988) and rabbit muscle (Combes and others 2004) during a heating process. For example, when rabbit meat was slowly heated from 50 to 90 ˚C, the shear force as measured by Warner–Bratzler (WB) probe significantly increased at 50 ˚C, then dramatically decreased to a minimum at 60 – 65 ˚C, and increased again reaching a maximum shear value at 80 – 90 ˚C (Combes and others 2004). This observation has been explained by texture changes due to collagen solubilization and denaturation of myofibrillar proteins (actin/myosin hardening) (Harris and Shorthose 1988). Specifically, the initial texture toughening is thought to be caused by denatuaration and thermal shrinkage of connective tissue with collagen solubilization leading to decreases between 50 to 65 ˚C. The second peak is probably due to hardening of the myofibrillar structure (Harris and Shorthose 1988).

The special features of fish muscle structure and the high temperature used in sterilization differentiate our study from previous ones, and thus the main mechanisms for textural changes might be different. Fish muscle contains very low amounts of connective tissue (2 - 3%) compared to that of terrestrial mammals (10 - 15%). Also, the collagen is much less heat stable than mammalian collagen (Dunajski 1979), so the influence of connective tissue on changes in texture following heating is likely to be smaller. The denaturation and melting temperatures of collagen are near 20 and 40 ˚C, respectively, and the denaturation of actin and

the other sarcoplasmic proteins occurs at 60 – 80 ˚C (Ofstad and others 1993). Therefore, the rapid heating to 121.1 ˚C in our study might denature the proteins rapidly, and the resultant thermal shrinkage of proteins, solubization and gelation of collagen, and dehydration might occur almost simultaneously. The two-peak feature in the shear force profile might have resulted from the following: in the first 5 min, the hardening effect caused by protein denaturation and dehydration was stronger than the tenderization caused by collagen solubilization, the net effect being an increase in toughening. At 5 min, most of the proteins had been denatured, thus collagen solubilization and gelation would become dominant with the net effect being tenderization. After 20 min heating, collagen liquefaction would have been almost completed; the continual dehydration and shrinkage of actin/myosin, protein aggregation in which covalent bonding might be involved, and the increased formation of heat-coagulated sarcoplasmic proteinaeous aggregates between myofibrils might be the main factors leading to an increase in toughness (Dunajski 1979; Hatae and others 1990; Gill and others 1992). At 60 min, the sarcoplasmic protein aggregates mixed with gelatin were able to hold water and/or plug the intracellular capillaries, thus reducing the amount of liquid being released (Ofstad and others 1993); the cook loss and the area shrinkage ratio thereafter did not significantly increase (Figure 3-10). After one hour of heating, the tissue gradually became softer due to heat induced muscle decomposition and fragmentation. However, more detail study on the changes of collagen content and microstructure, including sarcomere length, fiber diameter and density, would be helpful to fully understand the mechanisms involved.

Conclusions

The new multiple thin blade (MTB) texture device provided reliable measurements for salmon muscle tenderness. The computer vision system (CVS) served as a suitable mean for measuring the color and area changes of salmon muscle following heat treatments. Unlike shear force, color did not differ significantly along the length of raw salmon fillet. The content of red muscle in tissue samples influenced the force needed to shear raw salmon, but this effect diminished with heating. The tissue near the dorsal fin had less cook losses and shrinkage during heating, and yielded more reproducible results than tissue samples taken from the head and tail muscles, indicating that differences in morphology and chemical composition will have a significant affect on how heating affects. When salmon muscle was heated at 121.1 ˚C for 2 hours, the area shrinkage ratio and the cook loss increased to reach 25% and 20%, respectively. Most changes occurred within the first 10 min heating: the muscle color changed to the whitest, the shear force increased to the maximum, and 70% of total cook loss and shrinkage took place. The shear force profile had two-peaks, with the first peak in the 5 min and second peak at 1 hour heating time, indicating that longer heating does not necessarily decrease muscle toughness.

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CHAPTER 4. EFFECT OF SALT ADDITION ON THE QUALITY CHANGES OF SALMON FILLET (*O. GORBUSCHA*) DURING THERMAL PROCESSING

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Abstract

The effect of salt addition during commercial sterilization of pink salmon was evaluated. Sample cuts from salmon fillets were placed in sealed aluminum containers, and heated at 121.1˚C for 10, 30, and 60 minutes. Samples with 1.5% (w/w) salt addition were compared with those without added salt. Processing come-up-time and heating uniformity were predicted. Salt addition reduced cook loss, area shrinkage, shear force of heated fillet and resulted in a slightly darker color. Differences in thiamin loss, degree of lipid oxidation, and fatty acid profile were not significant. Peroxide (PV) and thiobarbituric acid (TBA) values showed a slight increase within the first 10 min of heating, followed by a significant decrease as heating progressed. No measurable loss of polyunsaturated fatty acids (PUFA) was observed. Canned salmon investigated were a valuable source of omega-3 PUFA with EPA values ranging from 52 to 71 mg/100 g product and DHA ranging from 258 to 340 mg/100 g of product.

KEYWORDS: Pink salmon; food thermal process; salt addition; seafood quality; fatty acids; thiamin; cook loss; area shrinkage

Introduction

A substantial portion of pink salmon (*Oncorhynchus gorbuscha*) is thermally processed in cans or flexible pouches. Sodium chloride is commonly used as an additive in the canned products although some salmon products are bland, with no added salt. The Commercial Item Description (CID) classifies canned and flexible pouched salmon products into four categories based upon salt level: regular (no more than 1.5 percent salt), no salt added, very low sodium (35 milligrams or less sodium per serving), and low sodium (140 milligrams or less sodium per serving) (*1*). Salt functions as a flavor enhancer and increases the perception of fullness and thickness, and enhances the perception of sweetness (*2*). From a sensory viewpoint, 1.5 - 2.0 percent of salt in canned muscle foods is the optimum level for consumer acceptability (*2*). The effect of salt addition affects cook yield, texture, and lipid oxidation (*3-5*). There is, however, a lack of information on the influence of salt addition on the quality of canned salmon, particularly when newer production methods such as dielectric based commercial sterilization processes are applied.

The most important changes that occur to the physical quality attributes of muscle foods during commercial sterilization processes include color, texture, and cook loss. Area shrinkage ratio is also an important quality indicator of muscle foods for it quantifies the amount of muscle shrinkage caused by heat-induced protein denaturation (*6*). Heating may also cause losses of nutrients such as thiamin, which is one of the least thermostable of the water soluble vitamins.

Canned salmon is an important source of thiamin, and its quantification provides a good indication of the nutritional integrity of the product.

The high content of the polyunsaturated fatty acids (PUFA) in marine lipids is thought to play a positive role in reducing the risk of cardiovascular diseases (*7*). However, high temperature processing can damage PUFA generating secondary lipid oxidation products that lead to rancidity and other off flavors in foods (*8*). The extent of lipid oxidation in muscle foods is widely evaluated by measuring the primary oxidation products using peroxide value (PV) method and secondary products using TBA method that determines the content of 2-thiobarbituric acid reactive substances, primarily malondialdehyde from the oxidation of ω-3 fatty acids. Studies on the changes of lipids during thermal treatment of fish have been reported (*8-10*), but reported data on changes of pink salmon lipids during modern canning process is limited.

The objective of this study was to investigate the changes in the quality of pink salmon during commercial sterilization at 121.1 ˚C at two salt levels as part of a larger study to optimize processing parameters for new developed dielectric heating protocols for salmon products.

Materials and Methods

Materials

Wild fresh whole grade A pink salmon (*Oncorhynchus gorbuscha*) were obtained from a seafood processing plant on Kodiak Island (Alaska) in August 2005, and immediately transported on ice to the Fishery Industrial Technology Center (FITC) pilot plant. Salmon used in this study were post-rigor and less than 48 hours post-mortem. Seine caught fish, sampled the same day and from the same harvest grounds (Kodiak Island, Alaska), were transported to the processor in a fishing vessel that used a conventional fish holding tank fitted with a standard recirculation chilled seawater system. To avoid heterogeneity of fish quality due to gender, only female fish were selected for this study. Fish (n=12) were 1350 ± 100 g in weight, 370 ± 10 mm in length, and 120 ± 10 mm in width. Fish were gutted, frozen and stored (-31 ˚C) at the FITC pilot plant and shipped overnight to Washington State University, Pullman, WA.

Sampling and heating

The sampling and heating of fish fillets followed methods previously described (*6*). To reduce the influence of heterogeneity of fish muscle on the cooking properties, only the white muscle in the dorsal area were sampled. Small disk shaped samples (6 mm in thickness and 30 mm in diameter) were used to reduce come-up-time and improve uniform heating. The samples were taken using an electrical food slicer (Model 632, Chef'Choice Int., CITY, Germany) and a 30 mm diameter corer. The samples were loaded into a custom-designed cylindrical aluminum test cells having a 35 mm inner diameter, 6 mm inner height, and 2 mm wall thickness and hermetically sealed. Prior to sealing, the tip of a 0.1 mm diameter copper-constantan

thermocouple (Type-T) was placed in the geometrical center of the sample for temperature monitoring and recording. Samples were subjected to heat in an oil bath (Model HAAKE W13, Thermo Electron Corp., Germany) operated at 121.1 ˚C using glycerol as the heating medium. During heating the signals from the thermocouple junctions were transferred to a computer equipped with a DLZe type data logger (DELTA-T Devices, Cambridge, England, UK). Time for the sample center temperature to reach within 1 ˚C of the total temperature rise was 2.5 - 3 min. After heating and upon removal from the oil bath, sample cells were immediately placed in an ice bath. Temperature dropped to below 20 ˚C within half a minute for all samples, and the thermal effect of cooling step on the product quality was not considered to be significant. After cooling, samples were dried with a filter paper, weighed in an analytical balance (Ohaus Analytical Plus, Pine Brook, New Jersey), and stored overnight in a cooler (4 ˚C) for further analysis.

Experimental design

Samples taken from twelve fish were pooled and equally divided into two groups, and salt was added to one of the sample groups. Salt addition was performed using food grade sodium chloride (1.5% w/w) that was evenly distributed on the sample surface immediately before sealing each test cell. This simulated industrial canning practices for salmon (*11*). Samples were heated at 121.1 ˚C for 10 min, 30 min or 60min. Twelve sample replicates were used for each experimental treatment.

After heating, the physical properties (color, shear force, cook loss and area shrinkage) were determined on each sample. Chemical analyses (thiamin, TBARS, PV and fatty acid

analysis) were conducted in pooled homogenized samples that were subjected to identical heating conditions.

Physical analysis

A multiple thin bladed Kramer type texture fixture (MTB) developed by Kong et al. (*6*) specifically for fish muscle was used to determine shear force of the raw and heated samples. Compared with standard Kramer shear cell, the MTB used much thinner blades (0.5 mm compared to 3 mm for the standard Kramer shear cell) reducing compression when cutting through the samples, thus leading to results that better correlated with actual shear force measurements (*6*). The cell was fitted to a Texture Analyser TA-XT2 (Stable Micro Systems Ltd., Surrey, England, UK) equipped with a load cell of 5 Kg. Raw and heated samples were allowed to equilibrate to room temperature before conducting shear force measurements. Samples were placed on a support base with blades being positioned perpendicularly to the muscle fibers and the traveling speed of the blades was 1 mm/s. The force-time graphs were recorded using the Texture Expert for Windows v.1.15 (Stable Micro Systems Ltd., Godalmine, UK). The shear force was measured as the peak height in the force-time profile.

A computer vision system (CVS) described by Kong et al. (*6*) was used to capture color images of fresh and cooked samples. Color images were downloaded and the color parameters (CIE L^*, a^* and b^*) determined using the Photoshop CS2 v.8.0 (Adobe, San Jose, California). Sample area was determined using the Vision IMAQ Builder v.6.1 (National Instruments, Austin, Texas). The area shrinkage ratio was calculated as the percent area reduction of cooked compared the corresponding raw sample. Cook loss was calculated as the percent weight reduction of the cooked compared to the raw sample.

Thiamin determination

Thiamin content in the raw and heated samples was determined using the Thiochrome method (AOAC 942.23, 2000): ca. 3 - 5 g samples were taken in duplicate and subjected to acid extraction, enzymatic hydrolysis, column purification and subsequent oxidation. The fluorescent intensity of thiochrome-isobutanol extract was determined in a fluorometer (Model FluoroMax-3, Jobin Yvon Inc., Edison, New Jersey) at an excitation wavelength of 365 nm and an emission wavelength of 435 nm. Thiamin content was determined by comparing fluorescence intensity of the extract of the oxidized test solution with that from the oxidized standard solution of thiamin hydrochloride (0.2 μg/ml).

Lipid extraction

Lipid content (percent wet basis) was determined using the Folch method (*12*) as described by Iverson et al. (*13*): ca. 12.00 g ground sample were mixed with 20 ml methanol and 40 ml $CHCl_3$, and incubated at room temperature for half an hour. The extract was vacuum filtered, and the residue was extracted one more time. The two extracts were combined, and added with 28 ml 0.88% (w/v) NaCl solution. The mixture was shaken for 30 sec, transferred to a separatory funnel and let stand for 1 hour. The lower phase of the mixture was filtered through Na_2SO_4 anhydrous to remove residual water and collected in a round flask. Solvent was removed from the lipid extract at 35 ˚C using a rotary evaporator (Rotavapor R-114, Büchi, Basel, Switzerland).

TBARS and PV analysis

TBARS assay was performed as described by Lemon (*14*). Ground sample (ca. 8.00 g) was homogenized with 1:2 (w/v) extracting solution containing 7.5% trichloroacetic acid, 0.1% propyl gallate and 0.1% EDTA. The extracts were separated using a small laboratory centrifuge at 2000 rpm for 20 min. 5 ml extract were mixed with 5 ml 0.02M TBA reagent. The mixture was heated in a boiling water bath for 40 min to develop pink color and cooled with running tap water. The absorbance of the supernatant was measured at 532 nm using a diode array spectrophotometer (Hewlett Packard 8452A, USA). A standard curve was prepared using 1,1,3,3-tetramethoxypropane. Results were represented as mg malondialdehyde (MDA) equivalents/Kg of muscle tissue.

Peroxide value (PV) of the oil extracted from raw and heated muscle tissue were conducted using AOAC method (965.33, 2000) as modified by Regulska-Ilow and Ilow (*15*): ca. 0.2 g extracted oil were dissolved in 15 ml chloroform in a conical flask, followed by addition of 15 mL ice-cold acetic acid and 1 mL saturated potassium iodine solution. The mixture was shaken for 1 min, and left in dark for 5 min. After that, 40 mL distilled water was added, along with 3 drops of 1% (w/v) starch solution as indicator. The released iodine was immediately titrated with 0.001 M sodium thiosulphate solution, with the sample being constantly stirred. Titration end point was indicated by the disappearance of the blue color.

Fatty acid profile

Fatty acid methyl esters were prepared according to the procedure of Maxwell and Marmer (*16*) using C23:0 as an internal standard and quantified as described by Bechtel and

Oliveira (*17*). Briefly, a GC model 6850 (Agilent Technologies, Wilmington, DE) fitted with a DB-23 (60 m x 0.25 mm id., 0.25 μm film) capillary column (Agilent Technologies) was used for this analysis. Hydrogen was used as carrier gas at a constant flow of 1.0 ml/min. Detector and injector were held at a constant temperature of 275 ˚C, and the split ratio was 25:1. The oven programming was: 140 ˚C to 200 ˚C at a rate of 2 ˚C/min, 200 ˚C to 220 ˚C at a rate of 0.5 ˚C/min and 220 ˚C to 240 ˚C at a rate of 10 ˚C/min for a total run time of about 62 min with autosampler injections of x μl. Data was collected and analyzed using the GC ChemStation program (Rev.A.08.03 [847]; Agilent Technologies 1990-2000, Wilmington, Delaware). All standards used in the identification of peaks were purchased from Supelco® (Bellefonte, PA). The standards used were Supelco 189-19, Bacterial Acid Methyl Esters Mix, Marine Oil #1, and Marine Oil #3. Individual fatty acids (FA) were reported in percent of the total fatty acids, and in mg FA/100 g of tissue.

Statistical analysis

The data for quality attributes of raw and heated samples were compared using analysis of variance (ANOVA) in the General Linear Models (GLM) procedure of the SAS System for Windows v.8.01 (SAS Institute Inc., Cary, North Carolina) (*18*). Differences between group means were analyzed by Duncan's multiple-range test. Statistical significance was set at a 0.05 probability level.

Results and Discussion

Cook loss

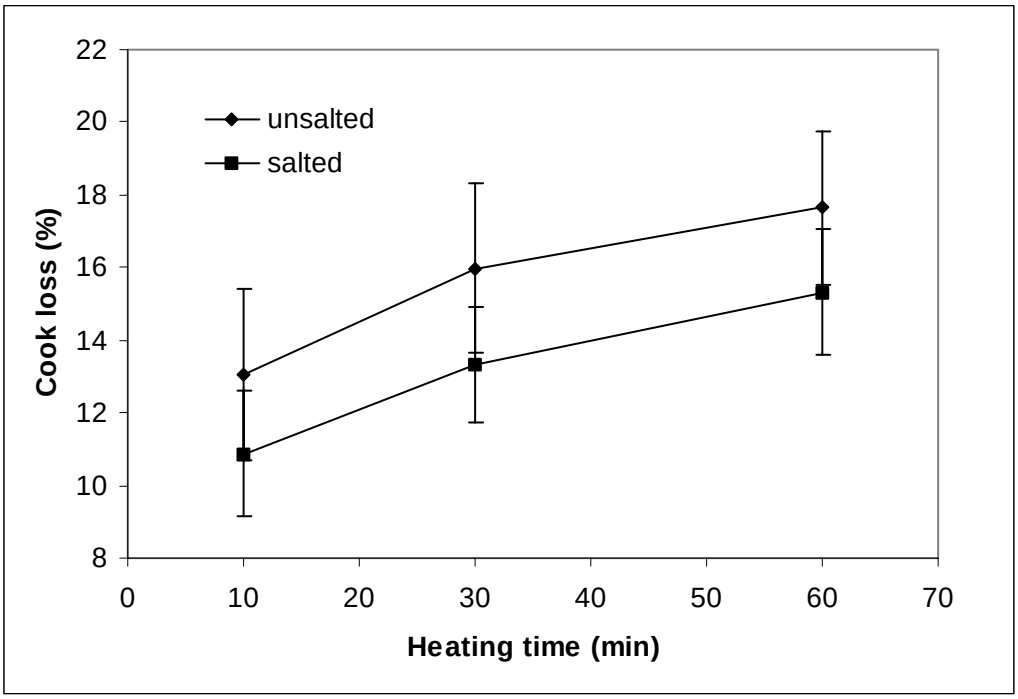

Figure 4-1. Comparison of the cook loss of pink salmon muscle at 0% and 1.5% added salt during heating at 121.1 ˚C (n=12). Error bars represent standard deviations.

The cook loss consistently increased as heating time increased from 10 to 60 min at 121.1 ˚C, and was significantly lower for samples with added salt than for the ones without (**Figure 4-1**). In general, the 1.5% salt addition resulted in approximately 2.4% less cook loss for the heated salmon meat: a 15.4% reduction compared to the control samples (no salt added). A reduction in cook loss from adding salt to samples has been reported for other food products such as meat emulsions (meatballs) (*19*), smoked rainbow trout fillets (*5*) and salted cod (*20*). A function of NaCl in meat products is to solublize myofibrillar proteins, which can result in an increased number of protein-protein and protein-water interactions. As salted meat products are heated aggregation occurs, through a gelation process, contributing to meat particle binding, fat

emulsification, and water-holding capacity (WHC) of the muscle tissues, thus reducing cook losses (*5*, *21*). It is important to determine the optimal level of salt addition as inadequately high levels of salt lead to an increase in cook loss. In a recent study of Thorarinsdottir et al. (*20*), dehydration occurred in cod treated at salt concentrations higher then 5% and was attributed to the phenomenon of salting-out of proteins and salt induced protein denaturation in muscle foods (*3*, *20*, *22*).

Baublits et al. (*4*) found a linear relationship for cook loss of solution enhanced whole-muscle beef decreased as the level of NaCl increased from 0 to 1.5%. In the present study, a linear model was developed to relate cook loss, salt content, and heating time at 121.1 ˚C for pink salmon:

$$y = -1.586 \cdot c + 0.08788 \cdot t + 12.63 \quad R^2 = 0.962 \qquad (1)$$

where y is cook loss (%), c is salt content (0 -1.5%), t is cook time (10 - 60 min).

Area shrinkage as affected by heating

During heating, the sample area shrunk consistently, with samples containing added salt showing less shrinkage than the ones with no added salt at all heating times (**Figure 4-2**). The average area shrinkage ratio of the added salt samples was 3.5% less than the control, equal to a 24.6% reduction. Hsu et al. (*19*) reported similar finding for meat emulsions (meatballs) which had significantly higher product diameters when 1 - 3% salt was added. Wierbicki et al. (*23*) investigated effect of adding salt on water retention in lean pork meat, and found that addition of 1 - 3% salt reduced the meat shrinkage from 14 - 34%.

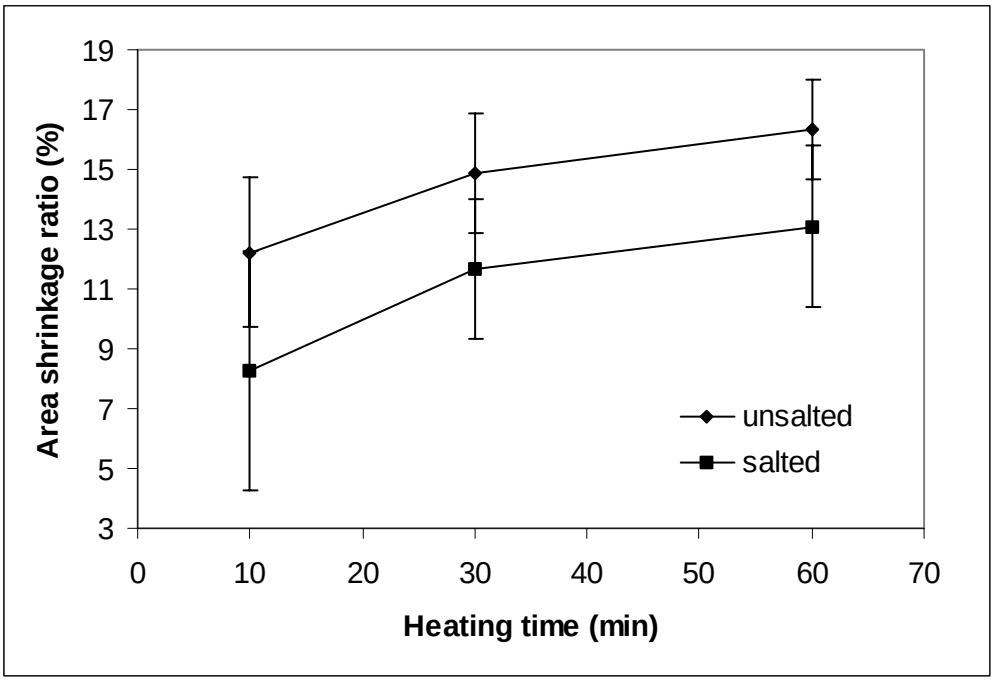

Figure 4-2. Comparison between area shrinkage ratio of pink salmon muscle at 0% and 1.5% added salt during heating at 121.1 ˚C (*n*=12). Error bars represent standard deviations.

Area shrinkage is a result of protein denaturation and liquid loss. Protein denaturation causes shrinkage of muscle fiber diameter and sarcomere length, this result in water, soluble proteins, and fats being expelled from the tissue (*25*). Salt changes the ionic strength of the fluids within the muscle tissues and this can lead to an increase in the denaturation temperature of proteins (*3*, *4*). The lower degree of protein denaturation and greater moisture retention in the tissues with salt addition favored a reduction in muscle shrinkage. The opposite effect caused by high salt level has also been reported (*23*). Wierbicki et al. (*23*) recorded a continuous increase of the meat shrinkage with increase of the salt addition from 5 - 10%. High salt concentrations enhance muscle shrinkage due to the formation of stronger protein – protein bonds and subsequent protein denaturation and dehydration (*24*).

Similar to cook loss, a linear equation relating area shrinkage ratio, salt content and heat time was developed:

$$y = -2.313 \cdot c + 0.08633 \cdot t + 11.60 \quad R^2 = 0.944 \qquad (2)$$

where y is area shrinkage ratio (%), c is salt content (0 -1.5%), t is cook time (10 -60 min).

Color

Figure 4-3 compares the color change with heating time between the samples with and without salt addition. As heating time increased from 10 to 60 min, the sample color became more brown, as reflected by a decreased CIE L^* and increased b^*, resulting from Maillard reactions between sugars, fish proteins or amines as well as protein-lipid reactions (*6*). For all sampling times, the samples with added salt were darker with lower CIE L^* and less yellow b^* values than the samples without added salt (**Figure 4-3**). No obvious trends were observed for CIE a^* between the two group of samples. Similar color effect has been reported on other heated muscle food products, such as frankfurters, emulsified meatballs and whole-muscle beef (*4*, *19*, *26*). Baublits et al. (*4*) reported the decreases in CIE L^* and b^* values of salt solution injected beef muscle as the salt level increased, and suggested that the darker surface color of salted samples may have been caused by greater water retention.

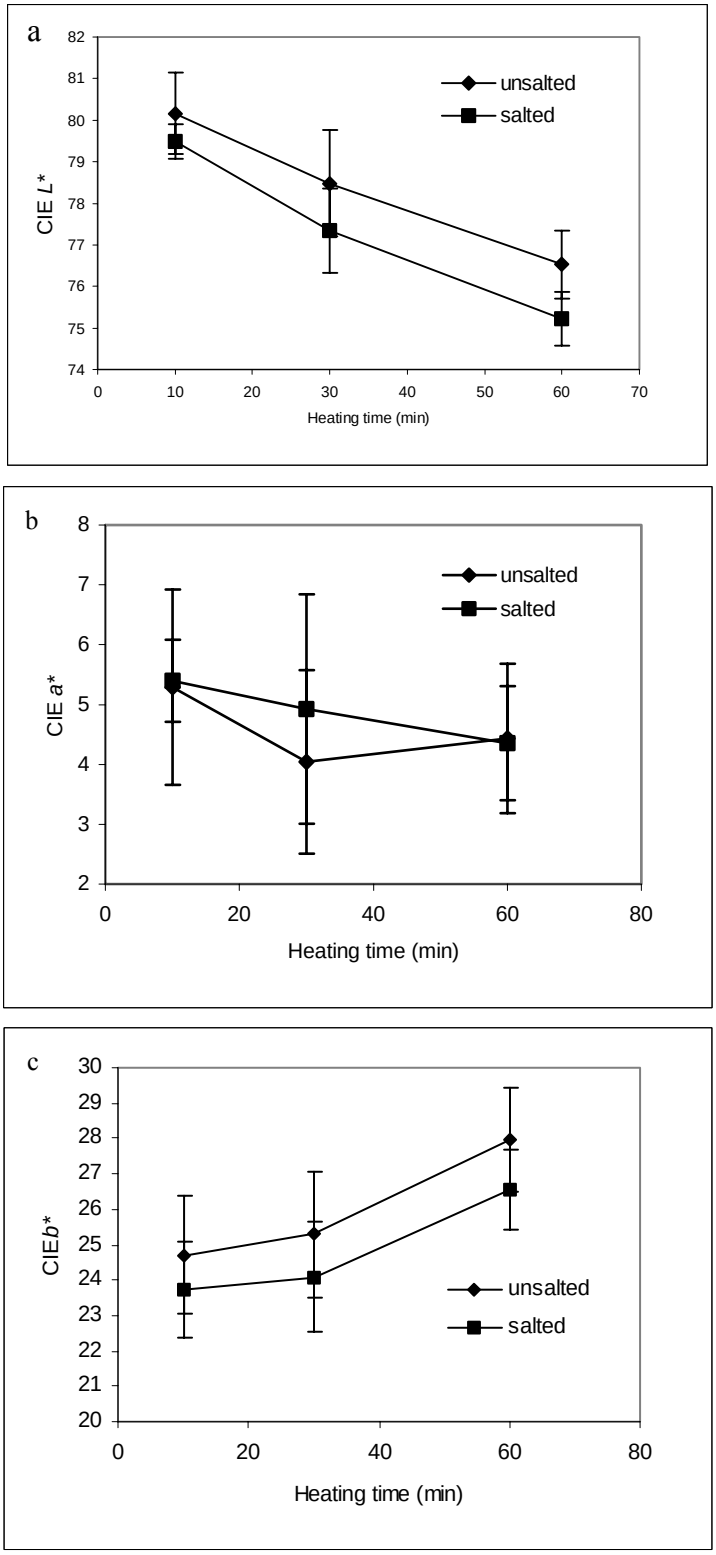

Figure 4-3. Comparison of the CIE color values (n=12) of pink salmon muscle at 0% and 1.5% added salt during heating at 121.1 ˚C: a) CIE L^*, b) CIE a^*, c) CIE b^*. Error bars represent standard deviations.

Shear force

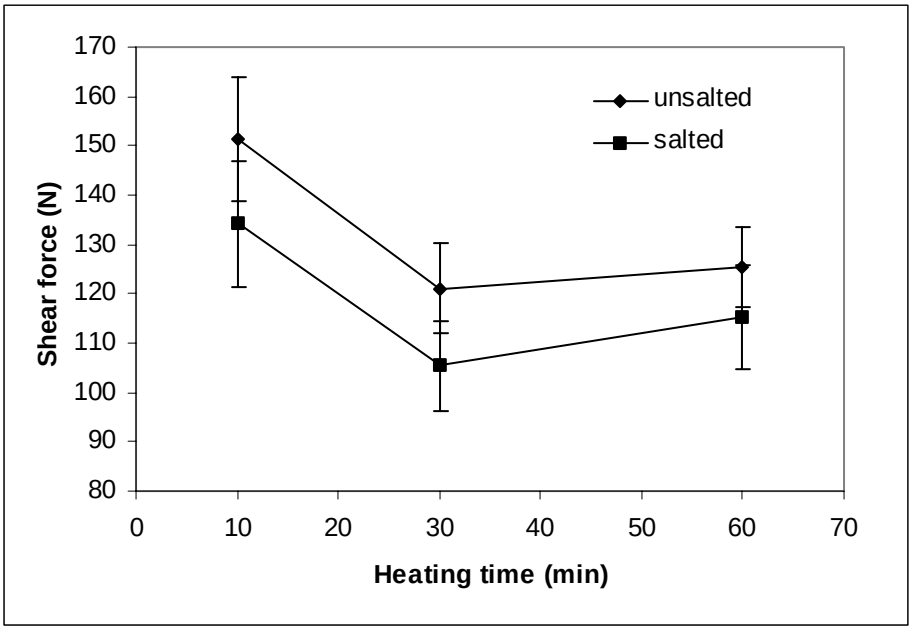

Figure 4-4. Comparison of the shear force of pink salmon muscle at 0% and 1.5% added salt during heating at 121.1 ˚C (n=12). Error bars represent standard deviations.

Figure 4-4 shows the changes of shear force with heating time for samples with and without salt addition. For both groups the shear force decreased from 10 to 30 min and then increased from 30 min to 60 min. This confirmed our previous finding that the change of shear force in canned pink salmon included two peaks, the first approximately at 5 min and the second at 60 min (*6*). Shear force was significantly lower in the samples with added salt (**Figure 4-4**). Salt increases protein solubilization and water retention, forming a protein–water matrix in the muscle that can reduce cook loss (*4*), and increase lipid retention. A reduction in shear force was reported for pork chop and whole-muscle beef when salt was added (*4*, *27*). Baublits et al. (*4*) reported a linear relationship for shear force in salted beef decreasing as the level of NaCl in the solution increased from 0% to 1.5%. In our study, the salt addition (1.5%) resulted in a 10 - 18 N decrease in the shear force for heated samples, corresponding to an average reduction of

10.8%. These results confirmed those of others that fish higher in lipid or moisture content were softer in texture (*5*, *28*).

Thiamin

Table 4-1. Summary of lipid content, thiamin content, PV and TBA values (n= 2) in pink salmon muscle at 0% and 1.5% added salt during heating at 121.1 ˚C (values are means ± standard deviation (SD)) [a]

Salt (%)	Heating time (min)	Thiamin (μg/g tissue)	Oil content ($\times 10^3$)	TBA (mg MDA/Kg tissue)	PV (meq/Kg fat)
Raw		2.03±0.07 [a]	1.24±0.01 [b]	4.59±0.11 [a]	30.3±3.6 [c]
0	10	1.44±0.08 [b]	1.52±0.02 [ab]	4.84±0.20 [a]	65.3±6.9 [b]
	30	0.73±0.02 [c]	1.72±0.16 [a]	1.22±0.08 [bc]	39.2±6.5 [c]
	60	0.38±0.06 [d]	1.70±0.14 [a]	0.97±0.02 [cd]	31.4±1.8 [c]
1.5	10	1.42±0.05 [b]	1.34±0.09 [b]	4.75±0.16 [a]	87.0±2.8 [a]
	30	0.69±0.02 [c]	1.81±0.14 [a]	1.32±0.07 [b]	34.0±9.2 [c]
	60	0.35±0.07 [d]	1.79±0.24 [a]	0.84±0.03 [d]	32.8±6.4 [c]

[a] Different letters within a column indicate significant differences ($p < 0.05$).

The effect of heating and salt addition on the thiamin content in thermally processed pink salmon is shown in **Table 4-1**. Thiamin content decreased from 2.03 μg/mg in fresh muscle to 0.35-0.38 μg/mg in cooked muscle after 60 min heating. The thiamin loss was mainly due to heat-induced thiamin oxidation and the loss of thiamin in the exudates. The thiamin degradation rate is a function of temperature, time of heating, and the pH of the medium (*29*). No significant difference was observed in thiamin loss at the salt content tested in this study. Thiamin loss in heated products is most likely product dependent. Szymandera-Buszka and Waszkowiak (*30*) found that addition of salt increased the loss of both free and bound thiamin in ground turkey burgers during cooking and storage by approximatelly 6%; while Stepanov et al. (*31*) found loss of thiamin in legumes was slightly reduced by cooking with salt.

Lipid retention

The low lipid content determined for raw salmon samples is not unusual for spawning female fish due to the mobilization of muscle lipids to the maturing eggs (*32*). The total lipid content in the dorsal muscle of pink salmon caught in the Gulf of Alaska in May 1999 was between 0.8 - 6.5% (*33*). The fat content of the belly flap is higher than in the flesh, and in Atlantic salmon, the dorsal white muscle contains only one tenth of the oil as the belly flap tissue (*34*). **Table 4-1** show that the lipid in the salmon fillet was retained for samples with and without added salt, and no significant differences between treatments were observed. The increase of total lipid content from 1.24 % (raw muscle) to 1.70-1.79%, after 60 min of heating time, was due to losses in the moisture content and exclusion of the exudates from the pooled samples used for chemical analysis (*35*). Exudates were excluded from the analysis because generally this portion of the product is not consumed, thus not contributing to the nutritional value of the food item.

Lipid oxidation

To assess the level of lipid oxidation of salmon meat during canning, both primary (PV) and secondary oxidation products (TBA) were measured. The PV significantly increased from 30.33 meq/Kg fat in the raw muscle to 65 - 87 meq/Kg fat after 10 min heating (**Table 4-1**), indicating the initiation of lipid oxidation. This was followed by a decrease in PV to 31-33 meq/Kg fat after 60 min of heating time indicating decomposition of peroxide free radicals to secondary products as lipid oxidation progressed. At temperatures above 100 °C, the initial hydroperoxides formed exist only transiently and decompose rapidly into volatile and non-volatile products (*36*). Saghir et al. (*35*) investigated the influence of braising on lipid oxidation

of beef fillets, and found that the PV decreased and *p*-Anisidine value increased simultaneously, suggesting a progressive stage of lipid oxidation. Secondly, the low oxygen availability in the sealed sample container may have slowed the oxidation process by limiting the conversion of lipid radicals to peroxyl radicals (propagation step). Andreo et al. (*37*) found that the meat emulsions packaged in bags with low oxygen permeability of 15 cm^3 m^{-2} d^{-1} showed a PV decrease from 8.68 to 3.63 meq/Kg fat after 35 day in chill storage, while the PV of those packaged in bags with high oxygen permeability 2000 cm^3 m^{-2} d^{-1} increased from 8.68 to 53.83 cm^3 m^{-2} d^{-1}. Additionally, it is possible that some of the lipid oxidation products may have been lost into the exudates not recovered for measurement (*8*, *35*).

The formation of thiobarbituric acid reactive substances (TBA) followed a similar trend as PV, with a slight increase in the first 10 min heating from 4.59 mg MDA/Kg raw muscle to 4.75-4.84 mg MDA/Kg cooked muscle, followed by a significant decrease to 0.84-0.97 mg MDA/Kg muscle at the end of 60 min heating (**Table 4-1**). TBA values reflect the amount of certain secondary oxidation products generated from the degradation of polyunsaturated fatty acids and the production of carbonyl and carboxyl compounds that create unpleasant aroma and flavor in oxidized foods. The decrease of TBA values during heat-treatment was previously reported for muscle foods but not for canned pink salmon. Huang and Greene (*38*) reported that beef subjected to high temperature and long heating times developed lower TBARS numbers than samples subjected to lower temperature and shorter time processes. Aubourg et al. (*8*) found that TBA values in canned tuna decreased from 8.13 to 1.70 mg MDA/Kg muscle after 60 min of heating at 115 ˚C. The secondary products (aldehydes, ketones, epoxydes, and carbonyl compounds) tend to react with amino acids, phospholipids and the amino groups of nucleic acids during heat treatment, and this may lead to the observed decrease in TBA value (*39*).

Addition of salt to meat products is known to greatly enhance lipid peroxidation (*2*). However, in this study, both PV and TBA values showed no significant differences between the salt levels tested (**Table 4-1**), and may be due in part to the low levels evaluated. As stated earlier, the low oxygen availability in the sealed containers may have contributed to the production of fewer oxidation products. Reduced oxygen packaging has been commonly used as an effective method to prevent foods from developing rancid flavor.

Fatty acid profile

Table 4-2 shows the fatty acid profiles of the raw and heated salmon samples reported as percent total fatty acids, as well as the total amount of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The changes in the content of fatty acids after heating were marginal and no significant differences in the total amount of SFA, MUFA, and PUFA were observed between raw and heated samples, or between samples with and without added salt (**Table 4-2**). These results confirm that thermal process, when optimized to reduce the changes in the nutritional profile of foods, may not lead to a significant change in the fatty acid content of the product (*9*, *10*).

The docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were the predominant PUFA accounting for 33.6 - 36.8% and 6.6 - 7.1% of total fatty acids, respectively (**Table 4-2**). Other abundant fatty acids were palmitic acid (16:0), stearic acid (18:0), cetoleic acid (C22:1ω11), gadoleic acid (C20:1ω11) and oleic acid (C18:1ω9 *cis*) (**Table 4-2**). In the raw samples, the SFA, MUFA and PUFA accounted for approximately 22%, 29% and 47% of total fatty acids. The ω–3 and ω–6 ranged about 45 - 48%, and 2.6 - 2.7%, respectively. SFA, MUFA and PUFA determined in our study were comparable to the values reported by the United States

Department of Agriculture (*40*). According to USDA nutritional data, raw pink salmon contains approximately 19.6%, 32.8% and 47.6% of SFA, MUFA and PUFA, respectively (*40*).

Table 4-3 shows the fatty acid contents represented as mg/100 g of product. No significant difference was observed between salt-added and no-salt added samples in the level of retained lipid. Per 100 g product (after 60 min heating) contained about 210 mg SFA, 240 mg MUFA, 440 mg PUFA, 320 mg DHA and 65 mg EPA. The ω–3 and ω–6 were 420 and 24 mg/100 g product, respectively. The raw fish contained 0.27 g DHA + EPA/ 100g product, while the cooked products provided about 0.4 g DHA + EPA/ 100g (DHA/EPA ratio 5.1). Nutrition experts recommend a daily intake of 500 mg of EPA and DHA to reduce the risk of coronary heart disease (*7*). Our result indicates that consuming 130 g of canned pink salmon is sufficient to obtain the recommended amount of EPA + DHA. It is notable that this number is based on dorsal muscle of female pink salmon that contains much less oil content than other tissues or other wild Pacific salmon species. Furthermore, **Table 4-3** shows a high ω 3/ ω 6 ratio in the raw and cooked fish (16.6 - 18.4%). The ω 3/ ω 6 ratio has been suggested to be the best index when comparing relative nutritional values of fish oils from different species (*41*), and ranged from 7.5 to 19.5 in marine fish species (*42*). Therefore, it can be concluded that canned wild pink salmon is a valuable source of these fatty acids.

Conclusions

Addition of 1.5% salt reduced cook loss of canned pink salmon by 15.4% and area shrinkage 24.6%. Product with added salt was more tender with shear force decreased by 10.8%. Salt addition also caused the product to be slightly darker. Salt addition did not result in significant changes in thiamin degradation, lipid oxidation and fatty acids composition. The canning process employed here did not result in a loss of ω -3 fatty acids making canned pink salmon, even when prepared from spawning female fish, a very good source of ω -3 PUFA, particularly with respect to DHA+EPA (~ 0.4 g/100 g tissue) and ω 3/ ω 6 ratio(> 16.6%).

Table 4-2. Results of fame analysis (n=2) for raw and heated pink salmon muscle (percent total fatty acids) **(values are means ± standard deviation (SD))**[a]

FA	Raw	0% salt			1.5% salt		
		10 min	30 min	60min	10 min	30 min	60min
C14:0	2.19±0.22	1.90±0.46	2.04±0.37	1.94±0.63	2.13±0.06	2.20±0.21	2.00±0.31
Iso 15:0	0.08±0.12	0.07±0.10	0.07±0.10	0.08±0.11	0.13±0.00	0.14±0.01	0.07±0.10
C15:0	0.53±0.09	0.51±0.08	0.53±0.07	0.51±0.09	0.53±0.03	0.54±0.03	0.52±0.05
C16:0	12.08±0.71	12.89±1.50	12.79±1.32	12.83±2.07	12.76±1.15	12.58±1.50	13.19±0.98
C16:1ω11 + C16:1ω13	0.30±0.04	0.29±0.02	0.29±0.01	0.29±0.02	0.30±0.01	0.31±0.02	0.30±0.01
C16:1ω9	0.13±0.01	0.11±0.00	0.11±0.00	0.11±0.00	0.12±0.02	0.12±0.02	0.11±0.01
C16:1ω7	1.67±0.09	1.50±0.13	1.62±0.15	1.51±0.23	1.71±0.09	1.77±0.10	1.62±0.07
C16:1ω5	0.26±0.05	0.26±0.07	0.27±0.07	0.26±0.07	0.29±0.11	0.30±0.13	0.27±0.08
Iso 17:0	0.13±0.01	0.00±0.00	0.13±0.03	0.06±0.09	0.13±0.03	0.13±0.03	0.00±0.00
AI 17:0	0.48±0.08	0.45±0.07	0.46±0.06	0.45±0.07	0.45±0.06	0.46±0.06	0.46±0.06
C17:0	0.68±0.06	0.63±0.03	0.67±0.04	0.64±0.05	0.67±0.07	0.65±0.06	0.70±0.04
C17:1ω9	0.37±0.07	0.36±0.05	0.39±0.09	0.35±0.06	0.39±0.04	0.39±0.03	0.37±0.05
C18:0	3.93±0.21	4.17±0.59	4.01±0.46	4.18±0.97	4.12±0.29	3.96±0.48	4.31±0.35
c18:1ω9 *trans*	0.93±0.27	0.91±0.37	0.94±0.35	0.94±0.42	1.01±0.19	0.95±0.40	0.96±0.38
C18:1ω9 *cis*	5.48±0.70	5.02±0.54	5.23±0.51	5.06±0.41	5.55±1.00	5.56±1.01	5.32±0.62
C18:1ω7	1.65±0.42	1.67±0.46	1.69±0.45	1.66±0.51	1.72±0.46	1.68±0.51	1.73±0.47
C18:1ω5	0.44±0.02	0.42±0.04	0.42±0.04	0.43±0.05	0.46±0.11	0.48±0.12	0.43±0.05
C18:2ω6 *cis*	1.03±0.03	0.96±0.09	1.01±0.07	0.97±0.13	1.05±0.04	1.09±0.03	1.00±0.04
C18:3ω3	0.91±0.18	0.88±0.18	0.91±0.20	0.87±0.15	0.98±0.31	1.02±0.35	0.91±0.24
18:4ω3	1.90±0.33	1.79±0.43	1.87±0.27	1.76±0.53	1.92±0.05	2.05±0.07	1.82±0.10
C20:1ω11	4.67±0.48	3.77±1.57	3.91±1.42	3.85±1.98	3.89±1.14	3.97±1.71	3.73±1.41
C20:1ω9	2.19±0.44	1.81±0.78	1.88±0.65	1.92±0.97	1.92±0.49	2.02±0.76	1.97±0.54
C20:1ω7	0.13±0.02	0.14±0.00	0.16±0.01	0.08±0.11	0.15±0.02	0.15±0.01	0.07±0.10
C20:2ω6	0.46±0.02	0.41±0.00	0.43±0.00	0.43±0.00	0.47±0.03	0.46±0.05	0.44±0.00
C20:4ω6	1.15±0.05	1.24±0.01	1.19±0.02	1.22±0.10	1.20±0.10	1.17±0.05	1.23±0.05
C20:3ω3	0.10±0.14	0.00±0.00	0.00±0.00	0.00±0.00	0.11±0.16	0.11±0.16	0.00±0.00
C20:4ω3	1.31±0.03	1.28±0.04	1.30±0.02	1.27±0.09	1.34±0.07	1.38±0.08	1.27±0.00

C20:5ω3 (EPA)	6.62±0.58	7.14±1.17	7.08±1.00	7.01±1.21	7.01±0.88	6.99±1.04	7.02±1.03
C22:1ω11	9.49±3.20	8.03±4.79	8.37±4.27	8.45±5.89	8.25±3.49	8.72±4.74	8.16±3.67
C22:1ω9	0.84±0.29	0.71±0.43	0.76±0.36	0.76±0.52	0.75±0.27	0.80±0.37	0.76±0.28
C24:0	2.34±0.33	2.42±0.44	2.37±0.37	2.39±0.44	2.40±0.33	2.36±0.38	2.40±0.35
C22:6 ω 3 (DHA)	33.77±2.70	36.83±5.03	34.89±3.51	35.33±6.95	34.52±1.14	33.63±2.94	34.72±3.27
C24:1ω9	0.73±0.20	0.72±0.24	0.86±0.19	0.92±0.23	0.75±0.14	0.90±0.17	0.89±0.15
∑FAME ID	98.96±0.14	99.29±0.43	98.64±0.50	98.53±0.79	99.17±0.25	99.05±0.40	98.75±0.11
SAT	22.44±0.81	23.04±1.85	23.07±1.60	23.08±2.54	23.31±1.72	23.01±2.14	23.65±1.20
MUFA	29.29±3.86	25.72±7.25	26.90±6.42	26.59±9.40	27.25±3.95	28.14±6.27	26.70±5.43
PUFA	47.24±3.18	50.53±5.83	48.67±4.32	48.87±7.65	48.60±2.48	47.90±4.53	48.40±4.34
ω 3	44.61±3.25	47.92±5.90	46.04±4.42	46.24±7.68	45.88±2.51	45.18±4.50	45.74±4.43
ω 6	2.63±0.06	2.61±0.08	2.62±0.10	2.63±0.03	2.72±0.03	2.72±0.03	2.66±0.09

[a] Abbreviations: FAME ID: fatty acids methyl esterds identified; SAT saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids; DHA docosahexaenoic acid; EPA eicosapentaenoic acid.

Table 4-3. Summary of fame analysis (n=2) results for raw and heated pink salmon muscle (mg/100 g of tissue)

(values are means ± standard deviation (SD))[a]

Salt (%)	Heating time (min)	SFA	MUSA	PUFA	DHA	EPA	ω 3	ω 6	ω 3/ω 6
raw		148.74±1.26^{c}	194.57±30.97^{a}	312.94±12.41^{c}	223.67±11.71^{b}	43.85±2.63^{b}	295.49±13.32^{b}	17.45±0.91^{d}	16.98±1.65^{a}
0	10	194.17±11.26^{ab}	217.66±66.02^{a}	425.65±39.53^{abc}	310.16±35.44^{ab}	60.13±8.48^{ab}	403.63±40.69^{ab}	22.02±1.16^{bc}	18.40±2.82^{a}
	30	219.83±25.81^{a}	254.36±48.80^{a}	463.92±63.37^{ab}	332.64±49.33^{a}	67.58±12.80^{a}	438.98±63.13^{a}	24.94±0.24^{ab}	17.59±2.36^{a}
	60	207.66±14.10^{ab}	241.51±94.82^{a}	439.17±50.40^{ab}	317.23±49.22^{ab}	62.97±8.28^{ab}	415.49±51.71^{a}	23.68±1.31^{abc}	17.63±3.16^{a}
1.5	10	173.71±17.72^{bc}	206.96±65.34^{a}	362.88±45.16^{bc}	258.14±36.75^{ab}	51.98±2.6^{ab}	342.43±41.37^{ab}	20.45±3.79^{cd}	16.85±1.10^{a}
	30	232.83±17.66^{a}	285.47±68.36^{a}	484.62±37.51^{a}	340.21±23.88^{a}	70.68±9.32^{a}	457.07±37.71^{a}	27.55±0.19^{a}	16.60±1.49^{a}
	60	219.19±20.51^{a}	246.12±39.7^{a}	449±59.41^{ab}	322.11±44.06^{a}	65.19±12.29^{ab}	424.35±59.15^{a}	24.65±0.26^{ab}	17.21±2.22^{a}

[a] Abbreviations: FAME ID fatty acids identified; SAT saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids;
DHA docosahexaenoic acid; EPA eicosapentaenoic acid.
Different letters within a column indicate significant differences ($p<0.05$).

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CHAPTER 5. KINETICS OF SALMON QUALITY CHANGES DURING THERMAL PROCESSING

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Nomenclature

t	time (min)
C, C_0, C_t ,C_∞	quality index value, quality index value at time zero and t, non-zero equilibrium quality value
T, T_{abs}	temperature (˚C), absolute temperature (K)
f	quality index
n	order of reaction
k	reaction rate constant, k^{-n}
k_0	frequency factor
E_a	activation energy (J/mol)
R	gas constant (8.314 J/mol.K)
m and T_c	constants (˚C $^{-1}$, ˚C)
$b(T)$ and $n(T)$	temperature-dependent coefficients
L^*, a^*, b^*	color dimensions
ΔE	color difference
TM, TM_0, TM_t	thiamin content (μg/g), thiamin content at time zero and time t
CL, CL_t, CL_∞	cook loss (%), cook loss at time t, equilibrium cook loss
AS, AS_t, AS_∞	area shrinkage ratio (%), area shrinkage ratio at time t, equilibrium area shrinkage ratio
MSE	mean square error
CVS	computer vision system
MTB	Multiple thin bladed Kramer type texture fixture

Abstract

The kinetics of reactions leading to changes in salmon quality during thermal processing were evaluated. Small samples (D 30 mm × H 6 mm) cut from pink salmon (*Oncorhynchus gorbuscha*) fillets were sealed in aluminum containers (internal dimension: D 35 mm × H 6 mm) and heated in an oil bath at 100, 111.1, 121.1, and 131.1˚C for different time intervals up to 180, 150, 120, 90 minutes, respectively. A fractional conversion model was used to describe the increase in cook loss during heating; and a quadratic relationship to correlate cook loss with area shrinkage ratio. Color changes (CIE L^*, b^* and ΔE) involved whitening and browning phases. In the browning phase, the changes of CIE L^*, b^* and ΔE followed a zero order reaction. The progressive change of texture with time as indicated by shear force during heating went through four different phases, and the second (rapid tenderizing) and third phases (slow toughening) were modeled using a first order reaction kinetic model. The decay of thiamin during heating was modeled with two different relationships: a second-order reaction in which the temperature dependence of the rate constant followed an Arrhenius relationship; and a Weibull-log logistic model recently proposed.

Keywords: Pink salmon; food thermal process; quality changes; kinetics; shear force; cook loss; color; thiamin

Introduction

Salmon is one of the most important commercial fish species harvested in the United States. Thermal processing is commonly used in the fish industry to extend shelf life of salmon products. During thermal processing operations, salmon meat is hermetically sealed in cans or pouches and processed in steam retorts at high temperatures (around 120 ˚C) for various periods from 20 to 90 min depending upon package size and shape. The high temperature heating may cause severe quality deterioration, such as degradation in color and texture, nutrient loss, cook loss (weight loss) and area shrinkage, rendering the products less attractive to most consumers. The most drastic changes in salmon fillets during heating result from protein denaturation. In particular, denaturation of heme proteins and oxidation of carotenoid pigments darkens the products (Haard, 1992). The denaturation of the proteins also leads to reduced water holding capacity and shrunken muscle fibers, and subsequently leading to a harder and more compact tissue texture (Harris & Shorthose, 1988). Our previous studies demonstrated that when heated at 121.1 ˚C, the salmon fillet texture profile during the course of heating had two-peaks, with the first peak in the 5 min and second peak at 1 hour heating time (Kong, Tang, Rasco, Crapo, & Smiley, 2006). These are likely the results of different reactions including toughening caused by denaturation of myofibrillar proteins and muscle dehydration, and tenderization by collagen solubilization. Heating causes progressive shrinkage and disintegration of the myofibril; and water, soluble proteins, and fats are expelled from the tissue (Leander, Hedrick, Brown, & White, 1980; Offer, Restall, & Trinick, 1984; Bertola, Bevilacqua, & Zaritzky, 1994). Heating also caused loss of nutrients such as thiamin.

New thermal processing technologies such as microwave sterilization processes have been investigated as a means of producing commercially sterile food with higher quality (Guan,

Plotka, Clark, & Tang, 2002). To develop new thermal process and optimize existing retorting processes, knowledge of the kinetics associated with quality degradation is needed. Studies have been conducted on the kinetics of quality changes of food system during thermal processes. Zero-order and first order kinetics are used to characterize the quality changes of muscle foods (Bhattacharya, Choudhury, & Studebaker, 1994; Bertola et al., 1994). But information on salmon quality changes during thermal processing and associated kinetics are limited.

The classic Arrhenius model is commonly used to describe rate–temperature relations in food and biological systems. It is particularly suitable for systems over a broad range of temperatures without a major change of mechanisms that determine kinetic rate constants. But there are situations where changes in system parameters are only noticeable above certain threshold temperatures. For these cases, Peleg, Engel, Gonzalez-Martinez, and Corradini (2002) proposed using a Weibullian-power law model, $C(t)/C_0 = \exp[-b(T)t^{n(T)}]$, to describe its temporal degradation, and using a log logistic equation, $b(T) = \log_e\{1+\exp[k(T-T_c)]\}$, to describe the temperature dependence of the rate parameter. Oxidation reactions such as thiamin oxidation only occur at a noticeable rate at a high enough temperature (Peleg et al., 2002). In this study, an attempt was made to use this method to explain thiamin degradation, and its performance was compared with classic reaction order - Arrhenius equation model.

The objectives of this study were to investigate the kinetics of salmon quality changes during high temperature thermal process, including cook loss, area shrinkage, and deteriorations in color, texture and thiamin.

Materials and Methods

Materials

Wild fresh whole grade A pink salmon (*Oncorhynchus gorbuscha*) were obtained from a seafood processing plant on Kodiak Island (Alaska) in August 2005, and immediately transported on ice to the Fishery Industrial Technology Center (FITC) pilot plant. The fish weighed 1350 ± 100 g and were of similar size (370 ± 10 mm in length and 120 ± 10 mm in width). The fish were gutted, frozen and stored (-31 ˚C) at the FITC pilot plant and shipped overnight to Washington State University, Pullman, WA.

Sampling and heating

The sampling and heating of fish fillets followed methods previously described (Kong et al., 2006). Small disk shaped samples (6 mm in thickness and 30 mm in diameter) were taken using an electrical food slicer and a 30 mm diameter corer. Only the white muscle in the dorsal area were sampled in order to reduce the influence of heterogeneity of fish muscle on the cooking properties. The samples were hermetically sealed in custom-designed cylindrical aluminum test cells having a 35 mm inner dia, 6 mm inner height, and 2 mm wall thickness. A 0.1 mm diameter copper-constanstan thermocouple (Type-T) measured the temperature at the geometrical center of the sample. The cells were sealed at the top and the bottom with o-rings to prevent leakage of vapor during heating at or above 100^{o}C. The sealed cells were subjected to heat in an oil bath (Model HAAKE W13, Thermo Electron Corp., Germany) using glycerol as the heating medium. During heating, the signals from the thermocouple junctions were transferred to a computer equipped with a DLZe type data logger (DELTA-T Devices,

Cambridge, England). Time for the sample center temperature to reach within 1 ˚C of the total temperature rise was on the order of 2.5 - 3 min. Sample cells were immediately placed in ice upon removal from the oil bath. The sample temperature dropped to below 20 ˚C within 0.5 min, so the thermal effect of cooling step on the product quality was neglected. After cooling, the samples were dried with a filter paper, weighed in an analytical balance (Ohaus Analytical Plus, Pine Brook, New Jersey), and stored in a cooler (4 ˚C) overnight for further analysis.

Experimental design

Sixteen fish were used, providing about 240 samples. Weighed amount of sodium chloride (1.5% w/w) was evenly distributed on the sample surface immediately before sealing and heating to simulate industrial canning practice of salmon. The experiments were conducted at four temperatures (100, 111.1, 121.1, 131.1 ˚C). Table 5-1 shows the temperature and time conditions employed. Six replicates were used for each temperature and time combination.

Table 5-1. Experimental conditions

Temperature (˚C)	Heating time (minutes)								
100	0	3	10	20	30	60	90	120	180
111.1	0	2.75	5	10	20	30	60	90	150
121.1	0	2.5	5	10	15	20	30	60	120
131.1	0	2.5	5	10	15	20	30	60	90

Cook loss

The weight of raw and heated samples was recorded to calculate cook loss. Percentage of cooking loss was calculated as:

$$Cook\ loss = \frac{weight\ of\ raw\ sample - weight\ of\ cooked\ sample}{weight\ of\ raw\ sample} \times 100\% \quad (1)$$

Texture

A multiple thin bladed Kramer type texture fixture (MTB) developed by Kong et al. (2006) was used to measure shear force of the samples. The MTB consisted of upper part and lower part: the upper part had thin 10 blades, and the lower part was a support base with slots. It used 0.5 mm-thick blades with 40 mm blade length and 50 mm width. The probe was fitted to a Texture Analyser TA-XT2 (Stable Micro Systems Ltd., Surrey, U.K.) equipped with a 5 kg load cell. Before measurement, the raw and heated samples were allowed to equilibrate to room temperature (approximately 22 ˚C), which took approximately half an hour. The samples were carefully placed on the support base so that the blades were perpendicular to muscle fibers. The traveling speed for the upper blades was set at 1 mm/s. The force-time graphs were recorded by a computer and analyzed using the Texture Expert for Windows (version 1.15, Stable Micro Systems Ltd.). The shear force was measured as the peak height in the force-time profile.

Color and area measurement

A computer vision system (CVS) described by Kong et al. (2006) was used to capture color images of fresh and cooked samples for color and area measurements. Color images were downloaded into the computer for analysis. The color parameters (CIE L^*, a^* and b^*) were derived using Adobe Photoshop CS2 software (Version 8.0). For sample area determination, the Vision IMAQ Builder image processing software Version 6.1 (National Instruments, Austin, Texas) was used. The area of sample image was determined using the "area measurement" menu. The shrinkage ratio was calculated as:

$$Area\ shringkage\ ratio = \frac{area\ of\ raw\ sample - area\ of\ cooked\ sample}{area\ of\ raw\ sample} \times 100\% \qquad (2)$$

Chemical analysis

Samples subjected to a specific temperature and time combinations were homogenized in a blender. Moisture content was determined by vacuum drying a sample (3 – 5 g) at 65 ˚C to constant weight (Hart & Fisher, 1971). Lipid content was determined using modified Folch method (Folch, Lees, & Sloan Stanley, 1957; Iverson, Lang, & Cooper, 2001). Thiamin content in the raw and heated samples was determined using the Thiochrome method (AOAC 942.23, 2000), and the fluorescent intensity of thiochrome extracted with isobutanol was determined in a fluorometer (Model FluoroMax-3, Jobin Yvon Inc., Edison, NJ) at an excitation wavelength of 365 nm and an emission wavelength of 435 nm.

Data analysis

Generally, changes in quality factor "*C"* in an isothermal condition can be represented by (Wang, Lau, Tang, & Mao, 2004):

$$\frac{dC}{dt} = -k(C)^n \qquad (3)$$

where *k* is the rate constant, *C* is the quantitative indicator of a quality attribute at time *t*, and *n* is the order of reaction. The integrated form for 0, 1 and 2nd order kinetic models is listed in Equations 4, 5 and 6, respectively.

Zero order: $C_t = C_0 - k.t$ (4)

1st order: $\ln\frac{C_t}{C_0} = -k.t$ (5)

2nd order: $kt = \frac{1}{C_t} - \frac{1}{C_0}$ (6)

where C_0 represents the initial value at time zero, C_t is the value at time t, k is the rate constant. Fractional conversion model (a modified first-order kinetic model) has also been vastly used, in which a quality index, f, is used to express the extent of quality change at any time t:

$$f = \frac{C_0 - C_t}{C_0 - C_\infty}$$

For a 1st order reaction, the logarithm of (1-f) plotted against time is linear. The model becomes:

$$\ln(1-f) = \ln(\frac{C_t - C_\infty}{C_0 - C_\infty}) = -k.t \qquad (7)$$

where C_∞ is the non-zero equilibrium quality property after prolonged heating time.

The Arrhenius equation is usually applied to describe the reaction rate constant temperature dependence:

$$k = k_0 .e^{-\frac{Ea}{RT_{abs}}} \qquad (8)$$

If Equation 8 indeed applies to a reaction in consideration, a plot of the rate constant on semi-logarithmic scale as a function of reciprocal absolute temperature ($1/T_{abs}$) should give a straight line, and the activation energy can be determined as the slope of the line multiplied by the gas constant R.

Peleg et al. (2002) proposed using Weibullian power law model to describe isothermal degradation of food quality:

$$C(t)/C_0 = \exp[-b(T)t^{n(T)}] \qquad (9)$$

where *b(T)* and *n(T)* are temperature-dependent coefficients. If *n(T*) is more or less constant over a range of temperature for any particular thermal degradation process, then *b(T)* can be described by a empirical model (Peleg et al., 2002):

$$b(T) = \log_e \{1 + \exp[m(T - T_c)]\} \qquad (10)$$

where T is temperature (˚C), m and T_c are constants. The solver command in Microsoft Excel (2002) was used to derive optimal *b(T)*, *n(T)* , m and T_c to produce the lowest mean square error between predicted and observed values.

In our analysis on kinetics data, unless otherwise stated, the samples taken at the end of come-up-times (2.5 - 3 min) were used as the zero-time samples. This was the time when sample center reached the temperature within 1 ˚C from the set temperature. The quality values of zero-time samples were used as the initial values. The moisture, lipid and thiamin contents were determined in duplicate. All the quality data were analyzed by using the analysis of variance (ANOVA) in the General Linear Models procedure of the SAS System for Windows V8.01 (SAS User's Guide, 1996). Differences between group means were analyzed by Duncan's Multiple-Range Test. Statistical significance was set at a 0.05 probability level.

Results and Discussion

Effects of cook loss and area shrinkage

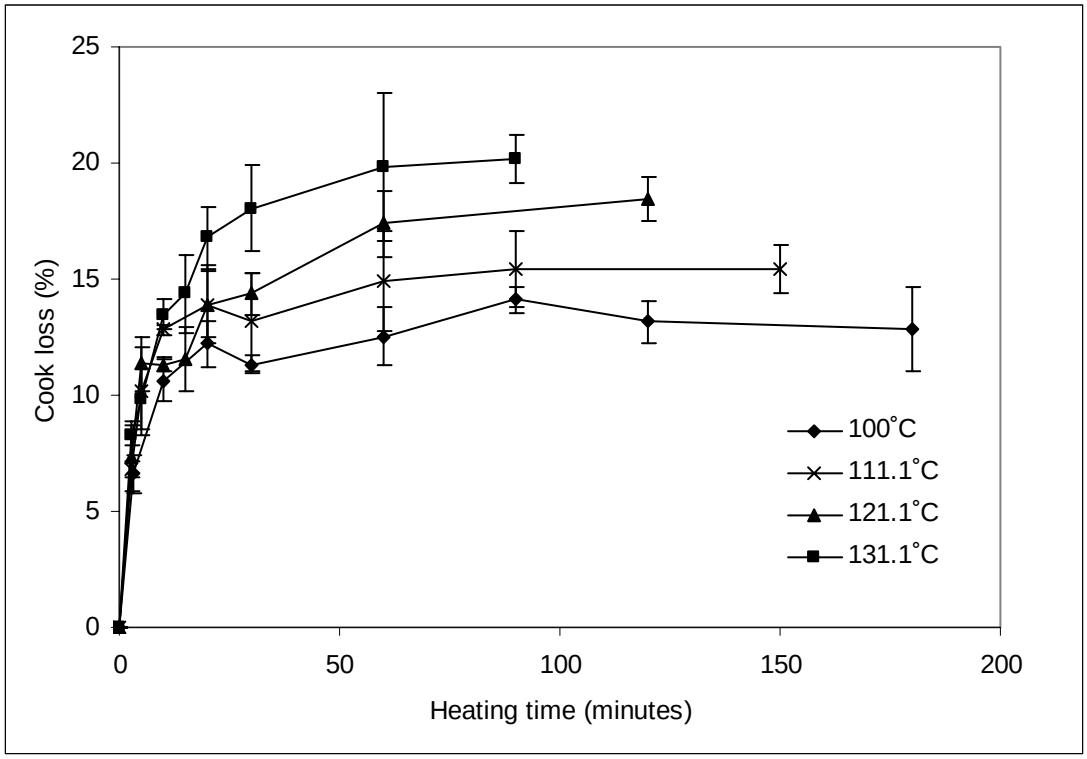


Figure 5-1. The change of cook loss of salmon fillet at different heating temperatures. Bars indicate the standard deviation from six determinations.

The changes in the cook loss with heating time at four different temperatures are shown in Figure 5-1. Cook loss increased significantly with increasing temperature. Although consistently increased with heating time, most of the cook loss occurred within the first 30 min. The first portion of the curves had steeper slopes. As the heating progressed, the slope gradually leveled off and the cook losses reached an equilibrium, which was 13.2%, 15.4%, 18.5% and 20.2% for 100, 111.1, 121.1 and 131.1.1 ˚C, respectively. The changes of equilibrium cook loss CL_∞ with heating temperature followed below equation:

$$CL_\infty = 0.2332T - 10.18 \quad R^2 = 0.988 \qquad (11)$$

Figure 5-1 shows a considerable cook loss (> 40% of the total cook loss) occurred during the first 2.5 – 3 min of heating that was within the non-isothermal stage. In kinetic modeling, the data for this period were combined to make models more widely applicable. The increase of cook loss with time was fitted to a fractional conversion model (Equation 7). The temperature dependence of the rate constant was expressed by the Arrhenius equation (Equation 8). The rate constants corresponding to the four temperatures, *Ea* and k_0 values, and coefficient of correlation are shown in Table 5-2. The activation energy of 36.98 kJ/mol is in the same range of beef that was reported to be 54.93 kJ/mol (Bertola et al., 1994). The frequency factor k_0 is 5761.2 /min. After incorporating the above values, the equation becomes:

$$k = 5761.2 \times e^{-\frac{36.98 \times 1000}{8.314 \times (273+T)}} \qquad (12)$$

Cook loss can then be estimated using equation 13 converted from Equation 7:

$$CL_t = (1 - e^{-kt}) \cdot CL_\infty \qquad (13)$$

Table 5-2. First-order kinetic parameters for cook loss of salmon fillets after heat treatments at four temperatures

T (°C)	k (min-1)×10^{-3}	R^2	E_a (kJ/mol)	k_0 (min^{-1})	R^2
100	36.0	0.891	36.98	5761.2	0.955
111.1	54.2	0.962			
121.1	79.6	0.931			
131.1	87.7	0.956			

This model gives a good fit to the cook loss data when samples were heated at 121 and 131 ˚C. It also fit well with the data of 100 and 111.1 ˚C after 30 min heating. Considering the heating time used in industrial canning practices for salmon is much longer (60 min at 111.1 ˚C and 773 min at 100 ˚C to achieve an equivalent lethality value (F_0) of 6), this model still has practical usefulness (see Figure 5-2).

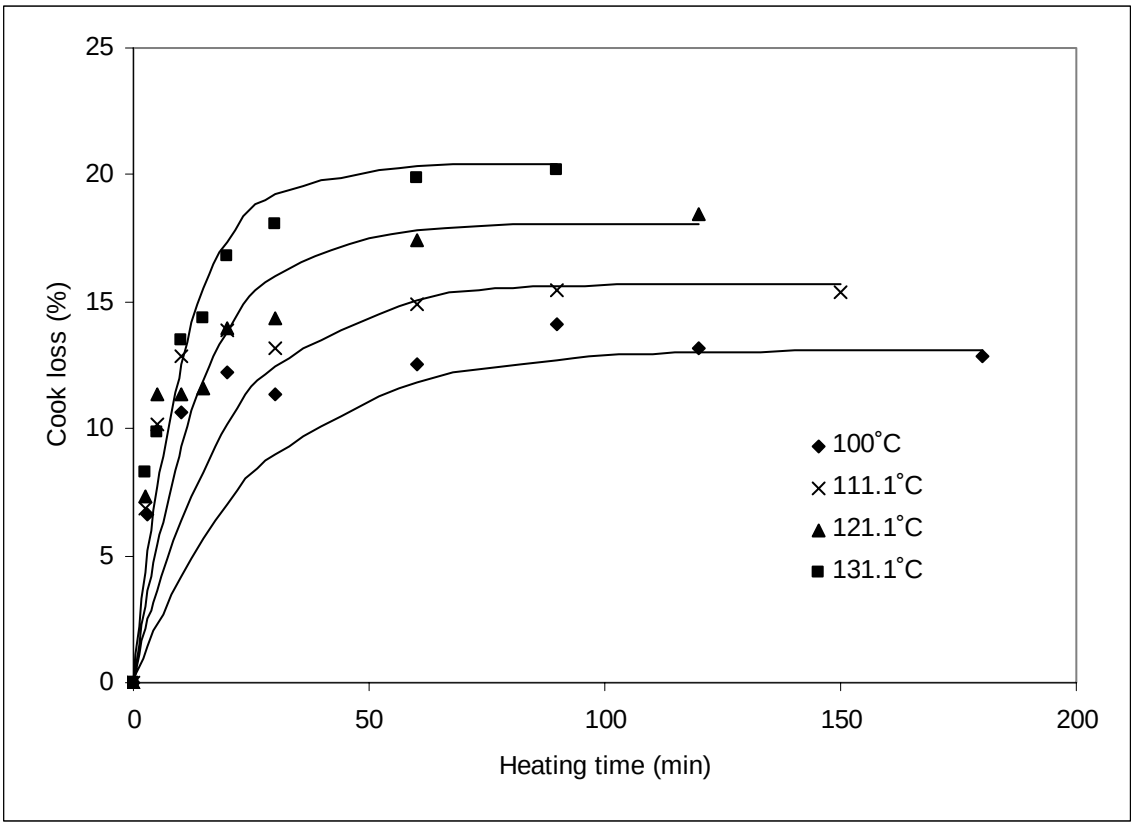


Figure 5-2. The change of cook loss of salmon fillet at different heating temperatures fitted with a fractional conversion model.

Most of the cook loss is water (> 85%), others are lipids and solids including collagen or gelatin, muscle fragments and coagulated sarcoplasmic proteins. Thermal denaturation of muscle proteins is the primary mechanism leading to the moisture loss (Bell, Farkas, Hale, & Lanier, 2001). Most water in meat is located within the myofibrils, in the narrow channels between thick and thin filaments (Offer et al., 1984; Bertola et al., 1994). Heating caused denaturation of myosin and shrinkage of myofibrils, and a subsequent expulsion of water (Ofstad, Kidman, Myklebust, & Hermansson, 1993). Heating caused moisture to decrease from the original level of 75.3% to 69.2 - 70.4% (wet basis) in the tested samples, and higher temperature treatment resulted in lower moisture products. Similar to cook loss, significant reduction in sample moisture ($P < 0.05$) occurred in the first 30 minutes. After that, the moisture

gradually reached equilibrium. The lipid recovered in the heated samples increased slightly from 1.46% to 1.55-1.71% (wet basis) indicative of a relatively higher loss of moisture from these samples compared to lipid. Similar observations were reported by Al-Saghir, Thurner, Wagner, Frisch, Luf, Razzazi-Fazeli et al. (2004).

Protein denaturation reduces the dimension of myofibrils and collagen, resulting in shrinkage of muscle fiber diameter and sarcomere length (Palka & Daun, 1999; Bell et al., 2001). Similar to cook loss, area shrinkage in salmon fillets increased sharply in the initial heating period and then gradually approached a relatively constant value (plateau) as the heating progressed beyond 30 min. The height of the plateau was positively correlated to heating temperature. For example, the largest shrinkage ratio measured for 100, 111.1, 121.1, and 131.1 ˚C was 10.34%, 15.6%, 20.1%, 24.4%, respectively. The plateau AS_∞ (%) values were linearly related to temperature T (˚C) as:

$$AS_\infty = 0.4523 \cdot T - 34.56 \qquad R^2 = 0.999 \tag{14}$$

A quadratic equation was used to relate area shrinkage ratio and cook loss as following:

$$AS = 0.0781 \cdot CL^2 - 1.0587.CL + 12.458 \qquad R^2 = 0.915 \tag{15}$$

where CL= 6.6 - 20.2%. The non-linear relationship between the cook loss and area shrinkage ratio could be explained by the fact that cook loss is a result of heat-induced volume change that is a function of both area and thickness reduction. Positive correlations between sarcomere shrinkage and cook loss have been documented in beef (Palka & Daun, 1999), pork (Barbera & Tassone, 2006) and fish muscle (Ofstad, Kidman, & Hermansson, 1996). Degrees of shrinkage and cook loss varied between the fish groups. Salmon muscle is more heat-stable than cod muscle, which likely results from the different collagen content (Ofstad et al.,1993). Cook loss and area change are also significantly affected by sample size and shape (Laroche, 1980).

Therefore, the equations 11-15 provide a rough approximation. Modifications are needed to determine the real effects of cook loss and area change for retorted product using industrial practices.

Effect of color

Figure 5-3 shows the change of CIE L^*, a^*, b^* with heating time. During the heating, the muscle color underwent a two-phase change: a rapid whitening phase followed by a slow browning phase. The whitening phase occurred within the first 10 min: the pink color of fish muscle quickly faded to whitish, with the L^* increased to a maximum (Figure 5-3a), and a^* and b^* decreased to a minimum ($P < 0.05$, Figure 5-3b&c) at all tested temperatures. The rapid whitening resulted from the quick denaturation of heme proteins (hemoglobin and myoglobin) and oxidation of carotenoids (Haard, 1992). Franklin, Crockford, Johnston, and Kamunde (1994) reported that roughly 50% of the haemoglobin in rockcod was denatured after 19 min at 50 ˚C. The whitening phase is concomitant with the formation of the cooked meat haemoprotein. The rate of cooked meat haemoprotein formation (measured as the rate of loss of myoglobin solubility) was found to obey a first order kinetic in beef and lamb (Geileskey, King, Corte, Pinto, & Ledward, 1998). Fish haemoglobins are considerably less stable than mammalian haemoglobins (Franklin et al., 1994).

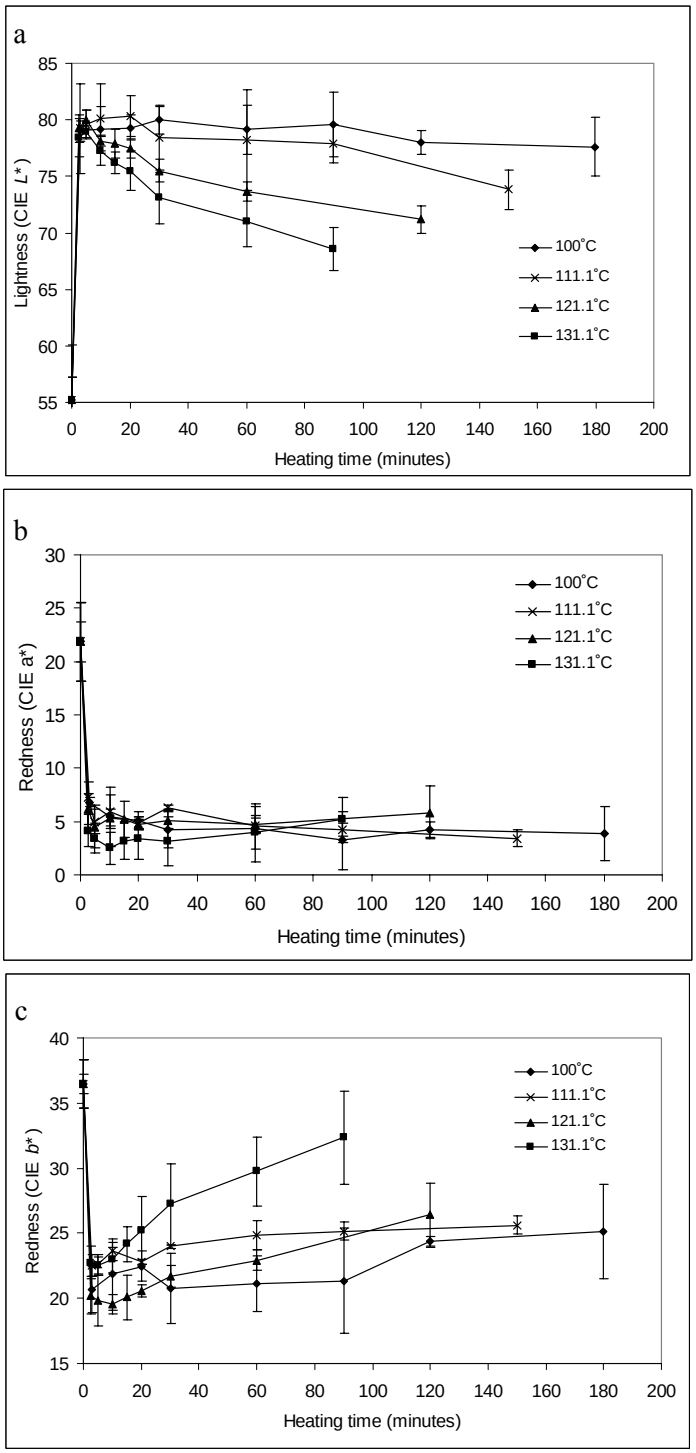


Figure 5-3. The change in the color of salmon fillet at different heating temperatures: (a) CIE *L**, (b) CIE *a**, (c) CIE *b**. Bars indicate the standard deviation from six determinations.

In the browning phase, Maillard reaction between sugars, fish proteins or amines as well as protein-lipid reaction dominated (Haard, 1992). As the processing temperature and time

increased, more browning products are produced (Whistler & Daniel, 1985), which is reflected by the decreased L^* value (Figure 5-3a) and the increased b^* values (Figure 5-3c). The L^*, b^* of the tissue were significantly affected by both processing temperature and time ($P < 0.05$); and higher temperature resulted in greater rates of decrease in those values. However, a^* generally did not show significant changes ($P > 0.05$, Figure 5-3b). To describe the total color changes of the salmon fillets in the browning phase, color difference (ΔE) was calculated using Equation 16 (Feng & Tang, 1998):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \qquad (16)$$

where L_0^*, a_0^* and b_0^* were for the samples were taken after 10 min heating, representing initial color values in the browning phase. The kinetics of changes of L^*, b^* and ΔE in the browning phase were analyzed. The samples taken at 10 min heating time, when the browning phase started, were used as time zero samples. Over the tested temperature range, the experimental data of L^*, b^* and ΔE were best fitted to a zero order reaction. Arrhenius equation was used to express the temperature dependence of the rate constants and calculate Ea values for L^*, b^* and ΔE. The results are summarized in Table 5-3. The Ea values fall into a range of 70-100 kJ/mol, which is within the range cited in the literatures for quality changes (60-120 kJ/mol) (Lund, 1977) and for brown chemical marker formation in foods (Lau, Tang, Taub, Yang, Edwards, & Mao, 2003; Wang et al., 2004; Pandit, Tang, Mikhaylenko, & Liu, 2006). Deterioration of color is affected by composition such as sugar and amino acid content, total solid content, pH, acidity and also the temperature range of the study (Haard, 1992; Hui, Cross, Kristinsson, Lim, Nip, Siow et al., 2006).

Table 5-3. Zero-order kinetic parameters for color changes of salmon fillets after heat treatments at four temperatures

Color index	Temp (°C)	k (min^{-1}) $\times 10^{-3}$	R^2	E_a (kJ/mol)	R^2
CIE L^*	100	0.204	0.669	87.99	0.949
	111.1	0.765	0.905		
	121.1	1.242	0.936		
	131.1	1.889	0.952		
CIE b^*	100	0.136	0.605	73.76	0.812
	111.1	0.114	0.788		
	121.1	0.383	0.985		
	131.1	0.715	0.961		
ΔE	100	0.458	0.587	99.46	0.977
	111.1	1.645	0.887		
	121.1	3.098	0.963		
	131.1	5.575	0.975		

Effect of shear force

Figure 5-4 shows the shear forces changes with time at the four tested temperatures. The changes in shear force with heating time exhibited two peaks at the four temperatures, dividing the profile into four phases: 1) rapid toughening, the shear force increased from raw muscle to the first peak; 2) rapid tenderization, the shear force decreased from the first peak to a minimum; 3) slow toughening, the shear force increased again to a second peak; and 4) slow tenderization, the tissue gradually became soft as heating time prolonged. The four-phase shear force changes could be explained by the net effect of different reactions (Kong et al., 2006). Thermal denaturation temperatures of fish muscle proteins take place between 40 to 80 ˚C (Bell et al., 2001; Ofstad et al., 1996). At the high temperatures used in this study, both the tissue-toughening caused by denaturation of myofibrillar proteins and tissue-softening caused by collagen gelation and solubilization might have been contributed to the observed changes in salmon fillet texture. As heating prolonged, the prevalent reactions shifted, resulting in a

fluctuation in shear force of the muscle. Figure 5-4 also indicates higher temperature expedited the texture changes. Shorter time was needed to complete the four phases when heated at higher temperatures: the rapid toughening phase lasted for 10 min for 100 ˚C, and 5 min for 111.1, 121.1 and 131.1 ˚C; the rapid tenderizing phase lasted for 50, 25, 15 and 10 min for 100, 111.1, 121.1 and 131.1 ˚C. The slow toughening phase lasted 60 min for 100 and 111.1 ˚C, 40 min for 121.1 ˚C and 15 min for 131.1 ˚C. Higher temperature induced faster denaturation of proteins, and subsequently more rapid textural changes. Protein denaturation rate increases about 600-fold for every 10 ˚C in temperatures (Anglemier & Montgomery, 1976).

Figure 5-4 shows that the shear force decreased as the temperature increased from 100 to 131.1 ˚C. For example, the maximum shear force (corresponding to the first peak) was 205, 170, 148 and 142 N for 100, 111.1, 121.1 and 131.1 ˚C, respectively. This phenomenon is opposite to the observed texture changes in Pacific chum salmon when heated at temperatures less than 100˚C (Bhattacharya, Choudhury, & Studebaker, 1993). These authors studied the effect of hydrothermal processing on the texture of Pacific chum salmon at 60 - 100 ˚C, and found that the hardness increased as the heating temperature increased. This discrepancy with our findings might be due to the coexistence and counteraction of the hardening and softening reactions at high temperature. Also, higher temperature promoted a rapid disintegration and fragmentation of the fish muscle, contributing to the decrease in the measured shear force. This effect can be also seen by an increase in the minimum shear force at higher temperatures: 120 N for the 131 ˚C, higher than the 105 N for the 111.1 and 121.1 ˚C.

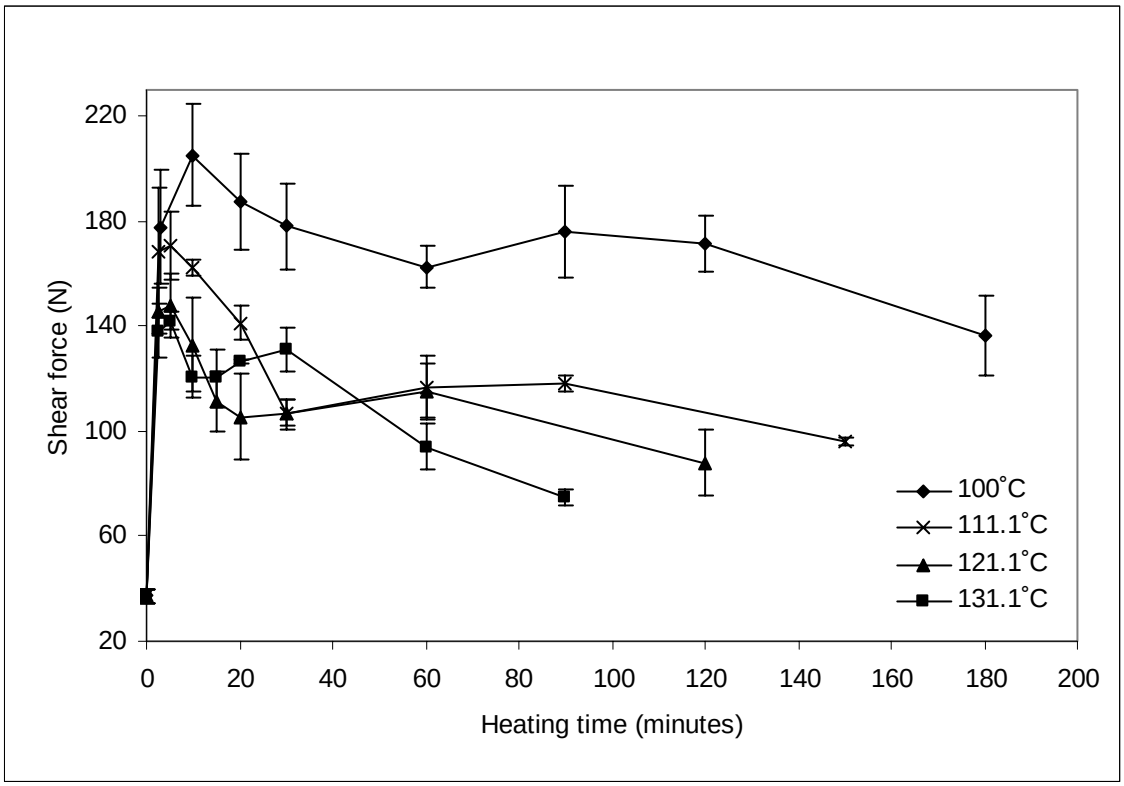


Figure 5-4. The change of shear force of salmon fillet at different heating temperatures. Bars indicate the standard deviation from six determinations.

The kinetics of the rapid tenderization and the slow toughening phases were evaluated. For the former phase, the shear force versus time curves at 100, 111.1 and 121.1 ˚C were used. The zones analyzed were from 10 to 60 min at 100 ˚C, 5 to 30 min at 111.1 ˚C, and 5 min to 20 min at 121.1 ˚C. For the latter phase, the shear force versus time curves at 111.1, 121.1 and 131.1 ˚C were used. The zones analyzed were from 15 to 30 min at 131.1 ˚C, 20 to 60 min at 121.1 ˚C, 30 to 90 min at 111.1 ˚C. First order kinetic model was used to fit the experimental curves, and an Arrhenius model was used to express the temperature dependence of rate constants. The resultant rate constants, activation energy and correlation coefficient were shown in Table 5-4. The *Ea* for the two phases are 100.47 and 70.04 kJ/mol, respectively, much lower than reported activation energies of protein denaturation (200-600 kJ/mole) (Anglemier & Montgomery, 1976; Bertola et al., 1993). Therefore, protein denaturation should not be the

contributing factor for the texture changes in these two phases: in the high temperatures used in this study, most proteins should have been denatured in the first phase. The dominant contributing factors might include dehydration, aggregation, and disintegradation. Employing Differential Scanning Calorimetry (DSC) analysis, and microscopic examination of collagen and muscle fibers might help reveal the mechanisms involved in the nature of these protein changes that occur during these four phases.

Table 5-4. First-order kinetic parameters for shear force changes of salmon fillets after heat treatments at four temperatures

Texture phase	T (˚C)	k $(min^{-1})\times10^{-3}$	R^2	E_a (kJ/mol)	R^2
Second phase (rapid tenderization)	100	4.34	0.926	0.949	100.47
	111.1	18.53	0.957		
	121.1	23.89	0.966		
Third phase (slow toughening)	111.1	1.66	0.817	0.931	76.04
	121.1	2.31	0.976		
	131.1	5.42	0.921		

Effect of thiamin retention

Salmon is an important thiamin source. Apart from the nutritional significance, thiamin content is commonly used as an index of quality of canned low acid foods. Thiamin is one of the least thermostable water soluble vitamins. The measured thiamin content in the raw salmon (TM_0) was 2.03 μg/g tissue, which is in agreement with literature values ranging between 2 - 3 μg/g tissue (Hui et al., 2006). After heating, it was reduced to 0.64, 0.34, 0.18, and 0.15 μg/g at the end of longest tests at 100, 111.1, 121.1 and 131.1 ˚C. The rate of thiamin losses increased with an increase in temperature. Heat treatment caused thiamin oxidation and leaching of this water soluble vitamin into expressed fluid.

Table 5-5. Second-order kinetic parameters for thiamin retention of salmon fillets after heat treatments at four temperatures

T (˚C)	k (min^{-1})×10^{-3}	R^2	E_a (kJ/mol)	k_0(min^{-1})	R^2
100	5.9	0.966	105.23	3.47×10^{12}	0.990
111.1	17.9	0.974			
121.1	44.4	0.995			
131.1	77.5	0.989			

The thiamin retention data was best fitted to a second order reaction, and the temperature dependence of the rate constant can be also expressed by the Arrhenius equation. Table 5-5 shows these kinetic parameters. The high correlation coefficients ($R^2 > 0.96$) suggest that this model is satisfactory for describing the thiamin degradation in salmon products. The *Ea* and k_0 values are 105.2 kJ/mol (z-value of 27.4 ˚C) and 3.47×10^{12}/min. Although most previous study shows a first order kinetic for vitamin degradation (Taoukis, Labuza, & Saguy, 1997), second order kinetics have also been reported (Viberg, Ekstrom, Fredlund, Oste, & Sjoholm, 1997; Kessler & Fink, 1986). Kessler and Fink (1986) explained the thermal decomposition of thiamin in cow's milk with a second order reaction kinetics, and found the *Ea* and k_0 values to be 100.8 kJ/mol and 5.14×10^{11}/min, respectively, quite close to the present results. Based on the kinetic parameters and equations 6 and 8, equations 17 and 18 were proposed to estimate rate constant k and thiamin retention (TM_t/TM_0) at a specific heating temperature T for a given time t. Figure 5-5 shows the experimental data of thiamin retention fitted with model curves, and the Arrhenius plot. A good fit can be seen.

$$k = 3.47\times10^{12}\times e^{-\frac{105.23\times1000}{8.314\times(273+T)}} \qquad (17)$$

$$\frac{TM_t}{TM_0} = \frac{1}{k\cdot TM_0\cdot t+1} \qquad (18)$$

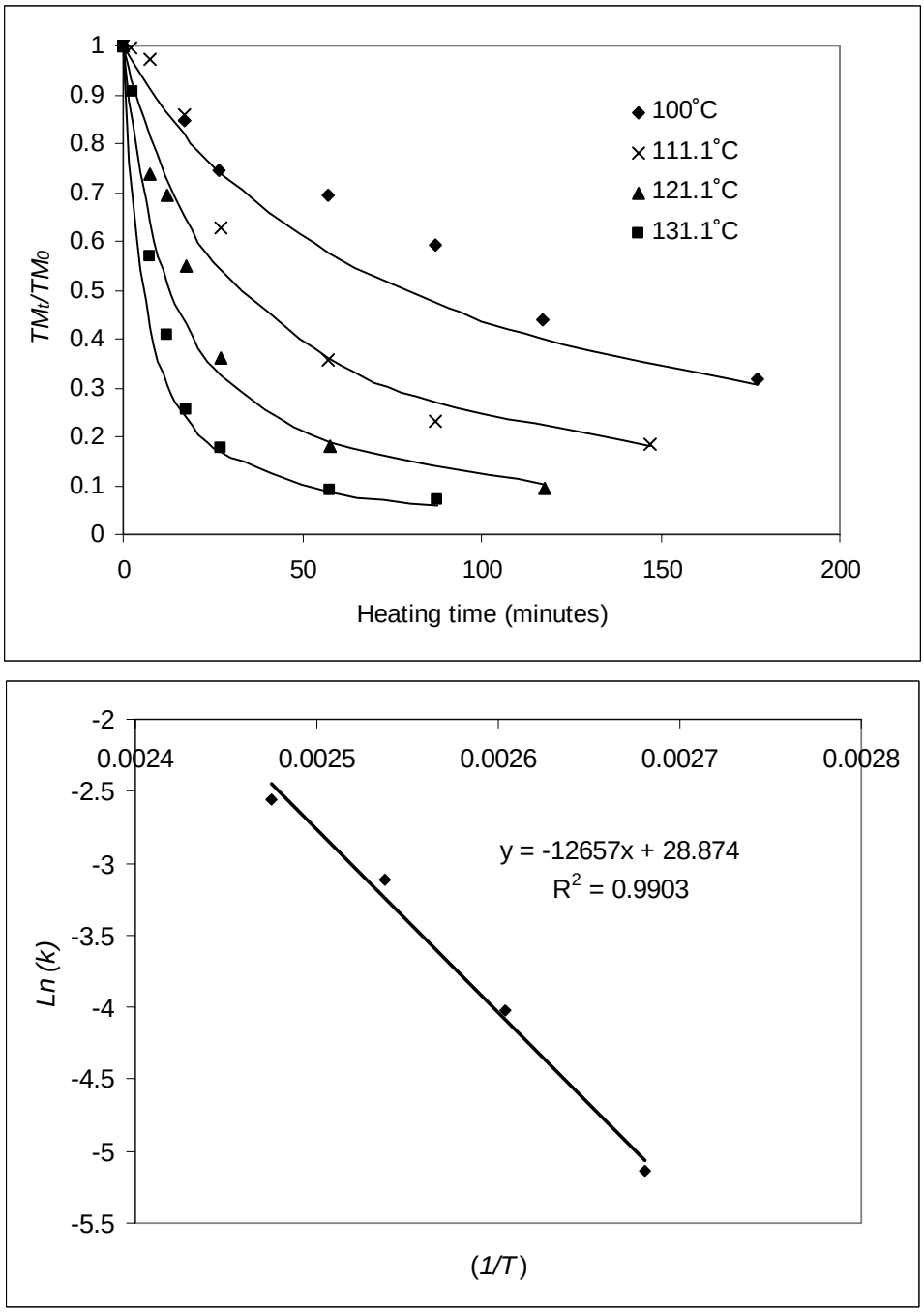


Figure 5-5. Top: The change of thiamin content of salmon fillet at different heating temperatures fitted with a second-order model. Bottom: Arrhenius plot showing the temperature dependence of rate constants.

Using a Weibull-log logistic model to model thiamin retention

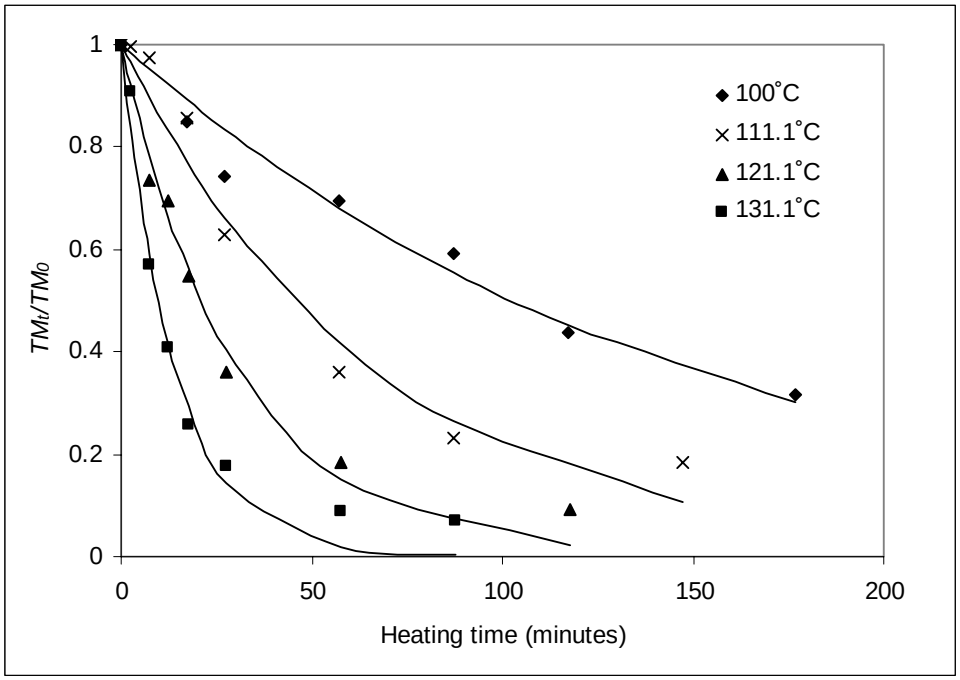
The thiamin degradation data were fitted to equations 9 and 10. At first, optimal *n(T)* and *b(T)* values corresponding to each temperature in equation 8 were derived using solver command in EXCEL software, and the four resultant *n* values were in a narrow range between 0.916 to1.007, with mean square error (MSE) in a order of 10^{-3} (Table 5-6). The MSE were comparable to literature values (Corradini & Peleg, 2004; 2006). When a fixed *n* value (1.003) was used for all temperatures, the corresponding MSE were very close to those for variable *n(T)*, indicating a possibility to further simplify the model. The normalized degradation curves and the fit of equation 9 using fixed power *n(T)* (1.003) are shown in Figure 5-6, also included is the fit of the temperature dependence of *b(T)* with the log logistic model. A good fit of the model can be observed, demonstrating that the Weibull-log logistic model was an adequate model for quantifying the isothermal decay pattern of thiamin in salmon fillets. Table 5-6 shows the degradation parameters using equations 9 and 10 as models. The *m* and T_c values are 0.078 $^{\circ}C^{-1}$ and 165 $^{\circ}C$, respectively.

Table 5- 6. Degradation parameters calculated with the Weibull-log logistic model

Temp (˚C)	*n(T)*	MSE $\times10^{-3}$	*n*	*b(T)* (min^{-n}))$\times10^{-3}$	MSE $\times10^{-3}$	*m* ($^{\circ}C^{-1}$)	T_c ($^{\circ}C$)	MSE $\times10^{-8}$
100	0.916	2.14	1.003	6.66	2.30	0.078	165	2.2
111.1	1.096	3.06	1.003	14.95	3.27			
121.1	0.973	2.55	1.003	32.57	2.56			
131.1	1.007	2.15	1.003	69.54	2.16			

The obtained parameters for the Weibull-log logistic model agree with reported studies on thiamin loss for other foods. Nisha, Singhal, and Pandit (2004) and Corradini and Peleg (2006) reported *n(T)*, *m*, and T_c of 0.83-1.05, 0.041-0.056 $^{\circ}C^{-1}$and 176 -190 $^{\circ}C$, respectively, depending upon pH values, for thiamin degradation in red gram splits (*Cajanus cajan L.*), an

important grain legume and a rich source of thiamin in India, during heating at temperatures between 50 – 120 ˚C. According to Peleg et al. (2002), T_c could be related to the minimum temperature (˚C) above which thermal degradation starts to accelerate, and m to the steepness of the rate–temperature relationship beyond T_c. Therefore, the higher m value and lower T_c in our study might indicate the thiamin in salmon fillets is more susceptible to thermal processing than in red gram splits.



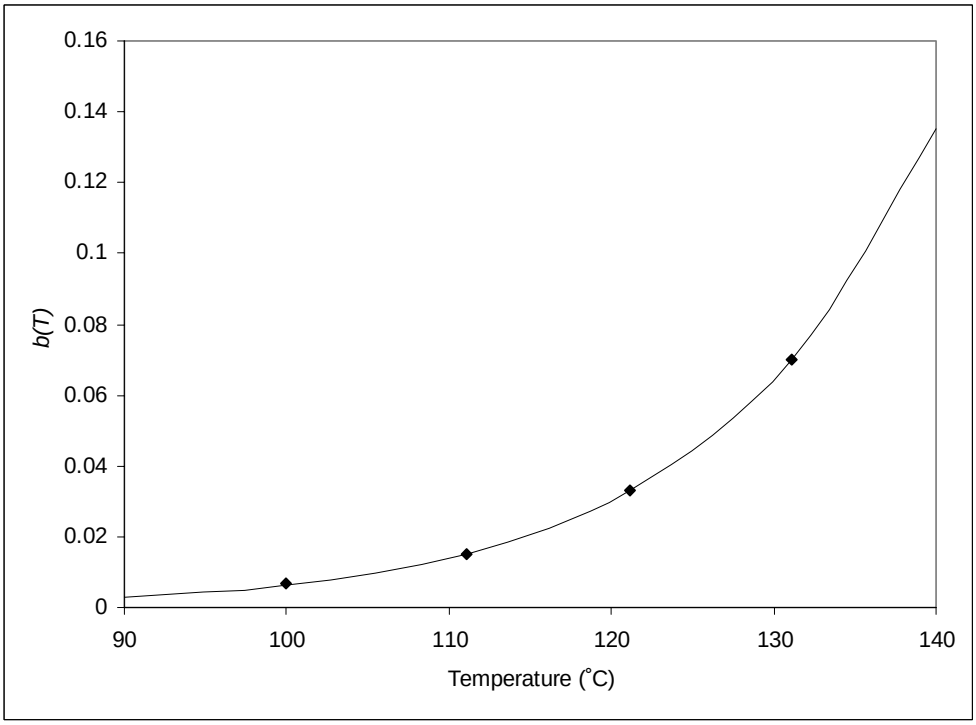


Figure 5-6. Top: The change of thiamin content of salmon fillet at different heating temperatures fitted with the Weibullian model. Bottom: The temperature dependence of *b(T)* fitted with the log logistic model.

Conclusions

The increase of cook loss of salmon fillet during high temperature heating followed a first order reaction, with the greatest amount of cook loss occurring within the first 30 minutes. High temperature caused the salmon muscle color to whiten in the first 10 minutes followed by browning as heating progressed, and the color changes in the browning phase can be fitted to a zero order reaction. There were four phases to the change in shear force observed as samples were heated at all the test temperatures, with the second and third phases exhibiting first order kinetics. However, further study is needed to fully understand the mechanisms involved. The degradation of thiamin in heated salmon fillets was second-order, with an *Ea* of 105.2 kJ/mol. Weibullian-power law and log logistic model provided adequate description of thiamin degradation data and the temperature dependence of *b(T)*, and the *m* and T_c values were 0.078 $^{\circ}C^{-1}$ and 165 $^{\circ}C$, respectively.

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CHAPTER 6. CHANGES IN THE TENDERNESS OF CHICKEN BREAST (*P. MAJOR*) AND SALMON (*O. GORBUSCHA*) MUSCLE DURING THERMAL PROCESSING: COOK LOSS, AREA SHRINKAGE, COLLAGEN SOLUBILITY AND MICROSTRUCTURE

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Abstract

Relationships among changes in chicken breast (*Pectoralis Major*) and pink salmon (*Oncorhynchus gorbuscha*) muscle were investigated for tenderness, cook loss, area shrinkage, collagen solubility and microstructure during thermal processing. Small white muscle samples (D 30 mm × H 6 mm) cut from pink salmon fillets and chicken breast were sealed in small aluminum containers (internal dimension: D 35 mm × H 6 mm) and heated in an oil bath at 121.1˚C for different time intervals up to 2 hours to simulate various thermal process durations. The changes in salmon tenderness had four phases (rapid toughening, rapid tenderizing, slow toughening and slow tenderizing), while that of the chicken breast only had two phases (rapid tenderizing and slow tenderizing). Twenty minutes was found to be a critical heating time in which > 85% collagen was solubilized and shear force reached a minimum. Cook loss and area

shrinkage were significantly ($P < 0.05$) correlated with shear force change for both the salmon and chicken, while collagen solubility was only significant for the chicken.
Keywords: Pink salmon; chicken breast; thermal processing; cook loss; area shrinkage ratio; collagen; tenderness; microstructure

Introduction

Thermally processed shelf-stable fish and chicken are an important food source, but they must be tender to achieve consumer satisfaction. Meat tenderness is defined as the ease of mastication, which involves initial penetration by the teeth, the breakdown of meat into fragments and the amount of residue remaining after chewing (Lawrie, 1998). Shear force value has often been used as an objective measurement of meat tenderness (Kong, Tang, Rasco, Crapo, & Smiley, 2006). Major proteins in muscle foods include myofibrillar proteins (myosin and actin), connective tissue proteins (mainly collagen) and sarcoplasmic proteins. The effect of heat on these proteins has a major influence on the resulting texture of the cooked meat, including denaturation, dissociation of myofibrillar proteins, transversal and longitudinal shrinkage of meat fibers, aggregation and gel formation of sarcoplasmic proteins and solubilization of connective tissue (Ofstad, Kidman, Myklebust, & Hermansson, 1993; Murphy & Marks, 2000; Tornberg, 2005; Wattanachant, Benjakul, & Ledward, 2005a, 2005b; Kong et al., 2006).

Factors affecting changes to meat tenderness during heating have been investigated by many researchers. Solubilization of connective tissue improved meat tenderness, while heat-denaturation of myofibrillar proteins generally caused toughening (Harris & Shorthose, 1988). Collagen characteristics, mainly content and solubility, determined the contribution of connective tissue to meat toughness. Shrinking of connective tissue exerted pressure on the

aqueous solution in the extracellular void and expelled water, and the cook loss was connected to tenderness and rigidity of tissue (Dunajski, 1979; Palka & Daun, 1999). Muscle fiber diameter and sarcomere length, as observed with scanning electron microscopy (SEM) and transmission electron microscopy (TEM), are closely related to flesh firmness (Hatae, Yoshimatsu, & Matsumoto, 1990; Hurling, Rodell, & Hunt, 1996; Sigurgisladottir, Sigurdardottir, Ingvarsdottir, Torrissen, & Hafsteinsson, 2001; Wattanachant, Benjakul, & Ledward, 2005a, 2005b). Denatured and aggregated sarcoplasmic proteins are also believed to contribute to firmness by forming coagulated interstitial material that obstruct or impede fibers movement (Hatae et al., 1990).

A previous study by the authors (Kong et al., 2006) showed that the shear force of pink salmon dorsal muscle during high temperature thermal processing has two peaks over a 2 hr treatment time at 121.1ºC, which were divided into four phases: 1) rapid toughening, when the shear force increased from raw muscle to the first peak; 2) rapid tenderization, when the shear force decreased from the first peak to a minimum value; 3) slow toughening, when the shear force increased again to a second peak; and 4) slow tenderization, when the tissue gradually became soft with prolonged heat. The four phases of shear force are due to a combined effect of different reactions (Kong et al., 2006).

It would be meaningful to examine if other types of meat exhibit the similar phenomenon during high temperature thermal processing and thus providing insights into the mechanisms behind these changes. We selected chicken breast muscle for the comparison, because it is somewhat similar to salmon dorsal muscle in that both are white muscle and contain lower collagen content than mammalian muscle tissue. However, compared to chicken breast muscle, fish contains lower amounts of collagen; the collagen is also significantly less cross-linked and

appears to be degraded more readily when heated (Bracho & Haard, 1990). In this study, the shear force of chicken breast muscle was compared with that of salmon subjected to the same heating conditions. The objective was to investigate tenderness changes in chicken breast and salmon during high temperature treatment, as related to changes in cook loss, area shrinkage, collagen and microstructure to gain insight into the mechanisms underlying heating-induced tenderness in muscle food products during thermal processes to produce low acid (pH < 4.5) shelf-stable products.

Material and Methods

Materials

Pacific pink salmon (*Oncorhynchus gorbuscha*) used for the study were female, from the same catch harvested in August 2005 near Kodiak, Alaska. The fish were 1,350 ± 100 g in weight, 370 ± 10 mm in length and 120 ± 10 mm in width, having similar color, firmness, odor and overall appearance. The fish were gutted, frozen, stored (-31˚C), shipped in cold storage (with ice pack) to Washington State University in Pullman, WA, and then stored in a freezer (-35˚C) until used in June 2006.

Fresh breasts (*Pectoralis major,* 180–250 g each) of Washington State grown broiler chicken were acquired from a local processing plant 3 days after the birds were sacrificed. The chicken breasts were frozen in a freezer (-35˚C) overnight before sampling was made.

Sampling and heating

To facilitate sampling, the frozen fish and chicken breast were partly thawed in a 4˚C refrigerator for 2 and 1 hr, respectively, so that the samples were softened enough to be cut off

easily using a slicer and a cutter. The sampling and heating of fish fillets and chicken breast followed the methods described in Kong et al. (2006). In brief, small disk-shaped samples (6 mm in thickness and 30 mm in diameter) were taken from the white muscle in the dorsal area of the salmon or the middle of the chicken breast muscle. The small size was chosen to reduce come-up time to improve uniform heating in an oil bath. The samples were taken using an electrical food slicer (Model 632, Chef'Choice Int., Germany) and a 30 mm diameter corer. They were then hermetically sealed in custom-designed cylindrical aluminum test cells with a 35 mm inner diameter, 6 mm inner height, and 2 mm wall thickness. Prior to sealing, a 0.1 mm diameter copper-constanstan thermocouple (Type-T) was inserted through a rubber gland in the lid of the container to measure the temperature at the geometrical center of the sample. Samples were then placed in a 121.1˚C oil bath (Model HAAKE W13, Thermo Electron Corp., Germany) using glycerol as the heating medium. During heating, signals from the thermocouple junctions were transferred to a computer equipped with a DLZe type data logger (DELTA-T Devices, Cambridge, England, UK). It took 2.5–3 min for sample center temperatures to reach within 1˚C of the total temperature rise. After heating, samples were immediately placed in ice upon removal from the oil bath. Since the sample temperature dropped to below 20˚C within 0.5 min, the thermal effect of the cooling step on product quality was considered insignificant. After cooling, the samples were dried with a filter paper, weighed on an analytical balance (Ohaus Analytical Plus, Pine Brook, NJ) and stored in a cooler (4˚C) for further analysis.

Experimental design

Samples taken from chicken and fish were pooled and heated at 121.1˚C for 2.5, 10, 20, 30, 90 and 120 min. Six sample replicates were used for each experimental treatment. After

heating, the physical properties (shear force, cook loss and area shrinkage) were determined on each sample. Representative samples were selected for SEM and TEM microscopy as described later. Samples subjected to the same heating conditions were pooled, homogenized in a blender and used for collagen and moisture analysis.

Physical analysis

Cook loss was calculated as the percent weight reduction of the cooked compared to the raw sample.

A multiple thin-bladed Kramer type texture fixture (MTB) developed in Kong et al. (2006) was used to determine the shear force of the raw and heated samples. Compared with a standard Kramer shear cell, the MTB used much thinner blades (0.5 mm vs. 3 mm), which reduced compression when cutting through the samples and led to more accurate and consistent results (Kong et al., 2006). The cell was fitted to a TA-XT2 texture analyzer (Stable Micro Systems Ltd., Surrey, England, UK) equipped with a load cell of 5 kg. Before shear force measurement, the raw and heated samples were allowed to equilibrate to room temperature, and then placed on a support base so that the muscle fibers were perpendicular to the blades. The traveling speed of the blades was 1 mm/s. Force-time graphs were recorded by a computer and analyzed using the Texture Expert for Windows (Version 1.15, Stable Micro Systems Ltd., London, UK), while shear force was measured as the peak height in the force-time profile.

A computer vision system (CVS) described in Pandit, Tang, Liu, & Pitts (2007) and Kong et al. (2006) was used to capture images of the fresh and cooked samples. Sample area was determined using Vision IMAQ Builder image processing software Version 6.1 (National

Instruments, Austin, TX). The area shrinkage ratio was calculated as the percent area reduction of cooked compared to the corresponding raw sample.

Longitudinal and transverse shrinkage ratios were determined as the percent reduction in the cooked sample length along and across the muscle fiber, respectively, compared to the raw sample. Maximum lengths of the raw and cooked samples at the two directions (Figure 6-1) were measured using the Vision IMAQ Builder image processing software, and the shrinkage ratios were calculated as follows:

$$Transverse\ shrinkage\ ratio = \frac{x1 - x2}{x1} \times 100\% \quad (1)$$

$$Longitudinal\ shrinkage\ ratio = \frac{y1 - y2}{y1} \times 100\% \quad (2)$$

where x1 and x2 were the diameters of the raw and cooked sample disks across the muscle fibers, respectively, and y1 and y2 were the diameters of the raw and cooked sample disks along the muscle fibers, respectively.

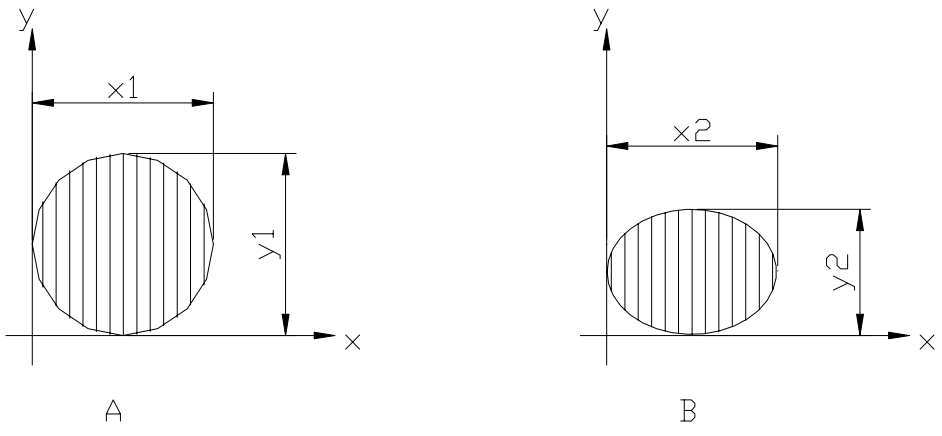

Figure 6-1. Raw and cooked sample shape. The parallel lines indicate muscle fibre. A: raw sample; B: cooked sample.

Moisture content was determined by vacuum drying a sample (3 – 5 g) at 65 ˚C to constant weight (Hart & Fisher, 1971).

Collagen

Total collagen (based on hydroxyproline content) was determined as described in Wattanachant, Benjakul, and Ledward (2004). Specifically, ground muscle samples (ca. 0.5 g) were hydrolyzed in 25 mL of 6 M HCI at 110°C for 24 hr. The hydrolyzed solutions were decolorized by activated charcoal and vacuum filtered through Whatman #2 filter paper. The filtrate was neutralized with 10 N NaOH, then diluted to 100 mL with distilled water. The hydroxyproline content in the hydrolysate was determined according to the procedure described in Bergman and Loxley (1963). The absorbance at 560 nm of samples and hydroxyproline standards was determined using a diode array spectrophotometer (Hewlett Packard 8452A). Hydroxyproline content was extrapolated from the standard curve and converted to collagen content using the following formula: collagen (mg/g sample) = hydroxyproline (mg/g sample) × 11.42 (salmon) (Eckhoff, Aidos, Hemre, & Lie, 1998) and 7.25 (chicken) (Wattanachant et al., 2004).

Soluble collagen for chicken was extracted according to the method of Wattanachant et al. (2004): 2 g muscle samples were homogenized with 8 mL of 25% Ringer's solution (32.8 mM NaCl, 1.5 mM KCl, and 0.5 mM $CaCl_2$). The 2,300 g homogenates were heated at 77˚C for 70 min and centrifuged for 30 min at 4˚C. The extraction was repeated twice, with supernatants combined. The sediment and supernatants were then hydrolyzed with 6 M HCl at 110˚C for 24 hrs. The collagen content of the sediments and supernatants were determined separately, with total collagen content as the sum of the collagen content in sediment plus that in the supernatant. The amount of heat-soluble collagen was expressed as a percentage of the total collagen.

As fish collagen can be degraded more readily when heated (Bracho et al., 1990), soluble salmon collagen was determined using a different method in which all operations were

performed in a 4ºC room (Eckhoff et al., 1998). The samples were subjected to preliminary extraction with a cold 0.1 N NaOH solution to remove non-collagen proteins. The alkali extraction procedures included homogenization of muscle with 10 volumes (v/w) 0.1 N NaOH, followed by centrifugation at 10,000 g for 20 min. The residue was added to 20 volumes of NaOH solution, stirred overnight and centrifuged at 10,000 g for 20 min. The NaOH addition and centrifugation steps were repeated three times. The final precipitate was washed with distilled water before centrifugation. Ten volumes (v/w) of 0.5 M acetic acid were added to the residue. The mixture was stirred for 2 days and centrifuged again at 10,000 g for 20 min, followed by collection of the supernatant and use as the acid-soluble collagen fraction (ASC). Pepsin-soluble collagen (PSC) was rendered soluble by limited digestion with porcine pepsin (Sigma-Aldrich, St. Louis, MO) at an enzyme: substrate ratio of 1:20 (w/w, wet weight) in 0.5 M acetic acid. Digestion was performed at 4°C for 2 days before another centrifugation at 10,000 g for 20 min. The final supernatant was the PSC, and the insoluble matter was the insoluble collagen (ISC). The collagen content of the ASC, PSC and ISC fractions were determined separately using the procedure described in Bergman et al. (1963), with total collagen content as the sum of the collagen content in ASC, PSC and ISC fractions. The amount of collagen in each fraction was expressed as a percentage of the total collagen.

Microscopy examination

Samples from raw and cooked salmon and chicken muscle (20 min and 120 min heating) were selected for microstructure examination using SEM and TEM. Pieces (2 × 2 × 2 mm) were excised from raw and cooked samples, and placed in a fixative containing 2.5% glutaraldehyde/2% paraformaldehyde in a 0.1 M phosphate buffer overnight at 4˚C. The specimens were then rinsed in phosphate buffer, post-fixed in 1% osmium tetroxide in phosphate buffer and dehydrated in a serial ethanol solution containing 30%, 50%, 70%, 95% and 100% ethanol for 15 min in each solution.

For SEM analysis, the ethanol in the dehydrated samples was removed with two changes of 100% acetone at 10 min for each, then another 10 min with a mixture of acetone and hexamethyldisilizane (HMDS) (1:1) followed by two changes of 100% HMDS. The HMDS was air-dried overnight in a fume hood. The samples were cut along and perpendicular to muscle fibers using a razor blade to produce longitudinal and transverse sections. The specimen fragments were then mounted on aluminum stubs, coated with gold and examined and photographed in a Hitachi S-570 SEM (Hitachi, Japan) using an accelerating voltage of 25 KV. Macrographs were taken at a magnification of 200× for transverse sections and 10,000× for longitudinal ones. Pictures of the transverse and longitudinal sections were analyzed using the Vision IMAQ Builder image processing software to characterize fiber diameter and sarcomere length. The total area of the 6–10 fiber bundles containing 10–20 muscle fibers each were added up, and the fiber diameters calculated from the fiber area. Sarcomere length was measured from randomly chosen muscle myofibers that contained ca. 100 sarcomeres.

For TEM analysis, the ethanol in the dehydrated samples was replaced with a mixture of acetone and ethanol (1:1) followed by 100% acetone. The samples were infiltrated with a

mixture of acetone and Spur resin (1 hr) followed by 100% Spurs overnight before being polymerized at 70°C for 24–48 hrs. The embedded material was sectioned (gold interference color) to around 80–100 nm using a Reichert-Jung ultratome, mounted on copper grids and stained using a solution of 4% uranyl acetate in ethanol for 10 min followed by an aqueous solution of Reynolds' lead (7 min). The stained material was then observed in a JEOL JEM1200EX-II TEM using an accelerating voltage of 100 k.

Statistical analysis

The data for quality attributes of raw and heated samples were compared using analysis of variance (ANOVA) and the general linear model procedure of the SAS System for Windows V8.01 (SAS Institute Inc., Cary, NC). Differences between group means were analyzed by Duncan's multiple-range test. Statistical significance was set at a 0.05 probability level. Correlation analysis between cook loss, shear force, area shrinkage and collagen solubility was conducted using the SAS program and the result expressed in a linear correlation coefficient matrix.

Results

Shear force

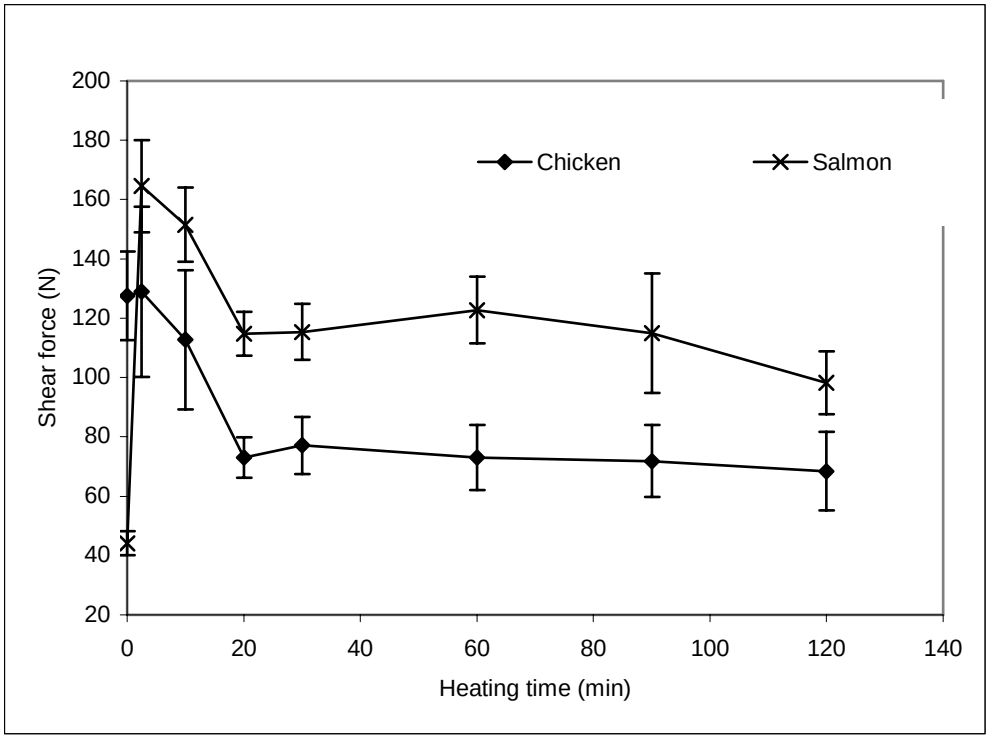

Figure 6-2. Shear force of raw and cooked chicken and salmon samples after heating at 121.1 ˚C for different time periods. Bars indicate the standard deviation for six determinations.

The effects of heat treatment on the measured shear force of chicken breast and salmon fillets are shown in Figure 6-2. For the raw muscle, the shear force of the chicken breast sample (127 N) was much higher than that for the salmon muscle sample (38 N). For both samples, changes in shear force were sharp in the first 20 min of heating and moderate thereafter. Shear force in the salmon experienced the four-phase changes of salmon fillets during high temperature thermal processing as reported previously (Kong et al., 2006). Specifically, the salmon muscle shear force rapidly increased from 38 N in the raw samples to a maximum of 165 N after 2.5 min heating at 121˚C (rapid toughening), then quickly decreased to a minimum of 115 N after 20 min (rapid tenderizing). After that, it significantly increased again to a second peak of 124 N after 1

hr of heating (slow toughening). The shear force then decreased consistently and reached 98 N after 2 hrs of heating (slow tenderizing). The shear force change in the chicken, however, showed only two phases, namely rapid tenderizing during which the shear force decreased from 127 N to 73 N (20 min), and slow tenderizing in which the shear force gradually ($P > 0.05$) decreased from 73 N to 68 N in 2 hrs.

Both fish and chicken samples had a minimum shear force after about 20 min; thereafter the shear force changed slowly, indicating that 20 min heating was a critical cooking time. Although the raw pink salmon tissue was more tender than the chicken breast, after heating, the cooked salmon was consistently tougher, with ca. 40 N higher shear force.

Cook loss

Figure 6-3 compares the cook loss of the chicken breast and salmon fillet samples. After heating, the cook loss increased rapidly to reach a plateau, which was higher for the chicken breast (30.6%) than salmon (23.4%). Most cook loss occurred within the first 20 min, which was 26.2% and 19.1% for chicken and salmon, respectively. An additional cook loss of 4.4% and 4.3% occurred after the first 20 min of heating, representing 14.4% and 18.4% of total cook loss for the chicken and salmon, respectively. Most cook loss was water. After 2 hrs of heating at 121˚C, the moisture in the chicken and salmon samples decreased from 73.43% (wet basis) and 74.15% in the raw muscle to 67.12% and 68.90%, respectively.

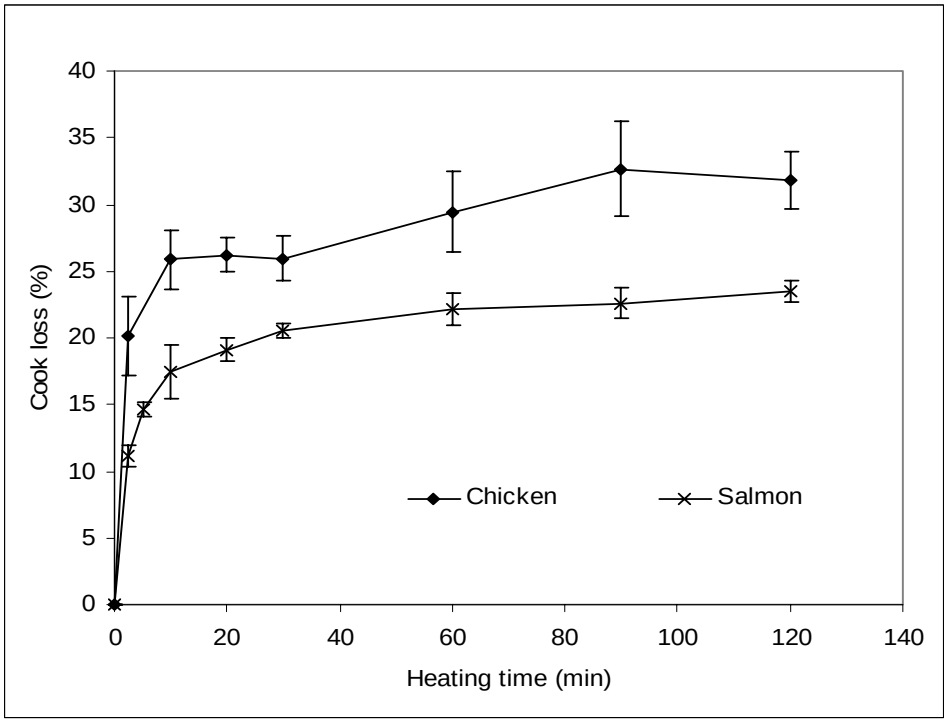

Figure 6-3. Cook loss of salmon and chicken breast muscle after heating at 121.1 ˚C for different time periods. Bars indicate the standard deviation for six determinations.

Area shrinkage ratio

Figure 6-4 shows area shrinkage for both salmon and chicken during heating at 121.1˚C over the 2 hr heating period. Similar to cook loss, the muscle shrank rapidly during the first 20 min, followed by a plateau. The area shrinkage ratio at the end of the heating was much higher in the chicken (37.3%) than the salmon (23.6%). Around 34% and 19% shrinkage for chicken and salmon, respectively, occurred in the first 20 min. An additional 3.3% and 4.6% shrinkage, representing 8.8% and 19.5% of the total area shrinkage ratio in the chicken and salmon, respectively, occurred after the first 20 min of heating.

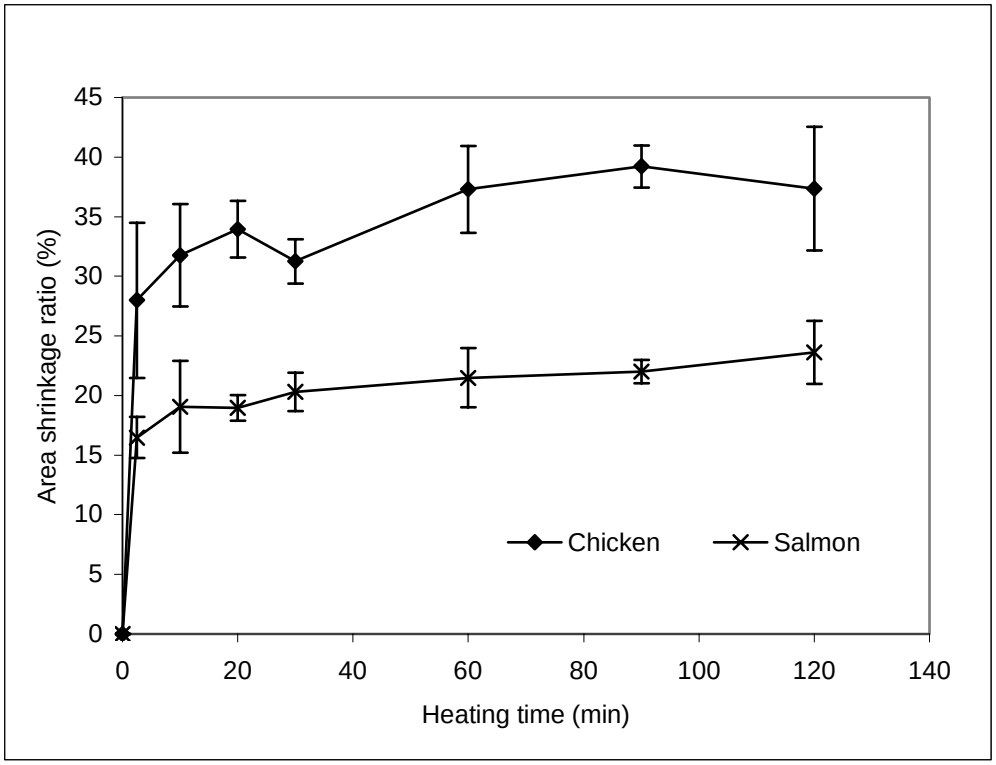

Figure 6-4. Area shrinkage ratio of salmon and chicken breast muscle after heating at 121.1 ˚C for different time periods. Bars indicate the standard deviation for six determinations.

Figure 6-5 shows changes in longitudinal and transverse shrinkage ratios for both salmon and chicken muscle samples, indicating the shrinkage mainly occurred along muscle fiber. For example, chicken had a 27% and 17% shrinkage ratio in longitudinal and transverse directions, respectively, after 2 hrs of heating at 121.1˚C. For salmon, 2 hrs of heating at 121.1˚C caused 20% longitudinal shrinkage, but only 2% transversally. These results suggest shrinkage is more parallel to fiber direction on salmon than chicken.

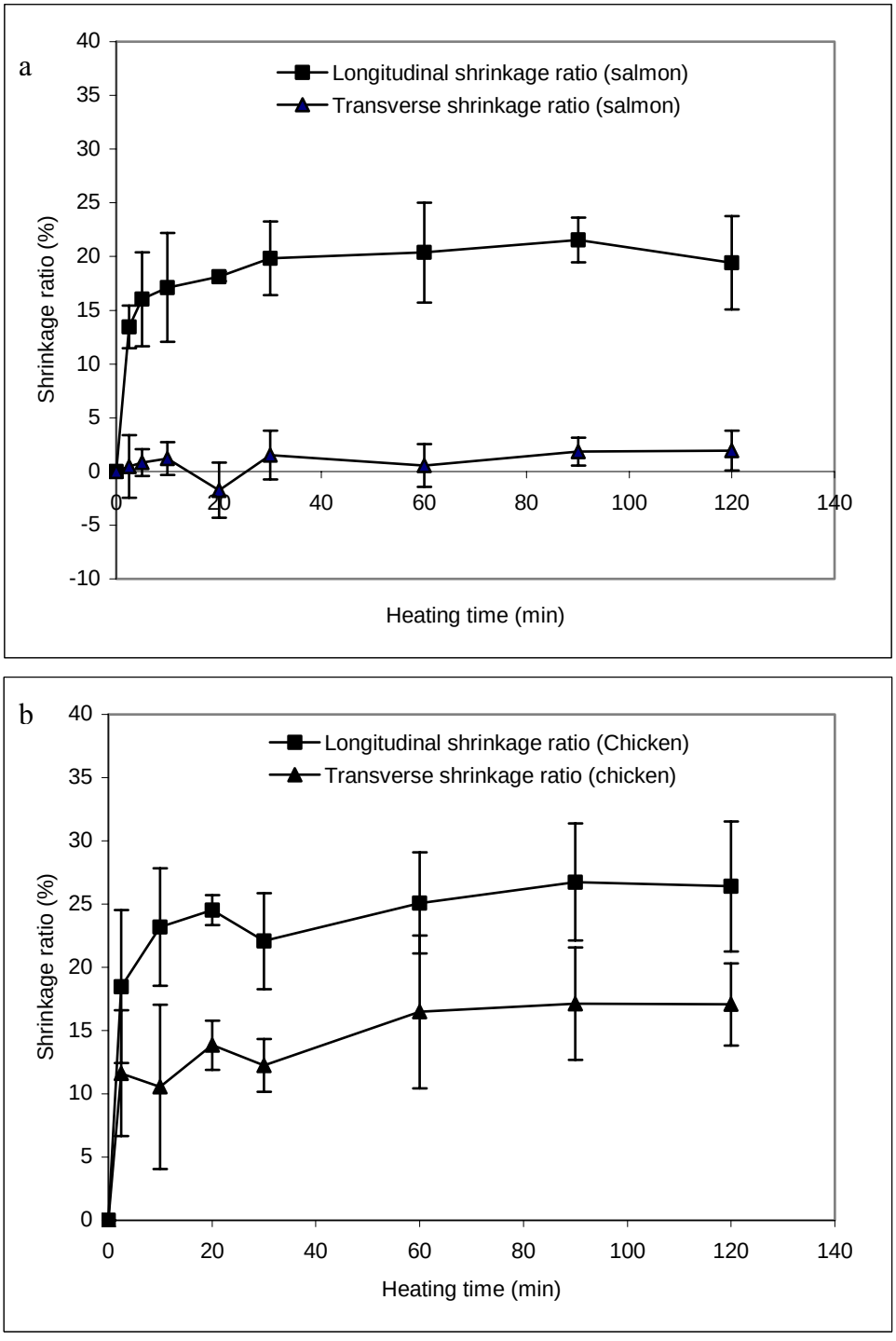

Figure 6-5. Shrinkage ratio at longitudinal and transverse directions in pink salmon (a) and chicken breast (b) after heating at 121.1 ˚C for different time periods. Bars indicate the standard deviation for six determinations.

Collagen

Table 6-1. Soluble collagen content (expressed as percentage of total collagen) in the samples of chicken breast and salmon fillet heated at 121.1˚C for different time [a]

Heating time (min)	Chicken breast (%)	Salmon dorsal muscle			
		ASC (%)	PSC (%)	ISC (%)	Total soluble (ASC+PSC)(%)
0 (Raw)	36.1±0.5[d]	5.13±0.29[a]	84.8±1.1[b]	10.1±0.6[a]	89.9±1.3[a]
10	69.0±0.9[c]	2.02±0.62[b]	88.7±1.8[a]	9.24±1.08[ab]	90.8±1.1[a]
20	82.6±2.2[b]	1.62±0.39[b]	90.0±1.8[a]	8.37±0.76[b]	91.6±2.6[a]
60	84.2±1.0[ab]	1.81±0.12[b]	91.9±0.3[a]	6.25±0.72[c]	93.8±0.2[a]
120	86.1±1.3[a]	1.91±0.46[b]	91.4±1.7[a]	6.70±0.15[c]	93.3±2.2[a]

[a] ASC: Acid-soluble collagen; PSC: pepsin-soluble collagen; ISC: insoluble collagen content. Different letters in the same column indicate significant differences ($P < 0.05$). Values are means (n= 2) ± standard deviation (SD).

Total collagen contents were 5.13 ± 0.22 and 2.60 ± 0.13 g/kg (wet basis) in raw chicken and salmon, respectively. The soluble collagen content in raw salmon was 5.1%, 84.8% and 10.1% in ASC, PSC and ISC fractions, respectively (Table 6-1). The total soluble collagen content (ASC + PSC) in salmon was 89.9%, which was much higher than that in chicken breast (36.1%), indicating that chicken collagen is richer in crossed bonds, and therefore of greater stability. The results confirm that salmon, like other fish species, have a unique collagen with less cross-links and higher solubility in dilute acid compared to the collagen in hot-blooded animals (Bracho et al., 1990; Aidos, Lie, & Espe, 1999). Similarly, Eckhoff et al. (1998) reported the collagen content of Atlantic salmon at 2.9 g/kg, and the ASC, PSC and ISC at 6%, 93% and 1%, respectively. In contrast, Wattanachant et al. (2004) reported 5.09 and 3.86 g/kg collagen content and 22.2% and 31.4% collagen solubility for Thai indigenous and commercial broiler chicken breast muscles, respectively.

The collagen solubility significantly increased with heating time for both chicken and salmon, and most changes occurred within the first 20 min (Table 6-1). The soluble collagen content in chicken muscle increased rapidly from 36.1% in the raw tissue to reach a plateau (82.6–86.1%) at 20 min, indicating that most collagen had been solubilized and gelatinized after 20 min of heating, with only a small proportion of the fiber matrix remaining (14–18%). For salmon muscle, heating caused reductions in the ASC and ISC fractions, but an increase in the PSC. The total soluble collagen (ASC + PSC) had a slight but insignificant increase (from 89.9% to 93.3%) over the entire heating time. During the first 20 min of heating at 121˚C, the ASC decreased from 5.1% to 1.6%, ISC decreased from 10.1 to 8.4%, but PSC increased from 84.8% to 90%. The significant increase in the PSC fraction indicates an increase in the proportion of non-helicoid regions vs. the helicoid ones as a result of heat denaturation on helicoid regions (Suárez, Abad, Ruiz-Cara, Estrada, & García-Gallego, 2005).

Microscopy examination

The changes in microstructure of raw and cooked muscles (20 min and 120 min heating) for both chicken breast and salmon fillet are presented in Figures 6-6 to 6-8. On the transverse sections (Figure 6-6), gaps between muscle fibres were visible in cooked samples due to solubilization and gelation of collagen (perimysium and endomysium) (Figure 6-6 E & F).

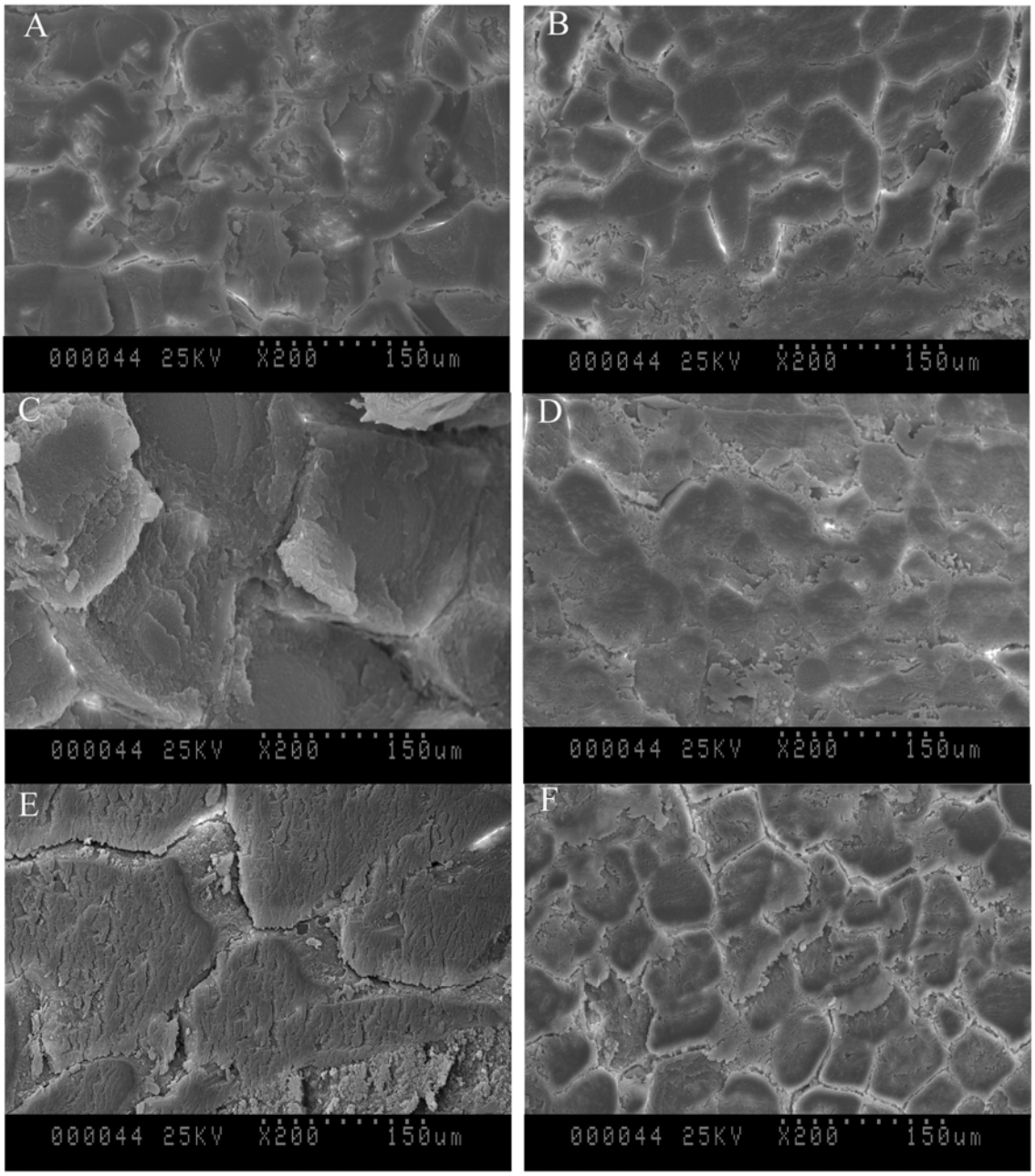

Figure 6-6. SEM (200X) images showing transverse section of raw and cooked muscle after heating at 121.1 ˚C for different time periods. A, C, E: raw, 20 min and 120 min cooked salmon muscle; B, D, F: raw, 20 min and 120 min cooked chicken muscle.

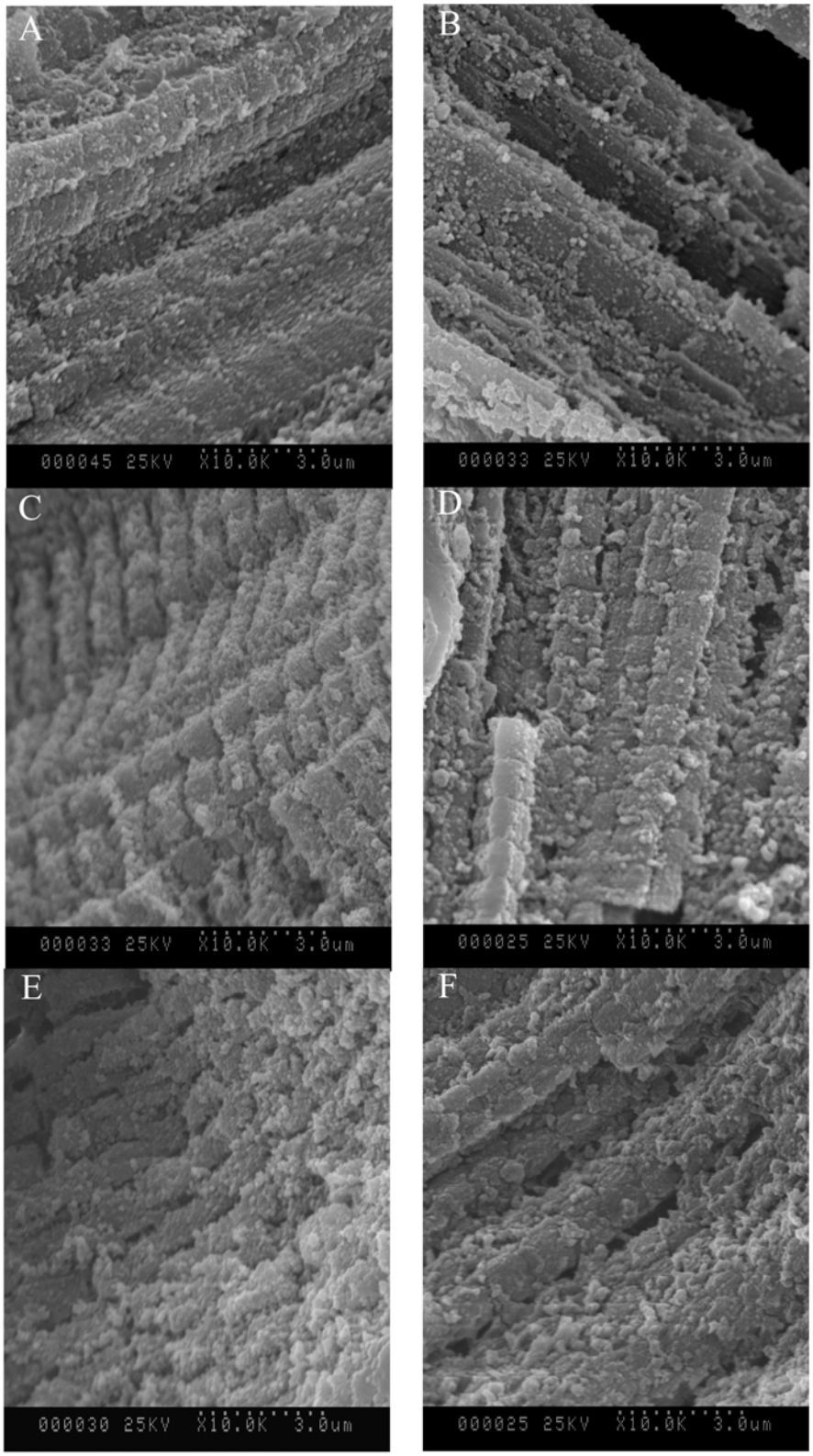

Figure 6-7. SEM (10,000X) images showing myofibre of raw and cooked muscle after heating at 121.1 ˚C for different time periods. A, C, E: raw, 20 min and 120 min cooked salmon muscle; B, D, F: raw, 20 min and 120 min cooked chicken muscle.

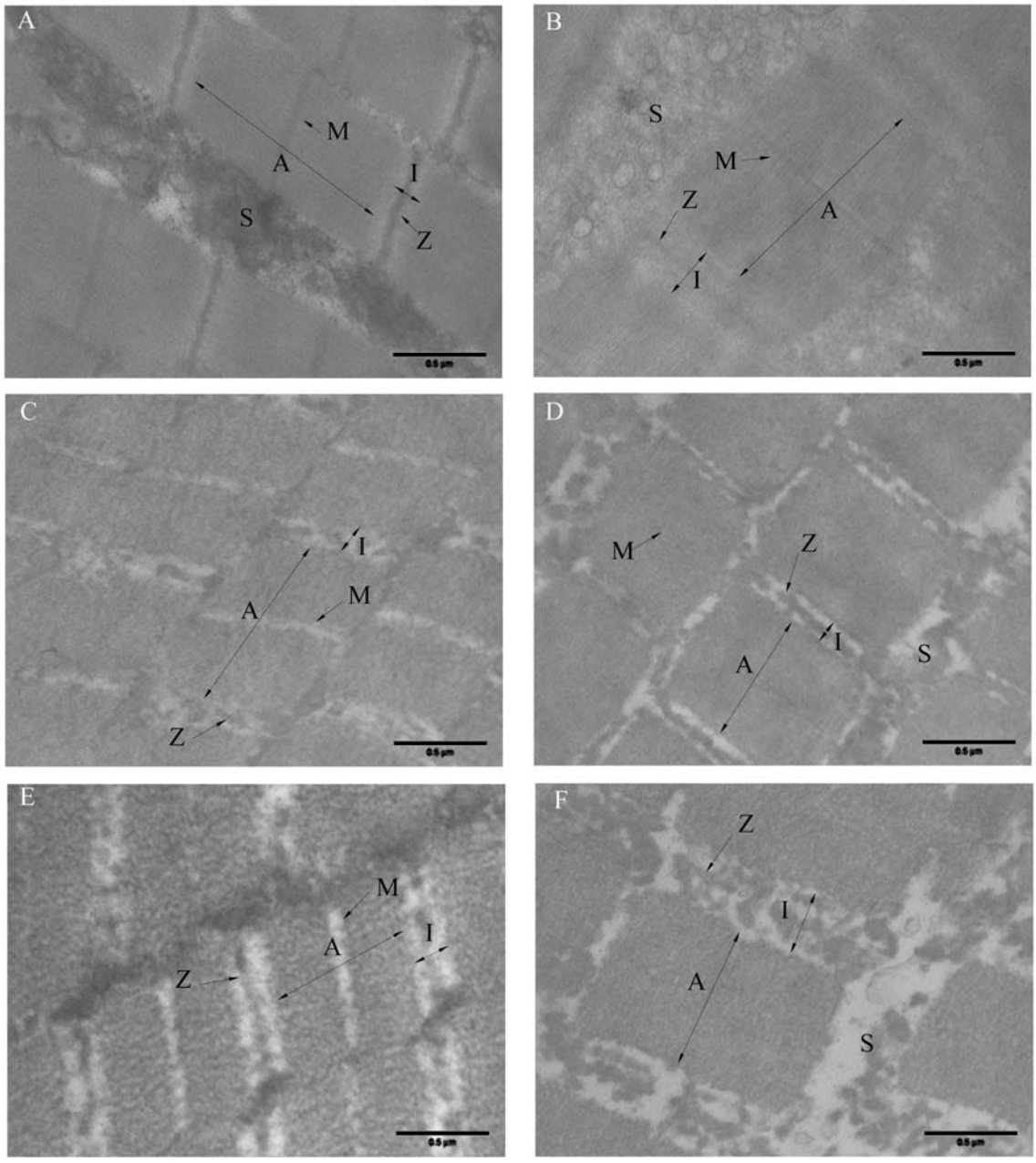

Figure 6-8. TEM (50,000X) image showing sarcomere of raw and cooked muscle after heating at 121.1 ˚C for different time periods. Bars indicate 0.5 μm. A, C, E: raw, 20 min and 120 min cooked salmon muscle; B, D, F: raw, 20 min and 120 min cooked chicken muscle. The black letters A, I, M, Z, S indicate A band, I band, M line, Z line and sarcoplasmic reticulum.

The fiber diameters of raw and cooked salmon and chicken muscle are shown in Table 6-2. A small increase (ca. 9%) was observed in the fiber diameter of chicken, from 49.7 μm in the raw tissue to 54 μm after 2 hrs of heating. A more significant increase was shown in the salmon muscle, in which the fiber diameter increased from 72.1 μm in the raw tissue to 127 μm after 2 hrs of heating, corresponding to a 76% increase. The swelling of fish muscle fiber can be clearly seen when comparing Figure 6-6A, C and E. The average diameter of white fibers in raw chicken muscle has been variously reported from 32.6 to 68.2 μm (Wattanachant et al., 2005a).

Table 6-2. Sarcomere length and fiber diameter in the samples of chicken breast and salmon fillet heated at 121.1˚C for different time[a]

Heating time (min)	fibre diameter (μm)		Sarcomere length (μm)	
	salmon	Chicken breast	Salmon	Chicken breast
0 (Raw)	72.14±8.48[b]	49.68±3.92[b]	1.60±0.07[a]	1.36±0.08[a]
20	101.2±10.3[a]	60.95±9.23[a]	1.09±0.13[b]	0.99±0.03[c]
120	127.0±24.7[a]	53.98±8.96[ab]	1.08±0.05[b]	1.11±0.10[b]

[a] Different letters in the same column indicate significant differences ($P < 0.05$). Values are means (n= 100) ± standard deviation (SD).

In the raw chicken and salmon muscle, myofibrils were closely packed and covered by a thin, thread-like pericellular layer surrounding the compact cells (Figure 6-7A and B). After cooking, the collageneous tissue denatured and melted, muscle cells ruptured, the sarcomere shrunk and the extracellular space and intracellular cavities and canals increased (Figure 6-7D–F). Granulates of protein aggregates appeared in the extracellular space (Figure 6-7D and F). After 120 min heating at 121.1˚C, myofibrils retained their peripheral ribbon-like shape (Figure 6-7E and F). Sarcomere lengths for the raw salmon and chicken muscle were 1.60 and 1.36 μm, respectively, but were significantly reduced to 1.08 and 1.11 μm after 120 min of heating, corresponding to a 32% and 24% shortening, respectively (Table 6-2). Most sarcomere shortening occurred within the first 20 min of heating. Sigurgisladottir et al. (2001) reported sarcomere length in raw fish muscle between 1.5 and 2.2 μm depending on fish species.

Wattanachant et al. (2005b) reported sarcomere lengths between 1.56 and 1.64 μm for the raw muscle of Thai indigenous and broiler chicken, and 0.99 to 1.35 μm after cooking at 80°C for 10 min.

The TEM images for longitudinal sections of chicken and salmon muscle are shown in Figure 6-8. Figure 6-8A and B indicates the ultrastructure of the fresh muscle has intact sarcomeres with clear A and I bands and M and Z lines, and sarcoplasmic reticulum surrounds the sarcomeres. The actin and myosin can also be distinguished. After heating, denaturation and aggregation of myosin and actin caused sarcomere shrinkage (Figure 6-8C–F) and the cooked meat to appear grainy. As a result of shrinkage of myosin and actin, I band was enlarged and A band shrunk, producing gaps and discontinuity between sarcomeres. After 120 min heating at 121.1˚C, the typical structure of the sarcomere was still recognizable and the A and I bands and Z lines still visible (Figure 6-8E and F). A large gap was formed along the M line in the sarcomere of salmon (Figure 6-8C and E), while this was not obvious in the chicken breast (Figure 6-8D and F). Coagulated sarcoplasmic proteins can be seen in both the inter- and intracellular spaces. Ofstad et al. (1993) have shown that aggregated sarcoplasmic proteins and collagen can form a gel that glues the fibers and fiber bundles together, holding water and/or plugging the intercellar capillaries to prevent water from being released. The stability of cooked meat relies on the gel network formulated with the melted collagen, denatured and aggregated myofiber proteins and sarcoplasmic proteins (Tornberg, 2005).

Discussion

Compared to the salmon, the cooked chicken samples had lower moisture content (67.12% vs. 68.90%), higher insoluble collagen (Table 6-1), a higher shrinkage ratio (Figure 6-4) and smaller fiber diameter (Table 6-2). Although these properties are correlated with tougher

meat (Dunajski, 1979; Tornberg, 1996), in our study, the cooked chicken was consistently more tender (ca. 40 N lower in shear force) than the cooked fish muscle. This was likely caused by 1) the different properties of myofibrillar proteins and 2) the higher sarcomere shortening in salmon (32%) than chicken (24%) during heating, which contributed to a larger shearing resistance in salmon muscle. According to Palka et al. (1999) and Wattanachant et al. (2005b), sarcomere contraction and shortening are positively correlated with meat toughness.

The change in sarcomere length mainly contributed to longitudinal shrinkage, whereas that in fiber diameter was more related to transverse shrinkage. As discussed earlier, the shortening of sarcomere lengths in salmon and chicken reached between 24 and 32% after 120 min heating at 121.1˚C (Table 6-2), which was in good agreement with the longitudinal shrinkage ratio of the samples (20–27%) (Figure 6-5). On the other hand, the same length of heating resulted in a fiber diameter increase in both chicken (9%) and salmon muscle (76%), while they shrunk transversely by 17% and 2%, respectively (Table 6-2 and Figure 6-5). The simultaneous occurrence of the increase in fiber diameter and transverse shrinkage for both chicken and salmon indicates that the shrinkage of collagen, particularly perimysium, might be the main reason for the transverse shrinkage. Perimysium constitutes some 90% of intramuscular connective tissue, and is believed to be the main factor affecting the contribution of connective tissue to toughness (Light, Champion, Voyle, & Bailey, 1985).

Figure 6-5 and Table 6-2 show much more shrinkage of salmon muscle along the fiber than transversally compared to the chicken muscle. According to Lepetit, Grajales, & Favier (2000), the extent of muscle fiber deformation during cooking depends on the compression stress applied by collagen fibers and the resistance of the muscle fibers to compression, while the compression force applied by collagen networks on muscle fiber bundles depends on the amount

of collagen present and its thermal solubility. Compared to chicken breast, cooked salmon muscle contains lower content of collagen with higher solubility, and the muscle fibers have higher resistance, resulting in more parallel shrinkage and transverse expansion along the fibers. Similarly, Wattanachant et al. (2005a) found the shrinkage in cooked broiler muscle more parallel to the fibers than transversally compared to that in Thai indigenous chicken muscle.

The shear force for the raw salmon muscle (38 N) tested for this study was much higher than that in the raw chicken breast (127 N), probably as a result of lower collagen content (2.60 vs. 5.13 g/kg wet tissue) and higher collagen solubility (Table 6-1) in the raw salmon muscle. After cooking, the apparent shear force change in salmon and chicken was a combined effect of different reactions: toughening caused by denaturation and aggregation of the proteins and the subsequent shrinkage and dehydration, and a tenderizing effect caused by solubilization and gelation of collagen. These changes were quantitatively characterized by measuring cook loss, area shrinkage ratio and collagen solubility in the cooked muscle.

Correlation analyses were conducted to evaluate the relationships among different variables including shear force, cook loss, collagen solubility and shrinkage ratios in the area and longitudinal and transverse directions. Except for shear force, the data used for correlation analyses of other variables covered the entire heating time from 0 to 2 hrs. Heating in the first 2.5 min was non-isothermal, and denatured most proteins, which had a rapid toughening effect on the salmon muscle. To simplify the correlation analysis of shear force, data from 2.5 min to 2 hrs of heating were used (excluding that for raw muscle), which corresponded to an isothermal heating period. Tables 6-3 and 6-4 show the correlation matrix results for both chicken and salmon muscle.

Table 6-3. Correlations among shear force, cook loss, shrinkage ratio and collagen solubility for chicken breast [a]

	Shear force	Cook loss	A-shrinkage	T-shrinkage	L-shrinkage	Collagen solubility
Shear force	1					
Cook loss	-0.8161*	1				
A-shrinkage	-0.8028*	0.9922****	1			
T-shrinkage	-0.7679*	0.9732****	0.9753****	1		
L-shrinkage	-0.8227*	0.9919****	0.9951****	0.9609***	1	
Collagen solubility	-0.9946**	0.9739**	0.9789**	0.9941***	0.97012**	1

[a] A-shrinkage: Area shrinkage ratio; T-shrinkage: transverse shrinkage ratio; L-shrinkage: longitudinal shrinkage ratio. * P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; ns = no significant difference.

Table 6-4. Correlations among shear force, cook loss, shrinkage ratio and collagen solubility for chicken breast [a]

	Shear force	Cook loss	A-shrinkage	T-shrinkage	L-shrinkage	PSC	ASC	ISC
Shear force	1							
Cook loss	-0.9118**	1						
A- shrinkage	-0.8869**	0.9667****	1					
T- shrinkage	ns	ns	ns	1				
L- shrinkage	-0.8297*	0.9746****	0.9812****	ns	1			
PSC	ns	0.9731**	0.9504*	ns	0.9535*	1		
ASC	ns	-0.9649**	-0.9696**	ns	-0.9877**	-0.9074*	1	
ISC	ns	ns	ns	ns	ns	-0.9251*	ns	1

[a] A-shrinkage: Area shrinkage ratio; T-shrinkage: transverse shrinkage ratio; L-shrinkage: longitudinal shrinkage ratio. * P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; ns = no significant difference.

From 2.5 min to 2 hrs of heating, the shear force of chicken breast muscle was negatively correlated with cook loss (r = -0.82), the area shrinkage ratio (r = -0.80) and collagen solubility (r = -0.99) (Table 6-3). The shear force of salmon muscle was negatively correlated with cook loss (r = -0.91) and the area shrinkage ratio (r = -0.89), but was not significantly correlated with ISC, ASC or PSC (Table 6-4). These results confirm that collagen is relatively unimportant in fish

after cooking compared to that in hot-blooded animals (Hatae, Tobimatsu, Takeyama, & Matsumoto, 1986). Following cooking, the muscle fibers themselves provide the main resistance to mastication (Dunajski, 1979).

This study showed that cook loss and area shrinkage were highly correlated with collagen solubility (Tables 6-3 and 6-4). For chicken, collagen solubility significantly affected cook loss ($r = 0.97$) and area shrinkage ratio ($r = 0.98$). For salmon, both cook loss and the area shrinkage ratio were positively affected by PSC ($r = 0.97$) but negatively correlated with ASC ($r = -0.96$). The higher cook loss and area shrinkage ratio in the chicken than salmon (Figures 6-3 and 6-4) might be explained by its higher amount of collagen with more cross-links that compelled more water out of the cooked muscle.

Tables 6-3 and 6-4 also indicate that the shear force of chicken was negatively correlated with both the longitudinal ($r = -0.82$) and transverse ($r = -0.77$) shrinkage ratios, while that of salmon was only significantly correlated with the longitudinal ($r = -0.83$) shrinkage ratio. For both chicken and salmon, cook loss was positively correlated ($r = 0.97$) with the area shrinkage ratio. These results confirm the significant correlations among cook loss, collagen solubility and shear force reported in the literature (Ofstad, Kidman, & Hermansson, 1996; Murphy et al., 2000; Sigurgisladottir et al., 2001; Kong et al., 2006).

The different shear forces we found in chicken and salmon muscle samples during heating were related to changes in cook loss, shrinkage and collagen solubility. For chicken muscle, as a result of the high content of strongly cross-linked collagen and low shearing resistance of denatured muscle fibers, the rapid solubilization of collagen (from 36% to 82.6% within the first 20 min of heating) dominated the reactions, so the net effect was a rapid tenderizing (Figure 6-2). At 20 min, most of the solubilization of collagen, cook loss and area

shrinkage were completed, so prolonged heating (from 20 min to 2 hrs) did not cause significant change in the shear force values for chicken.

The initial rapid toughening phase for the salmon muscle was characterized by a more profound toughening than tenderization after heating because of low collagen content, few cross-bonds and high shearing resistance of the denatured muscle fibers. After anywhere from 2.5 to 5 min of heating, most proteins in the salmon were denatured and the gelation and melting of collagen dominated the reactions, resulting in a rapid tenderizing phase (Figure 6-2). The third phase (slow toughening from 20 to 60 min), during which shear force slightly increased, resulted from substantial dehydration and area shrinkage. As stated earlier, compared to chicken, salmon had a higher proportion of shrinkage (19.5% vs. 8.8% of total area shrinkage ratio) and cook loss (18.4% vs. 14.4% of total cook loss) after 20 min, which could explain its toughening. However, after 60 min of heating, muscle disintegration and fragmentation softened the texture of both the salmon and chicken (Figure 6-2).

The critical heating time for both chicken and salmon muscle was found to be 20 min, as it marked the endpoint of the rapid tenderizing phase; thereafter the shear force (Figure 6-2), collagen solubility (Table 6-1), cook loss (Figure 6-3) and area shrinkage (Figure 6-4) did not change much. After 20 min, the denatured sarcoplasmic and myofibrillar proteins and melted collagen formed an aggregates gel that reduced cook loss (Ofstad et al., 1993; Tornberg, 2005).

Conclusions

Although raw salmon muscle is more tender than chicken due to lower collagen content and less collagen cross-linkage, after cooking it was found to be consistently tougher due to a higher shearing resistance of denatured muscle fibers. The critical heating time (20 min) in which shear force reached a minimum was due to the dominant tenderizing effect caused by collagen solubilization. The difference in the shear force of chicken and salmon muscle during heating was a combined effect of various interactions quantitatively characterized by cook loss, area shrinkage and collagen solubility. A significant increase in fiber diameter and decrease in sarcomere length were obtained for both salmon and chicken muscle during high temperature thermal processing, with salmon muscle exhibiting more shrinkage parallel to muscle fiber than chicken. The mechanisms that caused the different muscle fiber resistance in salmon and chicken remain to be studied.

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CHAPTER 7. CONCLUSIONS AND RECOMMENDATIONS

In this study, quality changes of salmon fillet muscle during thermal commercial sterilization processes were investigated. The quality properties studied included shear force, color, cook loss, area shrinkage, lipid composition and thiamin content. Small samples (D 30 mm × H 6 mm) were used to reduce come up time and improve uniform heating. WSU test cell and multiple thin blade (MTB) texture devices were developed for sample heating and shear force measurement. A Computer Vision System (CVS) was used to measure sample color and area. Quality variations along the longitudinal axis of salmon fillets (raw and heated) were examined. Effects of salt addition on quality changes were also examined. Kinetics of reactions leading to changes in salmon quality during thermal processing were determined. Relationships between the change in meat tenderness and cook loss, area shrinkage, collagen solubility and microstructure were investigated by comparing the changes of Washington State grown broiler chicken breast (*Pectoralis Major*) and Pacific pink salmon (*Oncorhynchus gorbuscha*) muscle during heating.

Conclusions

- The multiple thin blade (MTB) device provided reliable measurements for salmon muscle tenderness, and the computer vision system (CVS) served as a suitable mean for measuring the color and area of salmon muscle.

- The red muscle content and property in the raw salmon tissue was a crucial factor influencing the shear force of the raw muscle, but this effect diminished after heating.

- Muscle from the central dorsal region exhibited less cook loss and shrinkage during heating, and yielded more reproducible results than tissue samples taken from the head and tail regions of the pink salmon fillet.

- When salmon muscle (without added salt) was heated at 121.1 ˚C for 2 hours, the area shrinkage ratio and the cook loss increased to reach 25% and 20%, respectively.

- A fractional conversion model could be developed to describe the increase in cook loss during heating; and a quadratic relationship to correlate cook loss with area shrinkage ratio.

- Color changes (CIE L^*, b^* and ΔE) involved whitening and browning phases. In the browning phase, the changes of CIE L^*, b^* and ΔE followed a zero order reaction.

- The progressive change of salmon shear force during heating had four different phases (rapid toughening, rapid tenderizing, slow toughening and slow tenderizing), and the second (rapid tenderizing) and third phases (slow toughening) were modeled using a first order reaction.

- Salt addition (1.5% w/w) reduced cook loss, area shrinkage, and shear force of heated salmon fillet muscle and resulted in a slightly darker color.

- A slight amount of lipid oxidation occurred after heating at 121.1 ˚C reflected by increases in both peroxide values (PV) and thiobarbituric acid values (TBA) within the first 10 min of heating.

- Salt addition did not affect thiamin loss, degree of lipid oxidation, and fatty acid profile. No measurable loss of polyunsaturated fatty acid (PUFA) was observed during the thermal treatments.

- Canned pink salmon can be a valuable source of omega-3 PUFA with DHA values ranging from 258 to 340 mg/100 g of product, and EPA values ranging from 52 to 71 mg/ 100 g of product.

- More than 90% of the thiamin was destroyed after 2 hrs heating at > 100 ˚C. The decay of thiamin during heating can be modeled with a second-order reaction in which the temperature dependence of the rate constant followed an Arrhenius relationship as well as a Weibull-log logistic model.

- The raw salmon muscle is more tender than raw chicken due to lower content of collagen and less collagen cross-linkage, while cooked salmon muscle is consistently tougher than chicken breast due to a stronger resistance of the denatured muscle fibres of salmon to shearing.

- Cook loss and area shrinkage were significantly ($P < 0.05$) correlated with shear force change for both salmon and chicken, while collagen solubility was only significant for chicken not for salmon. The tenderness changes with heating of salmon and chicken muscle were a combined effect of different reactions that could be quantitatively expressed by cook loss, area shrinkage and collagen solubility.

- A significant increase in fibre diameter and decrease in sarcomere length were observed for both salmon and chicken muscle during high heat thermal processing, with salmon muscle exhibiting more shrinkage parallel to muscle fibre than chicken.

- Twenty min was found to be a critical heating time during which shear force reached a minimum, > 85% collagen were solubilized and > 80% cook loss and area shrinkage occurred.

Recommendations

This study was conducted by heating small size sample in an oil bath. Although this does not precisely simulate a commercial thermal process, the data should provide the food industry with useful information as to quality changes that would be expected to occur under different time and temperature regimes. However, the results presented in this dissertation, including the mathematical models, may need to be validated and modified for application in a commercial setting. For example, heating at 121˚C for about 20 min was shown to be an optimum heating time in terms of achieving the most tender salmon and chicken products within short time heating, but this is only true for container size and configurations similar to the ones used in this study which have very short come-up-time. However, a short come-up time is possible with microwave or RF heating indicating that the application of dielectric heating may be an appropriate technology for producing commercially sterile but higher quality and more tender products. Dielectric heating mechanisms are different with conductive heating used here and that may lead to different performance characteristics and a different range of quality changes during heating.

Measurments of microbial inactivation were not conducted in this study. Futher studies that validate the microbial lethality of the treatments evaluated here may need to be conducted to ensure that high quality shelf-stable products can be obtained.

The quality attributes of cooked salmon were determined shortly after heating. Storage may cause significant quality deterioration, and the deterioration rates during storage might vary depending upon the heating conditions used. For example, severe lipid oxidation in foods may occur in storage step, although this effect would not be obvious immediately after heating. Therefore, a study on the effect of heating regimes on quality following long term food storage

should be conducted. This is especially important when considering that the long term storage period for this product could be several years, which is possible in cases such as a Mars space mission.