ABSTRACT

RUILOVA-DUVAL, MARIA. Factors Affecting Water Holding Capacity and Texture in Cooked Albacore Tuna (*Thunnus alalunga)*. (Under the direction of Dr. Tyre Lanier).

The goal of this research was to determine if the thermal process parameters of precooking/cooling, prior to canning, might influence the activity of heat-stable endogenous proteases in albacore tuna such that canned meat quality and yield would be negatively impacted. Effects of meat location within the fish (dorsal, belly or tail), initial meat pH (which can vary with muscle location and among individual fish), and initial meat quality (as a function of prior handling procedures) were monitored as well. Proteolytic activity was measured as the rate of myosin heavy chain (MHC) degradation, determined by SDS polyacrylamide gel electrophoresis after heating over the range of 50-70°C for up to 180 min. MHC degradation during the subsequent cooling process was also measured by holding the heated meat isothermally at temperatures ranging from 30-65°C, representative of the temperature range that meat would experience during the cooling phase prior to manual cleaning of the fish. Textural quality of the meat was assessed by both Kramer shear press and sensory analysis.

Rate of degradation of meat from the belly location was higher than that for tail and dorsal meat. Meat from all three body positions degraded at a greater rate when precooked at temperatures lower than 70ºC, particularly at 50°C. However, even precooking albacore tuna at 70ºC did not totally inactivate proteolytic enzymes, since MHC of meat cooked to this temperature continued to degrade when the temperature was subsequently lowered, to simulate cooling. Degradation of MHC observed in precooked

albacore tuna was highly related to muscle textural properties. Belly and dorsal meat precooked at 50°C evidenced weaker texture, evidencing also a more gritty and grainy mouthfeel, and with more cook loss (lower post-cook moisture content) than meat precooked at 70°C. The gritty, grainy mouthfeel likely results from the greater moisture loss and smaller particle size of the degraded, cooked meat.

In a subsequent study, meat textural degradation, induced by precooking under conditions that favored autolysis of meat proteins, was found to influence the integrity of meat pieces, measured by tumbling to break up the meat and then sieving to examine the particle size distribution produced. The yield of meat canned in water, measured by both the standardized press test and drain weight, was also measured to determine whether such autolysis might affect this important factor. Meat precooked under conditions documented to cause extensive degradation of MHC produced a greater percentage of small flakes, and lower canned yields.

Based on the processing conditions used within albacore tuna canning plants for precooking, it seems likely that some portion of some fish (particularly larger fish) are exposed to conditions that will lead to degradation of meat proteins by autolysis, which could possibly have a measurable negative impact on meat quality and canned yield.

Factors Affecting Water Holding Capacity and Texture in Cooked Albacore Tuna (*Thunnus alalunga)*

by

Maria E. Ruilova-Duval

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APPROVED BY:

_____	_____
Dr. Brian E. Farkas	Dr. Josip Suminovic
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Dr. Tyre C. Lanier Chairperson of Advisory Committee	Dr. Andy Hale

DEDICATION

To my lovely and supportive husband, Luis D. Duval, and my exceptional and patient children, Jhostyn and Steven.

BIOGRAPHY

Maria E. Ruilova-Duval was born in Zaruma, Ecuador. She is the fifth of six children. She came from a farm family, where the main cultivated crops were bananas, sugar cane and coffee, and also cows for the local market. She enjoyed her childhood and school vacations visiting her parents' farm during harvesting season.

Maria gained her Bachelors degree in Biochemistry and Pharmacy from the University of Machala, Ecuador in 1986. After graduating, she was employed for five years as a Quality Control Manager at the Camarsa Seafood processing plant in Machala, Ecuador, where she trained and supervised employee groups to monitor the quality attributes and safety of seafood. Also, she trained all the employees in personal hygiene, Good Manufacturing Practices (GMP), cleaning, and sanitation procedures, as well as personal safety.

After five satisfying years at Camarsa Seafood International, Maria moved to the Casierra Seafood processing plant in the same country, to manage and supervise all stages in processing of raw headless and head-on shrimp in a variety of sizes and styles. After two years of working as Production Manager, Maria moved to Mayaguez, Puerto Rico, where she earned her Masters degree in Food Science in December 1995, under the direction of Dr. John Kubarik, studying the effect of temperature and humidity on survival of prawn (*Macrobrachium rosembergii*), transported in a non-water environment. After graduating with her Masters, she joined the Marine Science Department as a doctoral student under the direction of Dr. John Kubarik where she

stayed until May 1996 until she moved to Massachusetts, accompanying her husband. While living in Massachusetts, Maria learned English and taught Spanish at Worcester Polytechnic Institute. In January 2000, Maria moved to Raleigh, North Carolina accompanying her husband to accomplish his doctoral degree in Mechanical Engineering. While staying in North Carolina, Maria met Dr. Tyre Lanier while looking for an assistantship to complete her doctoral degree. Dr. Lanier submitted a proposal for a Sea Grant Industry Fellowship for Maria with StarKist Seafood as the industrial partner that was awarded for her to start as a doctoral student in the fall of 2002 in the Department of Food, Bioprocessing and Nutrition Sciences at North Carolina State University. In 2004, Maria was awarded another Sea Grant Industry Fellowship, this one's industrial partner was BumbleBee Seafoods.

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Special thanks to Dr. Tyre Lanier, Chairman of my committee, for being patient with my language limitations and overall for sharing his scientific knowledge that successfully led me throughout my doctoral accomplishments. I also extend my gratitude to the remaining committee members, Dr. Brian Farkas, Dr. Josip Simunovic and Dr. Andy Hale, for giving scientific direction when needed.

A very special recognition to Penny Amato, whose scientific, technical, and analytical expertise helped me accomplish my research objectives. Also, my gratitude goes to Lisa Nardelli and Lakendra Shepard who were extremely committed to helping me in my experiments. And finally to rest of the people from my lab, Alex Riemann, Dr. Patricio Carvajal, Dr. Bradley Wright, Natasha Evans and Vera Price who from the very beginning offered me their friendship making me feel part of the Seafood Lab family.

Thanks to Dr. Debra Clare and Evelyn Durmaz for helping with the some scientific methodology applied in this research. I also appreciate Dr. Pablo Coronel for helping me with the data analysis.

To my special friends from my church, Sacred Heart Cathedral, Father Jonathan Woodhall, Mela Alvarez, Fransisco Alvarez, Corazon Bilano, and Amelia Alba, thanks for their constant support and love.

Finally, my deepest gratitude to my husband, Luis, and my sons, Jhostyn and Steven, for their patience, sacrifices and support during all the time I spent on this work. And to my dearest mother, Rosita, and sisters Helena and Rosy, whose prayers and emotional support helped make these past few years a little more bearable.

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Introduction

The tropical tuna species (skipjack and yellowfin, which account for about 80 % of annual domestic canned consumption) are caught by the purse seine fleet and canned as light meat tuna. The remaining 20 % of tuna consumed in the U.S. are albacore tuna, a temperate water species, caught using troll (long-line) vessels and processed exclusively as premium grade white meat tuna. Albacore is the only tuna species that can be canned and labeled as white meat tuna in the United States (Economic Status of U.S. Fisheries, 1996).

Previously this laboratory conducted research on factors affecting the meat quality and canned yield of light meat (primarily skipjack) tuna (Stagg 1999). That work focused on how meat temperature/time history during the canning process affected meat autolysis. The present dissertation extends that investigation into canned albacore (white meat) tuna.

The texture and water-holding properties of canned albacore can be influenced by several factors. Important ante-mortem factors would include fish age and size, fat content and distribution, and harvest methods. Harvest and handling methods post-mortem can affect the rate and extent of pH decline, and thus the development and resolution of rigor mortis; these in turn can affect the rate and extent of meat autolysis (Dunajski, 1979) prior to freezing of fish at sea. Of course the whole frozen fish must be properly stored at low temperature (subfreezing temperatures; below -2°C) to maintain the meat quality until landed. During the thawing process carried out subsequently at the

cannery, if fish are allowed to warm considerably above 0°C then autolysis of the muscle proteins will be favored.

When fish are insufficiently thawed prior to precooking, and/or if fish are otherwise heated to an insufficient backbone temperature, the core of the fish may not subsequently receive enough heat treatment to ensure inactivation of endogenous proteases. These enzymes may thus remain active during the subsequent cooling and cleaning (deboning) of the fish, prior to being packed into cans and retorted.

It is presently unknown to what extent proteolysis could proceed in albacore tuna under these precooking and subsequent cooling conditions, and what effects such proteolysis might have subsequently on the texture or water-holding capacity of the canned meat. Thus the goals of this doctoral research project were to determine:

1. The extent of protein autolysis that occurs in portions of the tuna muscle during the precooking and cooling processes.
2. Whether texture and water holding ability of precooked meat might be affected by such autolytic changes.
3. Whether ultimately autolytic changes in albacore, and/or the water-holding properties of precooked albacore, will affect canned yield and meat quality.

Literature Review

The Albacore Tuna Fishery

Albacore, or long-finned tuna (*Thunnus alalunga*), range in size between 10 to 70 lbs (DeBeer, pers. comm.). These are classified as either "summer albacore" or "long–lined albacore."

Summer albacore are smaller, relatively higher in oil content because of their diet, and migrate during summers from Japan to California. These are caught from the surface of the sea by trolling lines (with hooks) (MacHale 2003). The effects of this harvest method with respect to fish stress and subsequently to meat quality are uncertain (Goodrick 1987). These fish are no longer canned by the major albacore processors because their higher fat content typically results in lower can yields as compared with long lined albacore (DeBeer, pers. comm.). However, smaller canners on the US west coast do can a limited amount of these fish for niche markets, and a good quantity is also utilized for sashimi.

Long-lined albacore are larger in size, and the meat is more lean. Very long lines, supported by floats and marked with flags, are released behind the fishing boats. Branch lines attached to each long line are sunk with baited hooks to depths of 55 to 150 meters, as these fish inhabit deeper water.

Albacore, as well as the lesser valued "light meat" species, skipjack and yellowfin, are warm-bodied fish, in that they maintain a body temperature about 10°C above the water temperature (Altringham and others 1997). Some research has suggested that muscle temperatures can exceed 20°C above ambient water temperatures during extreme stress (Goodrick 1987). This higher body temperature, in combination with the slow chilling rates often applied during post-harvest handling, could favor the activity of endogenous (to the muscle) proteolytic enzymes, particularly calpains. However, calpain-related softening is not commonly encountered in albacore, probably because the fishing method is more gentle and thus fish at harvest are typically not in a stressed condition. Fish taken from these lines are generally quiet and can be taken on board with relative ease and killed before physical activity or an elevation of body temperature occurs (Goodrick 1987).

The Tuna Canning Process

Canned tuna processed for the U.S. is available in a variety of packs, distinguished not only by the type of meat (white: albacore, or light: yellowfin or skipjack), but also varies according to the packing medium (water/broth or oil), and piece integrity (chunk, solid, flake/ grated). The chunk form in water/broth is the most popular light meat pack, although there still considerable demand for chunk light meat packed in oil. Albacore, however, is packed almost exclusively in water, most typically in the solid form (Economic Status of U.S. Fisheries 1996), and is sold as premium white meat tuna.

The type and order of the unit operations of the canning process that predominate the market have not substantially changed over the years (Bell 2000) (Fig.1). Frozen, raw, whole tuna are received from the ships which caught and froze the tuna at sea, then sorted by size and species. Sorted fish are placed into large containers called scows for storage in plant freezers until processing. Then approximately one ton of the whole frozen tuna are placed in each thaw tank, which is filled with potable water (28°C) that is constantly recirculated such that the water reaches an equilibrium temperature of approximately 19°C. This water is flushed and replaced approximately every 4 hour to prevent accumulation of fish contaminants (Lopez, pers. comm.).

Tuna are thawed until the backbone temperature reaches -2.5°C, and then the bins are moved to the butchering line for evisceration (removal of the fish viscera by hand cutting). Eviscerated tuna of the same size are placed into baskets, which are loaded onto metal racks. These racks are stacked inside a steam retort, wherein the fish are heated by atmospheric steam (100°C) for a time dependent on the fish size. Precook time is determined by the amount of time required to raise the backbone temperature into the range of 57 to 63°C. The racks of cooked fish are then moved to a cooling room where they are subjected to water sprays and forced (ambient) air circulation (DeBeer, pers. comm.) until they are cooled to a backbone temperature of approximately 30-35°C.

One purpose of precooking is to facilitate the manual separation of the edible meat from the carcass for canning. Loins are first prepared from the precooked tuna by

taking off the head and tail while the scales and skin are scraped from the body. The fish is split into two halves and the backbone is removed. The loins are produced by splitting the halves of fish along the median line into two roughly equal portions, the dorsal and belly loins. Red meat is then removed from each loin for processing into pet foods. Bones and heads, skins and viscera removed at this point are processed into fishmeal.

Mostly albacore, but also some premium packs of yellowfin, are sold in the solid packs, whereas mainly skipjack and yellowfin tuna are sold in chunk packs. Grated (flake) tuna are no longer common in the US, but can be of any species. The solid pack is achieved by a machine (Caruthers's Pak-shaper) that produces a cylinder of tuna loins of uniform density from which can be cut can-sized segments of uniform weight. Solid loins are placed on a continuous conveyor-belt, which carries the loins between vertical forming belts. These vertical forming belts mold fish into a cylindrical mass. As it emerges from the end of the molding belts this cylinder of fish is cut into can-sized cakes by a circular knife and pushed into the can (Jackson and others 1979; Lewis and others 1994). Similar to solid packs, the chunk packs are produced from loins, with the difference that the loins are cut on a moving belt by means of cutter blades. The blades may be of a straight edge type or star or diamond shapes. The cut loins are then filled and compressed into the can by conventional pocket filler machines (Jackson and others 1979).

Gated tuna is produced from broken tuna loins and flakes, which are packed by the same methods as the chunk pack. Often machines are used for continuous weighing of the cans with automatic elimination of those of low weight (Jackson and others 1979). The final step for canning process consists in adding to the open cans additives such as salt, vegetable broth, oil or water. The cans are seamed, retorted, cooled, then labeled and packaged in cases. Retort time depends on can sizes. Fill weight compliance for the amount of tuna packed into the can is determined by a press test method (FDA, 21CFR161.190).

Retortable pouches are other option for thermal processed tuna. This package offers advantages such as lightweight, reduced storage space, easy of opening and reduced heat exposure resulting in improved quality (Chia and others 1983).

A continuing goal of the tuna canning industry is to increase case yield (cases of canned product per ton of whole tuna fish processed) while maintaining an acceptable level of canned meat quality (in particular, good texture) (Bell 2000). Canned tuna yield and textural quality are affected by both the initial quality of the meat being canned, and by the parameters chosen for the processing steps to which it is subjected during canning. The remainder of this review will consider the broader aspects of fish meat composition and the factors affecting its raw quality, and also the effects of subsequent processing on its texture and water-holding ability. Special attention will then be given to how the process parameters of the canning process may affect tuna texture and water holding properties.

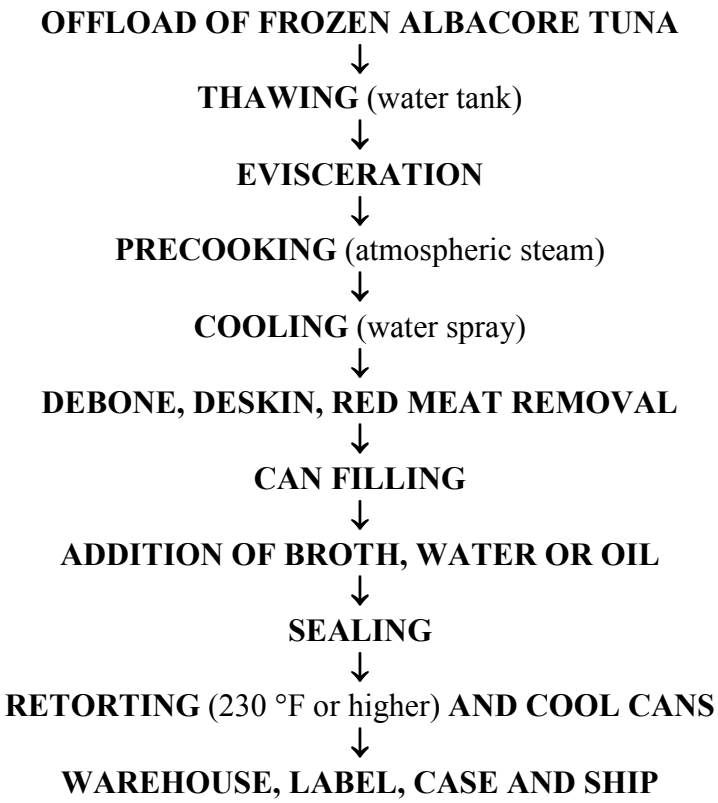

Figure 1: **Conventional tuna canning process**

Composition and Microstructure of Fish Meat

Muscle structure and composition

A fish fillet consists of the muscle mass from one side of the fish, of which the upper part is termed the dorsal meat and the lower part the ventral (or belly) meat. Two sets of bones extend from the spine on each side at right angles, dividing each fillet into two 'loins' (Carlson and others 1960).

The flakes of fish (Figure 2), evident when the fish is cooked, correspond to the myotomes, which are the bundles of muscle cells which extend between two adjacent sheaths of connective tissue (myocommata), more or less parallel to the long axis of the muscle (Huss 1995). In contrast, the muscle mass in mammals is elongated and narrows towards the tendons, which connect to the skeletal structure (Lampila 1990).

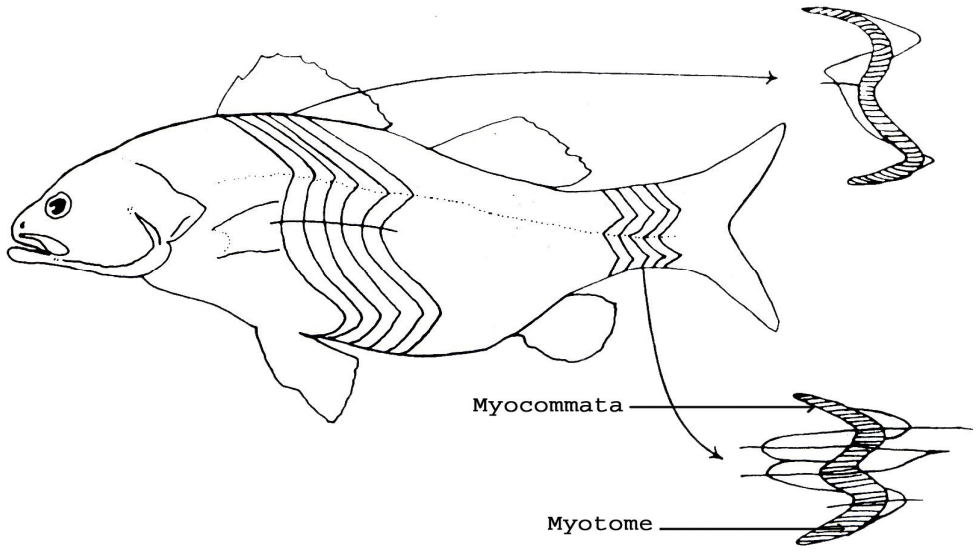

Figure 2: Myotome pattern of fish, with detailed lateral views of a single myotome (Fennema and others 1996)

Muscle is composed of fibers which constitute the cells of the muscle. Each muscle cell includes, within a sheath of connective tissue (mainly collagen) called the sarcolemma, sarcoplasma containing nuclei, glycogen grains, mitochondria, etc., and an array of parallel myofibrils. The myofibrils occupy about 70 % of the lean muscle volume (Van Laack 1999). Myofibrils are segmented into sarcomeres, which are composed of thin (actin) and thick (myosin) filaments, showing alternate arrangement of A and I bands bordered by Z-lines. The thick filaments (composed of myosin molecules), and thin filaments, consisting of double helical strings of actin, overlap and slide over one another during muscle contraction. These protein filaments are arranged in a characteristic alternating system making the muscle appear striated, similar to that of terrestrial animals (Venugopal and others 1996; Lampila 1990).

Muscle may be dark or light dependent on the relative content of heme proteins (mainly myoglobin) and according to their physiological activity they are classified as either fast-twitch or slow-twitch (Foegeding and others 1996). Dark muscle is slow twitch, for extended aerobic energy expenditure as in continuous swimming motion, whereas light muscle is fast twitch for rapid anaerobic action. The latter provides for short bursts of rapid swimming in pursuit or escape (Venugopal and others 1996). Thus the proportion of dark to light muscle varies with the relative activity of the fish species. In pelagic, migratory fish, i.e., species such as herring and mackerel that swim in the open sea, up to 48% of the body weight may consist of dark muscle (Love 1970).

Although tuna are very active and fast swimmers, these also contain a relatively high content of dark muscle because they also are migratory.

The relative quantity of dark muscle varies from species to species, and in its distribution along the fish body, usually increasing in percentage toward the tail (Love 1980). However, in skipjack the thickness of the band of dark muscle decreases toward the tail region (Figure 3a) (Love 1988). We have observed this same distribution pattern of dark muscle for albacore tuna. The dark meat is found near the spine and is surrounded by the white meat (Figure 3b).

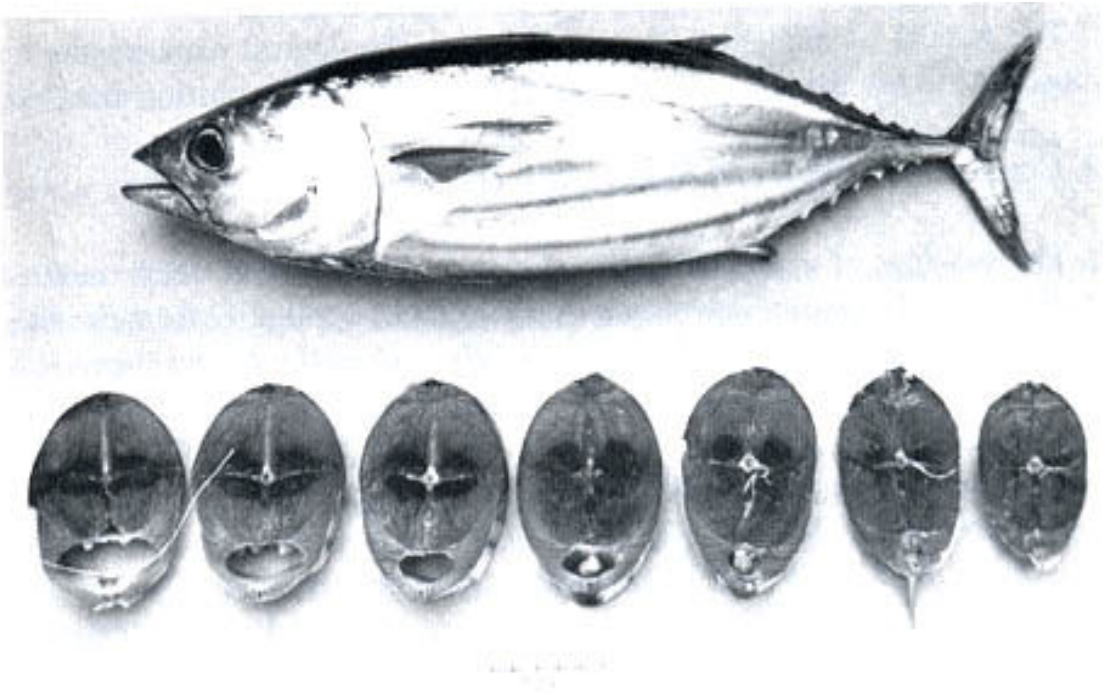

Figure 3a: Sections through the body musculature of albacore tuna, showing how the proportion of dark muscle changes along the body (Johnston and Brill, 1984)

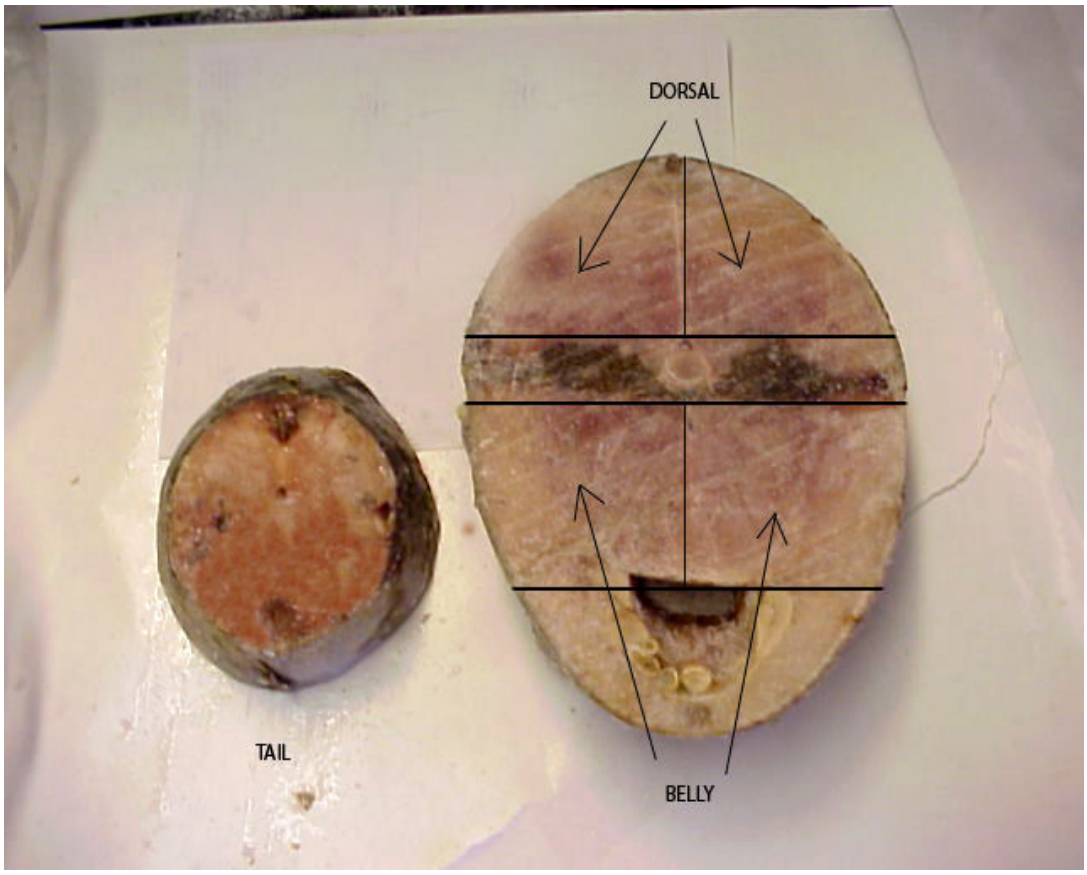

Figure 3b: Sections of the body of albacore tuna, showing how the proportion of dark muscle changes at different body parts.

The light muscle is very uniform in composition, at any location of the fish (Love, 1980). However, dark muscle varies in composition as a function of its location, containing more lipids in the anterior part (portion close to the head) of the fish and relatively more water and protein in the posterior part (portion close to the tail) (Love 1980).

Dark muscle typically contains less protein, less water, higher lipid content and more glycogen than white muscle (Love 1988). However, Murase and others (1996) has reported higher moisture content in dark muscle of albacore than in the dorsal ordinary muscle (fast twitch; white or light). Also, this author has found higher lipid content in ventral than dorsal ordinary muscle and dark muscle, with a markedly high fat content for small sized tuna. In fatty fish such as tuna most of the lipid reserves are found in the flesh. In contrast, non-fatty species, such as cod, carry most of their lipid reserves in their liver (Love 1992). The high lipid and heme protein contents of dark muscle can contribute to greater rancidity development during handling or storage (Huss 1995). The stronger flavor, dark color, and high propensity to rancidity (heme is a pro-oxidant) are reasons that dark meat is removed from precooked albacore before packing and canning. The dark meat of tuna is so high in heme content that it is commonly called "blood meat" as it seems also to have a highly developed vascular system (Regenstein and others 1991).

Muscle Proteins

The three major classes of muscular proteins, based on solubility characteristics, are the stroma, sarcoplasmic, and myofibrillar proteins.

Stroma proteins are those insoluble in water or salt solutions. These include both elastin and collagen. Elastin, a highly extensive fibrous protein, is present in connective tissue in small amounts, and in fish it and collagen are present in smaller amounts than in

muscle of warm-blooded animals (Dunajski 1979). The lower content of connective tissue in fishes is probably due to the water environment that supports their bodies; fish do not require strong connective tissues to maintain and support the muscles against gravity (Venugopal and others 1996; Foegeding and others 1996). Fish muscle typically contains only about 3% to 5% connective tissue, although some cartilaginous fish may contain up to about 10%.

Collagen is the main component of connective tissue. The myotomes of the muscle are separated by connective tissue; thus when collagen converts to gelatin during cooking and loses its connective quality the meat separates into flakes.

Fish collagens contain less proline and hydroxyproline than does mammalian collagen. The differences in the total content of these amino acids plays an important role in the protein structure and are responsible for fish collagen being less thermally stable and more easily soluble than mammalian collagen. Thus the temperatures at which thermal shrinkage and denaturation of fish collagen occur are generally lower than for avian or mammalian collagens (Dunajski 1979).

Other proteins of the flesh are the water-soluble or sarcoplasmic proteins, which include myoglobin, hemoglobin, globulins, albumins, and enzymes of the glycolytic pathway, lysosomes and various proteases as well as peptides. These sarcoplasmic proteins account for approximately 20-30% of the total protein content.

The myofibrillar proteins are classified as being salt-soluble (most readily soluble in solutions of higher ionic strength) although myosin, the primary myofibrillar

protein, also becomes quite soluble when the ionic strength approaches zero (it is largely insoluble at physiological saline). Myosin (thick filaments) and actin (thin filaments) are the two main myofibrillar constituents of the sarcomere. The sarcomeres are the linear units of myofibrils bordered by Z-disks(Fig.4), each of which connects the actin filaments of adjacent sarcomeric units. Actin is a smaller globular protein which exists in the thin filament as the polymeric form F-actin, a helical double strand of monomer units. Regulatory proteins, tropomyosin and troponin, are also located in the thin filament (Foegeding and others 1996).

Myosin accounts for 50% to 58% of the myofibrillar proteins (Venugopal 1996), and is composed of heavy meromyosin (HMM) and two fragments called light meromyosin (LMM) (Fig. 5). LMM consists mostly of the α-helical rod of the myosin molecule. HMM is comprised of the globular heads with light chains and the remaining rod section (Venugopal and others 1996). Fish myosin, particularly from white-meat species inhabiting cold water is apparently the least stable of all animal myosin, and is generally more susceptible to denaturation during frozen storage, coagulation during cooking, and degradation by proteolytic enzymes (Venugopal and others 1996; Foegeding and others 1996). Results of differential scanning calorimetry (DSC) study of fish myosin confirm a clear relationship between species of fish, habitat temperature and thermal denaturation temperature of myosin. Ogawa and others (1993) reported that the α- helical structure of fish myosin from ten fish species unfolded markedly in the temperature range of 30˚C to 40˚C, corresponding to high-temperature setting, and varies

from species to species of fish. Davies and others (1988) reported that snapper (tropical-water fish) myosin have greater stability (Tm= 52°C) than cod (cold-water fish; Tm= 42°C) myosin. In agreement with these results, other studies have shown also that, in cod and salmon muscle, both species living in cold water habitats, myosin denatured between 44°C and 45°C (Offstad and others 1996), while in skipjack tuna, species living in tropical water habitats, myosin denatured at temperatures (Tm) of 50°C and 52°C (Offstad and others 1996; Webb 2003; Bell 2000).

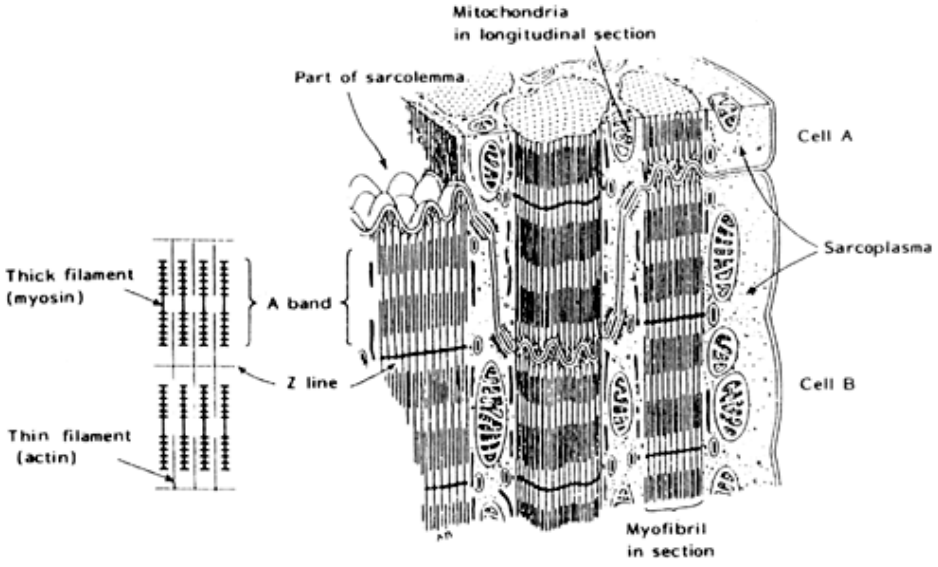

Figure 4: Section of a cell showing various structures including the myofibrils

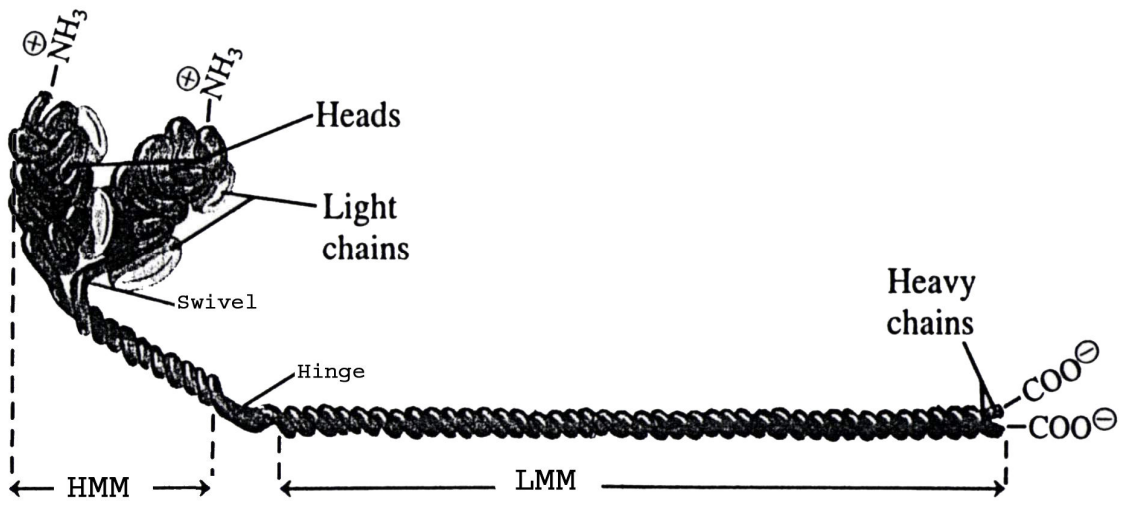

Figure 5: Schematic diagram of myosin molecule (from Rawn, Biochemistry)

Water Holding Capacity (WHC) of Meat

Definition and significance

Hamm (1960) has defined water-holding capacity (WHC) as the ability of meat system to hold its own and /or added water regardless of the application of force. This ability depends on the method of handling and the state of the system (fresh, cooled or cooked meat). As the state of meat and its treatment can differ considerably, the meaning of WHC varies widely (Honikel and others 1994). Therefore the water-holding ability of meats should be specified according to certain conditions: a) water that is held by fresh meat in chilled holding, when cut and wrapped in water impermeable film (loss is termed 'drip' or 'purge'), or when subjected to evaporative loss; (b) water that is held after freezing and thawing (loss is termed 'thaw drip'; or (c) water that is released during

cooking, whether in a saturated or dry environment (loss is termed 'cook loss') (Offer and Knight 1988a).

The amount of water in lean fish meat immediately after slaughter is about 75% (depending on species and fat content we measured 70% recently on albacore) and its retention during subsequent processing is of great economic importance since most forms of meat are usually sold by weight. Purge exudation of fresh meat is also unsightly if freely expressed in a tray-wrapped package. Drip losses are accentuated after freezing as damage is caused to the meat structure by freezing of ice crystals; this produces lateral shrinkage of the filament lattice (Offer and others 1988b). Empey and others (1954) mentioned that drip will vary among different muscles from the same meat. They pointed out that, apart from the effect of muscle pH, the percentage of fat, water, and connective tissue in the muscle, and the distribution of connective tissue, all influence the amount of drip. They also gave evidence that drip is greater from coarse-textured fish species than from those of finer texture.

Water retention as affected by meat microstructure and chemistry

Raw meat. In fresh meat the majority of water is present in the spaces between the thick (myosin) and thin (actin) filaments of the myofibrils. The amount of water immobilized within myofibrils depends to a large extent on the space available between filaments lattice spacing, which can vary due to changes in pH and ionic strength, or as a result of protein denaturation; factors that contribute to the shrinkage of myofibrils post mortem.

Sarcomere length, which is determined by muscle contraction during rigor mortis, also affects water retention; as sarcomere length increases so also does water-holding capacity (Offer and Trinick 1983; Offer and others 1988a). Cold shortening and thaw rigor are recognized to shorten the sarcomere length. Cold shortening is caused by too-rapid lowering of meat temperature prior to onset of rigor mortis, whereas thaw rigor results when meat is frozen prior to onset of rigor mortis. In both cases, muscle contraction is caused by sudden release of Ca^{2+} into the sarcoplasm and may cause a physical shortening of 80% of original length of unrestrained muscle. Considerable shortening may also occur in muscle removed from the skeleton while still in the prerigor condition (Hedrick and others 1994; Dunajski 1979). All these conditions generally produce a tougher texture, and lower water holding capacity, in the meat.

The pH of avian and mammalian meats falls postmortem from about 7 to about 5.5 (near the isoelectric point for the proteins) due to the conversion of glycogen to lactic acid. Muscle pH, as well as ionic strength, influences the net charge of proteins and filaments. At pH away from the isoelectric point (which also is influenced by ionic strength) the greater net charge leads to repulsion between adjacent protein molecules, and between myofilaments, allowing more space for water retention (Toldrá 2003; Offer and Knight 1988a). The increased electrostatic repulsion thus can also result in swelling of the myofibrils in the presence of sufficient externally available water. Added salts and/or phosphates may also contribute negative charge responsible for increasing the electrostatic repulsion between filaments such that water holding additionally increases

(Offer and Trinick 1983). The electrostatic repulsion due to salt (NaCl) addition has been explained as resulting when chloride ions bind preferentially to the thick and thin filaments surface (Offer and Trinick 1983; Hamm 1960).

If meat reaches the isoelectric point of its proteins, where the net charges are zero and electrostatic repulsive forces between filaments are eliminated, water holding capacity will typically attain a minimum because the filaments come in close proximity to one another (Van Laack 1999; Offer and others 1989). During the conversion of living muscle to meat, protein denaturation can occur if the meat pH rapidly declines to near the isoelectric point (5.4-5.5) after slaughter while the carcass is still warm. This additionally diminishes the water-binding capacity because of lateral shrinkage of denatured myofibril proteins (Aberle and others 2001; Van Laack 1999; Offer and Knight 1988b) and such meat is known as Pale Soft & Exudative (PSE). Certain pork and poultry species are the most susceptible to such a condition, seemingly a response to preslaughter stress that is predicated by genetics. The most obvious characteristic of this condition is loss in color intensity which results from this protein denaturation: incident light is strongly scattered by the tighter muscle structure such that it penetrates to a shallower depth, and so is absorbed to a lesser extent by myoglobin (Offer and Knight, 1988b). Additionally, because the color of meat also depends on the concentration of myoglobin, its partial denaturation under these conditions also contributes to the meat paleness (Offer and Knight 1988b).

In some fish species the ultimate pH (lowest pH attained) of the meat post-rigor can be as low as is typical for mammalian or avian muscle; for example large mackerel, for which the ultimate post-rigor pH may be as low as 5.8 to 6.0, or tuna and halibut, which can reach a post-rigor pH as low as 5.4 to 5.6 (Huss 1995). Perez-Villareal and others (1990) and Price and others (1991) reported that the postmortem pH of fresh muscle albacore was as low as 5.90± 0.12, when stored in ice over 33 days. Apparently enzymes naturally present in the flesh cause hydrolysis of glycogen to lactic acid after the fish dies, resulting in a fall in pH from about pH 7.0 to pH 6.0-6.8 (Connell 1980; cited in Price and others 1991).

While albacore are typically caught by the less stressful long lining technique, skipjack and yellowfin are caught by purse seining (Goodrick 1987). The strenuous struggling and restricted oxygen supply associated with this harvest method can rapidly bring about acidosis (formation of lactic acid in fish muscle as a result of anoxic metabolism; Sikorski and others 2000) in the muscle, resulting in a typical meat pH near 5.9 in yellowfin and skipjack caught by both methods bait boat and purse seine (Barret and others 1965). Acidosis is manifested as a hardening of the muscle proteins and an increase in drip from the muscle (Dunajski 1979).

Exhaustive exercise leading to the increased production of lactic acid in the muscle may also favor rapid autolysis post-mortem, because lactate is a potential activator of acidic lysosomal proteolytic activity (Sikorski and others 2000). Among these lysosomal enzymes, cathepsins B, D, L and H have been purified and characterized

from fish and shellfish muscles (An and others 1994; Aoki and others, 2000*)*. Some of the cathepsins are heat-stable such that they cause intracellular protein breakdown when the meat is thawed and subsequently elevated in temperature during cooking (Visessanguan and others 2001). Makinodan and others (1984) found that acid protease (cathepsin D) activity was higher in small and medium-size fish. Cathepsin D has been associated with post-mortem softening of sea bass due to myosin degradation (Ladrat and others, 2003). Visessanguan and others (2003) reported cathepsin L as the protease with maximum activity in arrowtooth flounder heated at 60°C and at pH 5.5.

Cooked meat. Cooking denatures meat proteins, inducing shrinkage of collagen and the myofibril structure that can squeeze water from the meat (Offer and Knight 1988a). Such water losses can negatively affect the texture, juiciness, and soluble nutrient content of meat.

Meat typically loses up to 40 % of its weight upon cooking. Lateral shrinkage of myofibril begins between 40 °C and 55 °C for avian and mammalian species, the temperature range over which the myofibril proteins denature and aggregate (Offer and Knight 1988a; Hamm 1977). Although this aggregation results in shrinkage of the muscle fiber, the water released is initially retained within the endomysial sheath (Foegeding and others 1996). As the temperature is increased to between 60°C and 80°C an additional shrinkage occurs with denaturation of the collagenous endomysium and perimysium. At these temperatures, denatured myofibril proteins and connective tissues shrink in a longitudinal manner, causing shortening along the meat fiber by decreasing

the sarcomere length. This shrinkage generates a tension, which exerts pressure on the fluid between the fiber and sheath and consequently forces water out of the intra and extra-cellular spaces (Offer and Knight 1988a; Hamm 1977).

In fish meats the collagen contains less proline and hydroxyproline than mammalian collagen and is therefore less thermally stable. Thus, the temperatures at which thermal shrinkage and denaturation of collagen occur are generally lower than in avian or mammalian muscle (Dunajski 1979). Offstad and others (1993) found that the main structural changes in the connective tissue of cod and salmon appeared at 5-40°C. He concluded that water loss at these temperatures is probably due to denaturation and melting of collagen, since he observed that at these temperatures the collagenous layers completely lost their linear appearance. The maximum water loss in these fish species that could be attributed to myosin denaturation was observed at 45°C for cod and 50°C for salmon (Fig. 6). In agreement with this author, Bell (2001) reported that moisture and mass loss of skipjack steam-cooked resulted primarily from thermally induced denaturation of muscle proteins and subsequent shrinkage. Sigurgisladottir and others (2001) reported that in salmon smoked (it includes salting step) at 20°C the sarcomere length did not significantly change: probably at this temperature only the collagen was denatured and the myosin was not, and the weight loss was attributed to loss of lipids as well as dehydration.

As has been found in the studies mentioned above, apparently water loss achieved a maximum at 45- 50ºC. In addition, Ofstad and others (1993) have reported that at

temperatures higher than 50ºC water loss in cod and salmon decreased (Fig. 6), which agreed with Bell (2001) results, who reported that moisture loss of skipjack tuna decreased notably above 48ºC backbone temperatures. This reduced water loss at higher temperatures (50 - 70ºC) has been attributed to the aggregates of sarcoplasmic proteins at the interstices between the sarcolemma and the muscle fiber (Ofstad and others 1993). This result coincided with work done on thermally processed skipjack tuna which reported that aggregation of denatured sarcoplasmic proteins and collagen are formed in the interstices between the shrunken muscle fibers and the sarcolemma at 60 ºC (Lampila and others 1986). These aggregated and coagulated muscle proteins components deposited between shrunken muscle fibers apparently create physical barriers, which reduce the flow of water out of the meat structure *(*Offstad and others 1993). The changes have been also described as increased tortuosity in the muscle structure, which enhance the resistance to moisture flow in the exterior muscle region (Bell and others 2001).

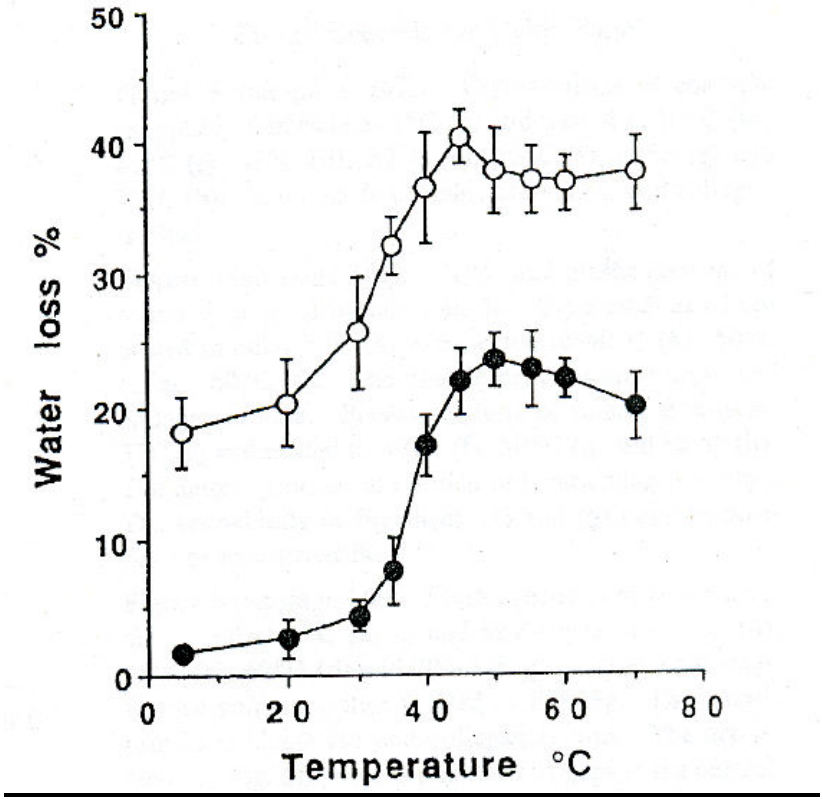

Figure 6: Water loss (% by weight) as a function of heating temperature of coarsely chopped salmon (solid circles) muscle and cod muscle (hollow circles) according to the net test (Offstad and others 1993)

Measurement of the water-holding capacity of meat

According to Hamm's (1960) definition of water holding capacity, the meat should hold the water regardless of force applied. Methods for measuring WHC vary according to whether the force is simply gravity or a greater application of force (pressure) to the meat surface.

Applying no force. To this group of methods belong the measurements of free drip, evaporation and weight loss. These methods are very sensitive but time consuming, requiring from one to several days to measure an equilibrium value.

The measurement of drained weight of product after cooking or canning also belongs to this group. In the case of canned tuna, however, this is not necessarily a measure of cook loss because the meat has already been cooked ('precooked') before canning. However this method does measure the ability of the canned meat to retain its initial and/or some percentage of the added liquid, since it quantifies the amount of canned meat retained after draining the liquid. In Canada and Europe this is the legal standard for can fill, and should be no less than 70 % of the can net weight. In the US, however, the standard for can fill is the pressed weight, which requires the application of force to the meat surface (Bumble Bee Foods, pers. comm.).

The moisture content of meat (AOAC 1995) at any stage of processing can also serve as a measure of its water-holding capacity. Bell (2001) measured moisture content from whole skipjack tuna at different cook temperatures (steam cooking) by using the standard method (AOAC 1995).

Applying mechanical force. In this group of WHC measurement techniques are centrifugation methods (Hamm 1960; Wierbicki and others 1957), the filter paper press method and the capillary volumeter method (Honikel and others 1994; Hamm 1960). With these methods the amount of water released is higher than with methods wherein no force is applied to the meat surface.

The legal standard of fill weight compliance for canned tuna in the US is the pressed weight method (21 CFR161.145). Again, because the tuna is cooked prior to can filling, this is not a true measure of cook loss but only an indication of moisture held/absorbed/lost by the meat during retorting.

Pressed weight is determined by applying pressure to the can contents via a plunger, roughly the same in diameter as the internal diameter of the filled can, slowly and at uniform rate, so that a full minute is used to reach a peak pressure of 384 pounds per square inch. This pressure is held for 1 additional minute and released, and the can contents weighed (Bell 2000). Results are reported as % pressed weight (based in net weight). The presumption is that the method simulates a consumer squeezing the liquid out of the meat structure by pressing down on the opened lid. However, the gap between cylinder and plunger is very small as compared with an opened lid, such that fine meat particles, if present, can obstruct the plunger gap and thus lower the water loss from the meat mass. Bell (2000) found that skipjack meat of smaller particle size, actually yield higher press weight measurement after canning than meat of larger piece size.

The pressed and drained weight tests also can be used to determine the effects of adding substances which enhance the water uptake and retention of canned tuna. It is for this reason that many varieties of tuna of canned in a ‘vegetable broth’, which contains soy proteins and other ingredients known to enhance water uptake and retention.

Centrifugation, filter paper press and capillary methods are also based on measuring the loose of water liberated by applying pressure on the muscle tissue. These

methods are rapid and simple, and applicable for intact and ground meat (Honikel and others 1994).

Meat Texture

The tenderness of meats is greatly affected by the content and cross linking of connective tissue, factors that vary according to species and muscle location among mammalian animals, while in fish it also vary according to season, and spawning (times of starvation causes an increase in collagen and collagen with a greater degree of cross-linking) (Lampila 1990; Foegeding and others 1996). In terrestrial animals, the collagen network becomes more highly cross-linked as age increases, which may explain why meat from older mammalian animals is tougher that from younger animals, although they contain more collagen. The opposite occurs in fish, where although old fish contain more collagen than younger fish, its collagen is weaker and has fewer cross-links than the collagen of younger fish (Foegeding and others 1996), which has been associated to its softer texture.

In general, the total collagen of fish is lower than for most avian and mammalian species. Apparently, the collagen content in raw fish contributes to the raw fish texture as has been shown by Hatae and others (1986). They reported that fish species with firmer raw meat texture contained higher collagen content than the species with softer texture. However, in cooked fish the textural properties of the muscle tissue depends primarily on the state of the myofibrillar proteins (Dunajski 1976) rather than the collagen content.

Of course, any meat when swelled with water will typically be more tender, irrespective of the type of muscle. In cooked meat, loss of water that occurs due to substantial shrinkage of denatured myofibril proteins is also associated with toughening of the meat (Offer and Knight 1988a).

Species and biological/compositional effects on fish meat texture. Textural differences among fish species may also be due to morphological or compositional factors that can vary, not only by species, but also according to age or size, and physiological factors, such as fish migration, spawning, sexual maturation and the nutritional state of the fish. For example, fluctuations in the meat water, protein and fat content, which are ultimately caused by cycles in the reproduction process, have a significant impact on the texture of fish meat (Dunajski 1979).

Size/Age Effects. Growth of the fish involves an increase in size of muscle fibers. Increased diameter and length of the fibers bring about an increase in the coarseness of the muscle. In a large adult fish, such as a 95 cm long cod, the fiber diameter varies from 150 to 300 μm depending upon the location along the fillet **(**Dunajski 1979). At present there are no studies supporting a fish size/tenderness relationship; however some researchers have proposed that fish fiber diameter is a key factor influencing the texture of raw and cooked fish. Differences in texture have been found between fish species where the common factor was that thin muscle fibers (fiber diameter of about 50 μm) were responsible for firmer texture than when muscle fibers were more thick (fiber

diameter of about 250 μm) (Hatae and others 1984, 1990; Hurling and others 1996; Kanoh and others 1988). Therefore, it could be inferred that the meat of young fish should be firmer in texture than adult fish.

Migration/Spawning/Sexual Maturation Effects. Migratory fish that use a large amount of energy for sexual maturation and for spawning migration may exhibit meat having a softer texture (Sikorski and others 2000). Such soft texture is attributed to hydrolysis of the nonhelical region in the collagen molecules as well as degradation of myofibrillar proteins (Yamashita and others 1991). It is frequently observed that yellowfin tuna females during spawning season (summer months) develop a soft meat texture and undesirable pale color, typically known as 'burnt' tuna (BST); a condition that has been compared to PSE in red meats. Burnt tuna is apparently caused by post-mortem activation of calpains and by the enhancement of the effect of these enzymes by high blood catecholamine (neuro transmitters-hormones) levels. The levels of catecholamines apparently increase in periods of intense physical activity or capture stress, spawning season and in female fish (Davie and others, 1986; Hochachka and others, 1987; Watson and others, 1988). Catecholamine and estrogen (reproductive steroid, which reaches a peak during spawning season) increase during the summer season. Apparently estrogen slows the clearance of catecholamine from the blood by competing for the same degradative enzymes in the fish gills; the organ responsible for the degradation of catecholamine (Watson and others 1988).

High autolytic activity has been found in Baltic herring and salmon during the spawning period (Sikorski and others 2000). Yamashita (1991) found that a specific protease (cathepsin L) is most probably responsible for a drastic muscle autolysis which causes extensive softening of the meat of mature salmon. Mommsen (2004) reported that lysosomal cathepsins, especially cathepsin D and sometimes cathepsin L, are responsible for the degradation of muscle proteins during fish migration, maturation and starvation. The author also pointed out that in the course of migration, the fish degrade almost all their lipid and about half their white muscle mass, replenishing the lost protein with water to maintain their mass and external shape; and fish higher in water content are softer in texture (Dunajski 1979).

Fat Content Effects. Fat content in muscle tissue may influence texture and autolytic activity in the pre-rigor state (Bonnal and others 2001). Several studies indicate that fish with higher lipid content are softer in texture and high lipid content also may enhance calpain-membrane interactions, calpain auto-activation, and sensitization to lower calcium concentrations (Dunajski 1979; Melloni and others 1996). Bonnal and others (2001) found that accumulation of lipid inclusions and phospholipid micelles induced by a high fat diet, and retention of these lipids by the cytoskeletal network, could lead to intense activation of calpains I and II. Smaller, summer-caught albacore are known to be both higher in fat content and softer in texture; it is possible these two factors could be related through calpain protease activity.

Post-harvest handling/treatment effects on fish meat texture

Harvest Conditions and Rigor Mortis Development. Rigor mortis initiates the conversion of muscle into meat. Biochemical and physiological regulatory functions operating in vivo cease postmortem, and the energy resources (ATP) in the muscle are depleted. When the level of adenosine triphosphate (ATP) reaches its minimum, myosin and actin become irreversibly interconnected, resulting in rigor mortis (Huss 1995). This ultimately results in the shortening of the muscle and stiffening of the meat.

Rigor mortis onsets almost immediately after death if the fish are starved, or if the fish have been highly stressed, because glycogen reserves are depleted (Dunajski 1979). Postmortem glycolysis results in the accumulation of lactic acid, which lowers the pH of the muscle. As the pH drops, the net surface charge on the muscle protein is reduced, causing partial denaturation/aggregation (more severe if the meat temperature is elevated) and a loss of some water-holding capacity. Meat in the state of rigor mortis loses its moisture more readily when cooked and thus becomes firmer in texture (Huss 1995).

If rigor mortis occurs at too high a body temperature in fish it may induce separation of muscle segments (myotomes) much as occurs during cooking, a condition known as gaping. The extent of disintegration of the fish flesh between the muscle segments is strongly influenced by temperature, pH and collagenase activity (Dunajski 1979). For Atlantic cod, it has been shown that upon reaching 17°C, gaping is inevitable presumably because of degradation of the connective tissue and rapid shortening of the muscle due to high temperature rigor (Huss 1995). Gaping decreases the market value of

fillets and makes processing difficult. The resulting damaged appearance makes the fillets difficult to sell and process, such as fillets cannot be mechanically skinned, hung for smoking or sliced (Love 1992).

Rate of Chilling and Proteolysis. It is apparent from the foregoing discussion that rapid chilling is important to minimize damage to meat induced by rigor mortis occurring at too high a body temperature. Fish that are chilled slowly upon capture can also exhibit considerable weakening of the texture due to autolysis from calpain (neutral; calcium-activated) proteases. MacDonald and others (2001) found that calpains are involved in loss of structural integrity of hoki muscle in the very early stages postmortem. They found that rapid chilling within the first 2 hrs after harvest to near 0°C reduced texture deterioration in hoki by inactivating calpains.

The rate of chilling of fish prior to onset of rigor or on rigor has different effects depending on the kind of meats. In the case of read meat, rapid chilling prior to onset of rigor mortis causes cold shortening (muscle contraction that affects water retention). However in fish, rapid shortening is caused when the fish is not effectively chilled (high body temperatures) after catch. Shortening in combination with weakening of the connective tissue causes gaping (separation of fish muscle segments) (Dunajski 1979).

Frozen Storage. Freezing provides an excellent means of storing fish for long periods of time by preventing or slowing the textural weakening of fish muscle that could occur postmortem. Although freezing is the most suitable preservation method for storing fish, it does not entirely prevent deterioration of fish quality.

Muscle proteins are susceptible to freeze-induced denaturation, whereby some of the water holding ability is lost (Haard 1992) and thus meat can become drier and tougher when cooked. During frozen storage fish muscle texture can change from soft, moist and succulent to become unacceptably firm, hard, fibrous and dry. These changes are mainly attributed to the denaturation and aggregation of the myofibrillar proteins, particularly myosin. As the myosin unfolds, non-polar amino acids are exposed so that hydrophobic interactions can take place with "like" groups in the vicinity – a phenomenon that usually leads to tough texture and drip loss (Jasra and others 2001). The same author showed that the main protein fractions showing denaturation during postmortem storage of carp fillet is the myosin light chains and α-actinin, while actin and tropomyosin are relatively stable. MacDonald and others (1992) reported that storage of headed and gutted hoki at –29°C resulted in significant loss of gel-forming properties of the flesh over time due to denaturation and aggregation of the myofibrillar proteins. This loss in gel-forming properties was also directly related to undesirable changes in meat texture.

Even though lowering the meat temperature slows enzymatic and chemical reactions, these processes may still proceed in frozen meat (Foegeding and others 1996). Jiang and others (1997) found that cathepsin B and L activity of mackerel surimi decreased after 4 weeks storage of fish at -40°C. However, there was still 82% activity left even after 8 weeks frozen storage, possibly because cathepsins B and L still existed in the lysosomes, which provided protection from denaturation during frozen storage. Another important fact is that freezing and thawing may cause lysis of mitochondria and

lysosomes and alter the distribution of enzymes, such as cathepsin D found in Baltic herring muscle (Karvinen and others 1982), and trimethylamine oxide (TMAO) demethylase commonly found in kidney, spleen and gall bladder of gadoid fishes (cod family) (Huss 1995).

Trimethylamine oxide (TMAO) demethylase, or TMAOase, can contribute to muscle toughening during frozen storage, especially in gadoid fishes like hoki, cod, hake and haddock. Most TMAOase has been reported as being membrane-bound, which becomes most active when the tissue membranes are disrupted by freezing. This enzyme is able to break down TMAO into dimethylamine (DMA) and formaldehyde (FA). Formaldehyde induces denaturation, aggregation, and cross-linking of the muscle proteins making the muscle tough and inducing loss of its water holding capacity (Huss 1995; Foegeding and others 1996). Apparently the most practical means of preventing the autolytic production of FA is to store fish at temperatures less than -30°C to minimize temperature fluctuations in the cold storage and to avoid rough handling prior to freezing (Huss 1995).

Cooking. Meat shrinkage that occurs during dry oven cooking (hot air), apart from reducing the water content and the size of the meat portion by surface evaporation , also contributes to toughening of the meat texture, which is usually explained as multi-step process (Offer and Knight 1988a). Shrinkage of denatured myofibrils is the first phase of moisture loss and meat toughening (about two to three-fold), which begins at low temperature range (40 - 55°C), with additional moisture loss, which relates to a further

two-fold increase in toughness as cooking temperature is increased (60 - 65°C), due to additional shrinkage of denatured collagen. However, at temperatures above 80°C, cooking for an extended period of time (e.g., stewing meat) in the presence of sufficient water will convert the collagen to gelatin, softening the cooked meat texture (Offer and Knight 1988a; Foegeding and others 1996; Dranfield 1994).

Thus the mechanism of mass and moisture loss during steam cooking depends primarily upon the denaturation of muscle proteins and the mechanical force produced by these changes, which during precooking of tuna transports moisture through and out of the tuna meat (Bell and others 2001).

Haard (1994) noted that the most adverse effects on fish texture are caused by heat stable proteases which cause excessive softening of fish meat during cooking. These proteases are activated at cooking temperatures in range of 50 - 65°C, which cause autolysis of myosin (the major structural protein) and other proteins with severe effects on fish meat texture.

Effect of Proteases on Meat Texture

Fish meat texture is generally more tender than that of land animals due to its lower connective tissue content, and an excessively soft or mushy texture can be caused by protease-induced breakdown of the contractile proteins (mainly myosin) prior to or during cooking. Such meat tenderization can be desirable in red meats, because they are generally tougher in texture and therefore must undergo this enzymatic tenderization in

order to develop an acceptable sensory quality. However, it is an undesired effect in the meat of most fish species, which are already inherently tender due to its lower connective tissue content.

Source, type and distribution of endogenous fish protease. Proteolytic degradation of fish muscle involves the autolytic activities of proteases endogenous to the muscle (cysteine proteases), which are active at the postmortem pH of fish**,** as well as to those in meat which may originate from the belly cavity (aspartyl proteases) or organ tissues, such as kidney (Haard 1994). Gildberg (1982) suggested that the leakage of pepsin and trypsin (digestive proteases), from fish viscera during stowage of fish in boat holds, can activate collagenases present in the connective tissue, initiating collagen degradation by digestive collagenases. Tissue
proteases may also originate from parasite infestation of the meat, such as with *Myxosporidian* parasites (Haard 1994).

Two classes of proteases, cathepsins and calpains, seem most involved in the softening of fish meat texture.

Cathepsins. Cathepsins are found packaged in tiny organelles called lysosomes, considered to be mostly not active in the living fish. They are released into the cell juices at the moment that the lysosomal membranes lose their integrity or upon physical abuse, freezing, and thawing of postmortem muscle. Cathepsins are mostly active at acidic pH, and can be distinguished by their active sites (aspartic, cysteine, and serine proteases) as well as by their substrate specificity and inhibitor sensitivity (Delbarre-Ladrat and others

2006). Sentandrew (2002) pointed out that cathepsin activity in postmortem muscle is controlled by factors such as pH, redox potential, extent of precursor activation, and presence of specific endogenous inhibitors (cystatins). Cathepsins include both exopeptidases (hydrolyze peptide bonds at the terminal end of the protein) and endopeptidases (hydrolyze peptide bonds in the interior of the protein). Cathepsins B, D, L and H are the major cathepsins within the fish muscle lysosomes (Aoki and others 2000).

Siebert (1973) found that muscle from the tail portion of cod reportedly exhibited about twice as much cathepsin activity as abdominal muscle, while muscle taken from the back (near the main fin) showed intermediate activity. Makinodan and others (1984) found that small and medium size carp tend to have higher cathepsin activities than large carp.

Calpains. Calpains are cysteine proteases, optimally active at neutral pH and at temperatures of 25°C. Calpains require calcium for their activation, which is internally released from the sarcoplasmic reticulum soon after death of the animal. For this reason, they are more active during the initial postmortem period, before lactic acid production decreases the pH (Ishiura 1981). Calpains are only active for a relatively short period as they are largely autolyzed within 24 hrs after capture (MacDonald and others 2001).

Calpains specifically attack certain proteins of the Z-line, such as desmin, filamin, and nebulin and primarily titin, which weakens the titin/∝-actinin interaction and results in the release of intact ∝-actinin from Z-lines (Koohmaraie 1992). Apparently, the initial

breakdown of these proteins also facilitates actin and myosin dissociation from the sarcomere, thus also possibly exposing actin and myosin to available cathepsins, which become active as the pH falls (Goll and others 1992; Jiang and others 1996). Contrary to this conclusion, however, Delbaree-Ladrat and others (2004a) concluded that the rate of hydrolysis by catheptic proteases subsequent to the action of calpains is unaffected.

In contrast with vertebrate muscle calpains, fish calpain digest myosin, specifically myosin heavy chain (MHC) (Muramoto and others 1989; Ho and others 2000). Muramoto and others (1989) demonstrated that fish calpains were more active at low temperatures than were mammalian calpains. By analogy, it seems likely that fish species adapted to colder environmental temperatures would also likely have calpains which were more active at even lower temperatures. Thus if fish were harvested into a similar ambient temperature, those fish from colder waters could be more susceptible to calpain autolysis than those from tropical waters. Calpain activity in postmortem meat is influenced by factors such as ultimate pH, free calcium ion concentration and presence of its endogenous inhibitor (calpastatin) as well by inactivation due to autolysis or denaturation (Delbarre-Ladrat and others 2004b). Although it is known that its maximum activity is at pH 7 and 25 °C in mammalian meat, Koohmaraie and others (1986) reported that some calpains retained 24-28 % of maximum activity at pH 5.5-5.8 and 5°C, which represent conditions similar to those of postmortem storage, associated with the tenderization process during meat aging.

Two types of calpains have been identified in mammalian meats, based on the calcium requirement for optimal activity: calpain I (μ-calpain) which requires 10 to 50 μM of calcium concentration for full activity and calpain II (m-calpain) requiring 300 μM to 1 mM calcium concentrations (Molinary and others 1997). Only calpain II was identified in fish muscle by Jiang and others (1991). However, Delbarre-Ladrat and others (2006) identified three different calpain-like activities in postmortem white muscle from sea bass: two similar to m-calpain and one to μ-calpain with biochemical properties similar to mammalian. These types were differently expressed throughout the year: μ-calpain was only detected during the spawning period of sea bass. This could be related to variations which have been noted, from season to season, of the extent or the rate of muscle degradation (Ladrat and others 2000).

Post-mortem meat tenderization by proteases. Both calpains and cathepsins had long been thought to be the major proteases causing tenderization of red meats during refrigerated storage; however, there are now doubts that cathepsins contribute to this process (Sentandreu and others 2002; Delbarre-Ladrat and others 2006; Herrera-Mendez and others 2006). For example, Whipple and others (1990) found that cathepsin B and B+L were not involved in meat tenderization. Koohmaraie and others (1991) also found that cathepsin L+B was not involved in pork tenderization, since no degradation of actin or myosin (the primary myofibril protein substrates for cathepsins) was detected. Uytterhaegen and others (1994) also concluded that cathepsins B, D, H and L did not play any role in meat tenderization, suggesting that calpains were the main proteases

involved. Mestre-Prates and others (2001) found that specific cathepsin inhibitors did not suppress postmortem proteolysis; however general proteases inhibitors that inhibit both calpains and cathepsins (E-64) prevented postmortem proteolysis on rabbit meat during refrigeration. These entire groups of researchers have concluded that calpains play the major role in postmortem tenderization of meat, since some of them reported desmin degradation and others the effectiveness of calpain inhibitor (calpastatin).

It has been suggested that meat tenderization is a multienzymatic process involving not only calpains and cathepsins but also proteasomes and caspases (Herrera-Mendez and others 2006). The 20S proteasomes exist as a latent form, possibly activated by heat and chemicals (SDS and oleic acid; Thomas and others 2004; Avila 1997). However, it has been found that proteasomes from rabbit, sheep and fish require high temperatures (60 °C) or addition of SDS (sodium dodecyl sulfate) or oleic acid to be activated (Otsuka and others 1998; Koohmaraie 1992; Mykles and others 1995); therefore its role in postmortem degradation might be of less importance.

At the present time research on capsases, of the cysteine protease family, has focused on their involvement in programmed cell death, where they function as vital executioner of apoptosis (Kemp and others 2006). Apoptosis is a genetically programmed, morphologically different form of cell death naturally occurring in a living organism that can be generated by a variety of physiological stimuli (Earnshaw and others 1999). The stimulus can be extrinsic, via activation of death receptors, or intrinsic in response to conditions very unfavorable to cell survive. Cell death in postmortem

muscle may be caused by deterioration of the mitochondria due to lack of the system to oxidize molecular oxygen, intense stress (e.g. oxidative stress) or overload with calcium (Herrera-Mendez and others 2006).

Some researchers have hypothesized that apoptosis might likely be the first step in post-mortem cell destruction (Herrera-Mendez and others 2006). Kemp and others (2006) found that the highest activity of caspases was at 2 h after slaughter in pigs. The same author also reported that alpha II spectrin and poly (ADP-ribose) polymerase, which are known caspase substrates, were degraded within the first 4 h after animal death. This was thought to be associated with the development of meat tenderization. They further suggested that caspases are most active in the early postmortem stages, and could contribute to early postmortem proteolysis and meat tenderization (Kemp and others 2006), possibly facilitating the later action of other proteases.

Effect of cathepsins and calpains on fish texture. While it thus seems clear now that red meat tenderness is mainly associated with calpain activity (Koohmaraie 1996), in fish both cathepsins and calpains seem to be involved in muscle proteins degradation during postmortem storage (Delbarrete-Ladrat and others 2006). These probably are complementary and even synergistic with the action of other proteases such metalloproteinases (responsible for collagen degradation), proteasomes and caspases. The role of proteasomes in postmortem degradation could be of less importance since they typically need heat and addition of SDS to be activated (Thomas and others 2004; Avila 1997). At this time there is no evidence of a direct role of caspases on myosin

degradation; however it should be considered that apoptosis could be the initial step during postmortem facilitating the action of cathepsins and calpain (Kemp and others 2006).

Both calpains and cathepsins seem to degrade myosin heavy chain, ∝-actinin and desmin, while actin and tropomyosin appear to be sensitive to cathepsins B, D, L (Muramoto and others 1989; Ho and others 2000; Delbarre-Ladrat and others 2004, 2006), although cathepsin B was found inactive against carp tropomyosin (Hara and others 1988). Ladrat and others (2003) reported that troponin T was degraded mainly by cathepsins B and L. Cathepsin L and H have been reported to breakdown myosin in meat ten and three times faster than cathepsin B, respectively (Bird and others 1980). Nielsen and others (2001) has reported that cathepsin D is the most important acidic protease involved in fish muscle proteolysis, since it attacks native proteins, including MHC, actin and tropomyosin.

Although, calpain has been identified in several fish species such as skipjack tuna, croaker, trout, tilapia, carp and red sea bream (Muramoto and others 1989), as well as hoki (MacDonald and others 1997), and mackerel (Ho and others 2000), little work has to date demonstrated a “cause and effect” relationship between calpain activity and instrumental measurements of texture (Huss 1995).

Cathepsin activity associated with changes in fish texture has seems to be affected most by factors such as spawning migration, postmortem meat pH, and storage and processing temperatures.

Role of proteases in fish muscle texture during storage. The physiological status of fish prior to harvest has been pointed out to influence proteolytic activity of meat, as during spawning migration and sexual maturation of chum salmon, Baltic herring and ayu, the cathepsin B, H, D and L levels are elevated and this activity was highly related to fish meat softening (Yamashita and others 1990; Mommsen and others 2004; Sikorski and others 2000).

Some studies have supported the fact that fish meat softening caused by cathepsins B, L, H and D is pH-related. Those studies have shown that each type of cathepsin is active at a specific optimum pH range, which encompasses from pH 3.0 to pH 7. The most critical cathepsins (B, L and H) exhibit an optimal pH range closer to that of postmortem muscle meat pH (pH 5.5 - pH 7.0) and calpain is optimal near neutrality (Jiang and others 1990, 1992; Makinodan and others 1982; Sentandreu and others 2002). It is also believed that cathepsins, which display a wide pH range of activity, such as cathepsin L (pH 3.0-6.5), play a greater role in the autolytic degradation of fish muscle than those having a relative narrow pH range of activity, particularly when it is far from the postmortem meat pH, such as cathepsin D (3.0-5.0) (Kirschke and others 1981; Delbarre-Ladrat and others 2006; Sentandreu and others 2002).

Role of cathepsins in fish muscle texture during processing. Thermo-stable proteases can contribute to fish meat softening since they can be highly active at temperatures achieved during cooking and at the postmortem/physiological pH (Visessanguan and

others 2003). Many studies have concluded that cathepsins B, D, L and L-like degrade myosin heavy chain (MHC) of fish optimally at 55 °C and pH 5.0 - 5.5 (Muramoto and others 1989; Jiang and others 1997; Stagg and others 1999; Ho and others 2000; Makinodan and others 1987). Visessanguan and others (2001, 2003) found that cathepsin L activity in arrowtooth flounder muscle increased as temperature was increased from 37 °C to 60 °C, being inactivated at temperatures over 60 °C. Of these, cathepsin L has been identified as most often responsible for excessive softening of many fish species muscle or fish gel products at cooking temperatures ≥ 55 °C, at slightly acid to slightly alkaline pH values; requiring 70 °C for inactivation (An and others 1994; Makinodan and others 1997; Porter and others 1995; Visessanguan and others 2001, 2003).

Texture degradation of Pacific whiting fillets and mackerel surimi at 20 -37°C was attributed to cathepsins B and H activity, which are typically inactivated at 50°C and 60°C, respectively (An and others 1994; Jiang and others 1997; Porter and others 1995). Cathepsin D is the only one highly active in chilled storage (4°C) (Jiang and others 1992).

Several studies have concluded that heat-induced softening in fish muscle could in part due to degradation of MHC caused by endogenous serine proteinases when fish muscle or gel is cooked at temperatures over 50°C (Toyohara and Shimizu 1988). Yongsawatdigul and others (2000) agreed that serine proteases were responsible for

texture degradation of tilapia surimi cooked at 65°C, as result of MHC degradation. Cao and others (1999) too reported that the optimum degradation temperature of myofibril-bound serine protease to MHC in myofibril and kamaboko gel were 55ºC and 60ºC, respectively. Recently, Cao and others (2005, 2006) showed that heat-stable serine proteases present in crucian carp and silver carp also degraded MHC in the temperature range 50-60°C, with a maximum MHC degradation at 55°C and reduced MHC degradation at temperature of 40°C and 70°C. Heat-stable serine proteases, as well as heat-stable cysteine proteases, has been categorized as those with pH optima in the alkaline range (8 to 9), and those which are optimally active at the postmortem pH of fish muscle (Wasson 1992). Chio and others (1999) and Cao and others (2005) reported that alkaline serine proteases with maximum activity at 55 °C and pH 8.0 were apparently responsible for softening of menhaden surimi gels and muscle silver carp, respectively. However, elastase (serine protease) from marine animals are apparently quite stable from pH 5 to 9 (Simpson 2000), although Sikorski and others (2000) reported that at pH 6.5 and higher, which prevails in the fish tissues postmortem, the trypsin-like enzymes play an important role in the degradation of myofibrillar proteins.

Additionally, several studies have identified that the main characteristic of alkaline proteases from fish muscle is that its activity is not detectable below 50°C, while considerable activity is observed around 60°C (Makinodan and others 1987). It has been confirmed by Boye and Lanier (1988) and Lin and Lanier (1980) whom reported that

maximum activity of alkaline proteases was at 55 °C-60 °C and at pH 7.5-8.0, while its activity decreased at temperatures bellow 45 ºC and when the pH was below 7.0 or above 8.0.

Measurement of meat texture

Determination of fish quality on the basis of color, odor, and appearance is common task encountered in sensory evaluation of raw fish (Gill and others 1987). However, texture is one of the most important sensory quality parameters of fish for producers, processors and consumers (Ali and others 2005). There are several definitions of texture commonly used by scientists (Bourne 1982); according to Coppes and others (2002) texture consists of a group of properties derived from the structure of the food, and can be described by physical properties (mechanical or rheological) and the physiological senses (feeling of touch mainly in the mouth and in some cases the hands).

Meat texture is thus largely evaluated in terms of tactile sensory properties and mechanical responses to stress. Mechanical properties include hardness (force required to bite or probe into the material), firmness (force required to compress), cohesiveness (degree to which sample deforms prior to rupture), and springiness (rate of return to original shape after some degree of deformation) (Meilgaard and others, 1991 cited in Ali and others, 2005). Tactile properties are sensed as a response to geometrical shape and size of particles (grainy, gritty, crystalline, and flaky), or oil/moisture release properties (moisture release, oiliness, moistness, dryness) by the tactile nerves in the surface of the hand, lips or tongue (Meilgaard and others 1991).

Both sensory and instrumental measurements have been used to assess various aspects of fish meat texture. There is a preference to use instrumental methods rather than sensory testing because of the limitations of the latter in terms of time, cost, measurement reproducibility (which improves with panelist training), and amount of sample required for testing (Casas and others 2006; Love 1988; Kolanowski and others 2006)

Firmness, toughness, cohesiveness, and juiciness are textural attributes which can be measured equally well by both sensory and instrumental methods, since these measurements seem to correlate reasonably well between both methods in a variety of products, such as cooked fish fillets, minced fish products (fish fingers, fish portions) and gels (Hamann and others 1986; Bosund and others 1972; cited in Howgate 1977; Barroso and others 1998; Sawyer and others 1984). Many of the methods used for measuring the instrumental texture of fish are modified from those originally applied to meat. However, several of those methods are not suitable for fish because of the low content of collagen in fish (Dunajski 1979). In choosing an instrumental method it is important to consider differences which can occur not only between fish, but also within a fish muscle, such as may occur due to differences in chemical composition, fiber diameter and muscle heterogeneity (Sigurgisladottir and others 1999; Hatae and others 1990; Kanoh and others 1988).

Sampling techniques must be chosen with consideration of the heterogeneous nature of muscle. Fish muscle in particular has a metameric structure, with muscle fibers parallel to the long axis of the muscle, such that uniform sampling is difficult (Dunajski

1979). It has been suggested that "a sample cut from the center region of the fillet is the most uniform (homogenous) in textural properties" (Segars and others 1986).

A variety of destructive mechanical tests have effectively been used to measure cooked fish meat texture. These include puncture, shearing and cutting devises, (such as the punch and die test), as well as shearing and cutting devises, such as the Warner-Bratzler Shear and the Kramer Shear-Compression Cells (Sigurgisladottir and others 1999; Sawyer and others 1984). The Kramer cell or compression test has generally been the preferred means of measuring the tenderness of fish muscle, since it overcomes some problems of samples heterogeneity (Dunajski and others 1979; Sigurgisladottir and others 1999; Barroso and others 1998; Borderias and others 1983). The other methods named above exert stress only a in localized area of the muscle and consequently variability between replicates depending on the number and orientation of the myotomes, can introduce considerable error (Borderias and others 1983; Barroso and others 1998). However, the Kramer shear method requires relatively large samples (Sigurgisladottir and others 1999) and can also be misleading in that it can be very influenced by the cohesiveness of the flakes (how well flakes adhere to one another) rather than the textural properties of individual flakes. Stagg (1999) found that the Kramer shear device was useful in evaluating the effect of cooking temperature and related proteolysis on textural quality of skipjack tuna.

Possible Effects of the Canning Process on Canned Tuna Texture and WHC

Thawing

Frozen tuna are generally thawed by immersion in recirculating tanks filled with water (28°C to 30°C). If the thawing process is not carefully controlled to assure that the core temperature of fish reaches no more than -2°C to 0°C, it may accelerate autolysis of muscle protein (Bell and others 2000; Farkas and others 2003). Stagg (1999) showed that skipjack tuna abusively thawed and pre-cooked at 55°C exhibited textural degradation.

If the meat interior remains frozen at the completion of the thawing process, this can lead to inadequate precooking, such that cathepsins or other proteases could be quite active, even remaining active into the cooling period prior to canning (Zhang and others 2001).

The traditional thawing process was recently improved (Farkas and others 2003) such that ambient water is more adequately circulated through the thawing tanks to effect better heat transfer. This system has helped canneries to reduce the thaw time by eliminating the presence of thermoclines (layering of water into a warmer surface zone and a colder deep zone) and thus decreasing the temperature difference between the surface and backbone of the fish.

Precooking and Cooling

One purpose of precooking is to facilitate the subsequent separation of edible meat, by manual labor, prior to packing into cans and retorting (canning). The more

desirable lighter colored meat is separated from the head, tail, scales, skin, bone and dark ("blood") meat. Precooking also reduces the fat content and accompanying strong taste of the tuna meat, and drives out liquid rich in sarcoplasmic proteins that otherwise often coagulate during the subsequent canning/retort step to form flecks of visible, unsightly curd in the canned product.

Because many fish proteases are activated upon heating, precooking could be a critical step affecting the texture and water holding properties of the product. The common industry practice is to precook round (whole) or split tuna (halved) in conventional (atmospheric pressure) steam pre-cookers to a backbone temperature range of 57 - 63 °C. At this temperature range many endogenous proteases may be most active (Stagg 1999). Besides possible autolysis that may occur during precooking, heating to this degree induces denaturation of the proteins and an accompanying decrease in the water-holding capacity of the meat (Bell and others 2001). Following precooking, the tuna is taken out of the large steel cookers and spread on racks, to be cooled by fans augmented by an ambient water spray for approximately 4-5 hours (depending on fish size) . Thus the meat temperature may linger in the temperature range of 60°C for some time, extending the period of protease activity in any portion of the meat which did not reach a temperature greater than 70°C to inactivate heat-resistant proteases (Porter and others 1995; Cao and others 2006).

An extended cooling time is more likely for a conventional, atmospheric steam precooking/cooling operation than when a more modern vacuum pre-cooker system

(FMC FoodTech) is employed, since the latter method reduces the cooling time to 30 minutes via a water spray applied prior to removal of fish from the cooker. However, cooking come-up time is often slower in the vacuum pre-cooking system (Bumble Bee Foods, pers. comm).

The cooking times for both methods vary for round or split tuna and also depend on tuna size. For round tuna the cook loss decreases as tuna size increases, while for split tuna the cook loss is higher than in round tuna ($P<0.05$) and does not increase with tuna size (Rovedo Clara; study conducted in Bumble Bee canning plant, Mayaguez). Bell (2000) studied the changes in mass and moisture content in skipjack tuna during precooking at atmospheric pressure. He concluded from this study that mass lost from the tuna meat during precooking was due to thermal denaturation of myofibril proteins and subsequent shrinkage of the myofibrils. Measurements of thermal denaturation temperatures of the skipjack muscle protein by differential scanning calorimeter (DSC), showed that myosin denaturation occurred around 51°C, collagen around 60 °C and actin around 70°C (Bell 2000; Webb 2003).

Effects of heating/cooling fish muscle on the activity of tuna muscle proteases

Stagg (1999) reported that skipjack meat tuna under acidic conditions, when precooked at 55 °C showed the most proteolysis, and thus concluded that the proteases involved were likely acidic heat-stable cathepsins. The same group of proteases might be

expected to be most active during precooking albacore tuna, since its postmortem meat pH is 5.9 ±0.12 (Perez-Villareal and others 1990).

Presently there are no studies, which have demonstrated proteolytic activity during the cooling step of tuna, as a result of insufficient precooking. However, there is a suspect that if the high temperature activated proteases are not completely inactivated during precooking, they may remain active during the cooling process.

Because the precooking/ cooling process is a critical step prior to canning, probably the selection of pre-cooker system is important. The newer vacuum precooking system reduces the chance for scorching the meat surface, but requires a longer time to reach the desired core temperature. Thus during the cooking phase meat in the core is exposed for longer time at temperatures between 50°C and 60°C (optimum temperatures for heat-stable cathepsins activity). The cooling time for this system is reduced (it employs a water spray within the cooker immediately upon cessation of heating) as compared to atmospheric steam cooking, but it would be expected that the greater proteolytic activity would come from the more heat-stable proteases near their optimum temperature of activity, rather than at the much cooler temperatures rapidly attained in the vacuum precooker after the water spray commences. Less heat-stable proteases, which might be more active at such low temperatures, should largely have been inactivated even if the desired core temperature was not reached.

Canning/Retorting

A main goal of the tuna canning industry is to increase case yield (defined as cases of cans meeting the pressed weight requirement for meat fill obtained per unit weight of fish being processed) while maintaining a nutritious, safe and acceptable level of canned meat quality (Bell 2000*).* This is a constant challenge since thermal processing can exert both positive and negatives effects on canned product quality and yield (Durance 1997). Certainly both nutritional and sensory quality can suffer if product is overprocessed (Seet and Brown 1983; Hamm 1977; Chia and others 1983).

Lampila and others (1986) observed muscle fiber shrinkage and the formation of small protein aggregates in the interstices between sarcolemma and shrunken muscle fibers in cooked skipjack tuna. These conditions were increased after canning and retorting as compared to just after precooking. Offstad and others (1993) similarly had observed the formation of interstitial protein aggregates in cod and salmon cooked to 50ºC –70ºC and postulated that these served to block the flow of water out of meat during cooking. Perhaps in concert with this observation, Stagg (1999) and Bell and others (2000, 2002) observed a higher drain weight yield of retorted canned skipjack tuna previously precooked at temperatures of 55ºC (which was shown to promote autolysis and loss of textural integrity) and attributed this at least in part to the creation (by meat autolysis) of a more tortuous path for the liquid to escape from the meat structure (Bell and others 2001).

Aubourg (2001) in a review pointed out that in canned fish, water losses during canning can vary from 9 - 28% depending on the severity of the commercial sterilization and the previous cooking, species, pH and other physiological factors. Kokuryo and others (1980) reported that when carp myosin was heated at 100 and at 120°C for up to 60 min, MHC degraded to many fragments with increased time. Tanaka and others (1988) retorted pouched bigeye tuna and halibut using different schedules to the same lethality and observed that at the highest retort temperature (124°C) the degree of MHC degradation (measured by SDS-PAGE) was less as compared to at a lower temperature (115°C) because processing time at the higher temperature was far less. This may explain in part why pouched products, which require less heat processing to attain sterility due to their thin profile, exhibit a firmer texture than canned products (Chia and others 1983). Tanaka and others (1985) also had concluded that myosin degradation during retorting leads to textural softening of canned mackerel.

Okazaki and others (1986) reported release of moisture simultaneous to MHC degradation during retorting of sea bass. Based on the studies mentioned above, this would suggest that faster processing at higher retort temperatures would likely also result in better moisture retention. However, Bell and others (2002) reported that canned skipjack tuna retorted at the lowest temperature, longest time process (110ºC, 98 min) lost less mass than canned tuna retorted at higher temperature, shorter time retort processes. Yet no significant moisture content changes were apparent in muscle chunks between high and low retort temperatures.

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Paper 1

Kinetics of Myosin Autolysis in Albacore Tuna (*Thunnus alalunga*) During Precooking and Cooling as Associated with Product Quality Deterioration

Ruilova-Duval M.E., Lanier T.C, and P.M. Amato

Department of Food, Bioprocessing and Nutrition Sciences

N.C. State University

Raleigh, NC 27695-7624

ABSTRACT

The effects of temperature during isothermal precooking/cooling, initial quality of the fish, location of the muscle within the fish, and initial meat pH were assessed on the rate and extent of autolysis of precooked albacore tuna. Autolysis was measured as the rate and extent of myosin heavy chain (MHC) degradation. Initial meat quality and postmortem pH of fresh albacore muscle did not affect the rate of heating-induced autolysis. The highest rate of MHC degradation occurred in belly meat precooked at 50 - 60°C**.** Proteases activated during precooking, even at 70°C, remained active during subsequent cooling at 30°C or higher. Belly and dorsal albacore tuna muscle precooked at 50°C was less firm in texture, as measured by Kramer press and sensory analysis, and had a lower cooked moisture content than meats precooked at 70°C. These also exhibited more grittiness and a more grainy mouthfeel. This weakening of albacore tuna texture and increased water loss is likely the result of endogenous heat-stable protease activity.

Keywords: Albacore tuna; myosin heavy chain (MHC) degradation rate (K-value); precooking process; cooling process, meat pH effect; meat texture; Kramer shear press; sensory analysis; SDS-PAGE.

INTRODUCTION

Tuna canned for the US market are typically precooked as whole or split fish, then partially cooled to facilitate manual removal of the meat for subsequent packing and canning. Stagg (1999) determined that autolysis of skipjack tuna myosin could occur during the precooking step if any portion of the meat remained in the temperature region of optimal proteolytic activity, about 55 °C, for a sufficient time. Proteolytic activity was evident at higher isothermal incubation temperatures up to 70°C, at and above which temperature the autolytic enzyme(s) responsible were rapidly inactivated. The temperature range of 50-70°C has been identified as ideal for meat autolysis of a number of fish species (Haard 1994; Visessanguan and others 2003). It was surmised that sufficient proteolytic degradation to induce meat softening and loss of piece integrity could occur if fish had been insufficiently thawed prior to the precooking step (thus slowing the heating process), and/or larger fish were cooked slowly without having reached a core temperature sufficient to inactivate proteases.

Albacore tuna are typically the largest of the tuna species canned for the US market, and are the only species permitted to be sold as premium white meat tuna (Hilderbrand 1999). These large fish are typically precooked to a core endpoint temperature of 60 °C. Thus it seemed likely that if autolytic activity, similar in nature to that detected previously in skipjack, were present in albacore meat then this could possibly exert a negative effect on meat quality at the core of these larger fish. Larger fish

would not only heat more slowly in the same precooking environment, but would also be expected to cool more slowly. Thus if autolytic activity persisted into the cooling stage further damage to meat integrity and water holding properties might occur.

The goal of this study was to determine the rates of myosin degradation in albacore tuna meat caused by endogenous proteases at temperatures corresponding to those typical during the precooking/cooling process. Trials were also conducted to determine whether textural properties of precooked meat might be affected by such autolysis. Presuming that cathepsins, with optimum activity under acidic conditions, might be a major source of autolytic activity in this meat (Stagg 1999), we also determined whether initial meat pH affected the rate of meat protein autolysis.

MATERIALS AND METHODS

Whole frozen (on the fishing vessel) albacore tuna (*Thunnus alalunga*) were obtained, having been harvested commercially in tropical Atlantic waters. These had been sampled from two different lots (denoted A and B), obtained from two fishing companies who harvest from different locations and employed somewhat different handling methods on board the catch and freezing vessel. Fish were sawed, while frozen, to obtain cross sectional samples, starting from behind the head through to the tail, of approximately 3 cm thick. Subsamples representing three fish body locations (anterior

dorsal, anterior belly, and posterior tail) were cut from these, vacuum-sealed and stored at -35°C.

Proteolytic degradation at precooking temperatures

Samples from two fish of each lot (lots A and B) were used for the myosin degradation trial, while only one fish of each lot were used for trials to determine the effects of tuna autolysis on texture and water holding.

Samples of dorsal, belly and tail locations were thawed under running tap water to a core temperature of -2°C. The pH of the thawed meat was measured directly with a Stainless steel micro probe with ISFET sensor attached to a model IQ 150 Digital pH Meter (I.Q. Scientific Instrument). Slices from each cross sectional sample, approx. 7 x 5 x 0.8 cm, weighing approximately 25 g, were made using a Hobart meat slicer (Troy, Ohio) then vacuum sealed in multi-layer "Seal and Meal " plastic bags (VSB2-6, Rival) whose size and shape was adjusted to fit the sample size. Two samples sealed in bags for each treatment combination were immersed in water at a temperature of 50, 55, 60 or 70°C for 0, 30, 60, 90, 120, 150 or 180 min. All treatments were then heated an additional 5 min in a 90 °C water bath to inactivate autolytic enzymes prior to cooling by immediate immersion in ice water. Sample equilibration to target center temperatures was measured with a type K thermocouple (10 cm in length with 0.3 cm in diameter) attached to a model HH21 Digital Microprocessor thermometer (Omega). Timing for treatments started after sample equilibration.

Proteolytic degradation during the cooling phase following precooking

Only the dorsal portion of albacore tuna was used in this study, because of the large numbers of samples required (about 115 per heat treatment for all the associated cooling temperatures). It was not possible to obtain this number of samples from the smaller belly and tail portions. Only albacore tuna from Lot A was used.

Albacore tuna cross sections from the dorsal portion were prepared as described in the precooking study. Samples were ramp heated in a water bath at 1°C per minute to temperatures of 55 °C, 60 °C, 65 °C and 70 °C, then held for 5 min at the end temperature. One sample for each treatment combination was transferred to an appropriate "cooling" bath held at 5 degree increments between 65 °C and 30 °C for varying lengths of time up to 150 min at 20 min intervals. All samples were then heated for an additional 10 minutes at 90 ºC to inactivate the enzymes, then cooled in ice water immediately.

Proteolytic degradation at different pH

Only the dorsal portion of albacore tuna was used in this study since the smaller portions of the tail and belly regions had insufficient area to facilitate homogenous injection of the pH adjusting solution. Fish samples from lot A were thawed by exposing the meat under running tap water until the core of the meat reached -2 C, then prepared by cutting the fish steaks (cross sections) into pieces of approximately 100 g (13 × 7 × 0.8 cm) using a Hobart meat slicer (Troy, Ohio). The thawed pieces of meat were adjusted to 3 different pH ranges (close to 5.6; 6.2; 6.7) using a mixture of two buffer solutions,

which consisted of 0.2 M boric acid and 0.05 M citric acid solution mixed 0.1 M sodium phosphate solution (Shugar and others 1990). The procedure consisted of injecting (20 % uptake by weight) tuna samples evenly along their total area with the respective buffer solutions (Shugar and others 1990). This procedure was conducted by manual injection of the buffer solution using a 22 gauge needle around the total are of the meat piece. These samples were left for approximately 16 hours immersed in approximately 100 ml buffer (enough volume to cover the meat in respective buffer) to obtain a homogenous meat pH. Samples were drained for 5 minutes and cut into small pieces of 5 × 3 × 0.8 cm, then vacuum sealed in multi-layer "Seal and Meal" plastic bags (VSB2-6, Rival) before cooking in a water bath at 50°C for 0-150 min at intervals of 30 minutes followed by an additional 5 min in a 90°C water bath to inactivate the enzymes and subsequent cooling in ice water. The pH of each sample prior to and after injection and marination was determined by inserting a Stainless steel micro probe with ISFET sensor attached to a model IQ 150 Digital pH Meter (I.Q. Scientific Instrument) into the tuna pieces.

Measurement of myosin autolysis by SDS-PAGE

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted according to the manufacturer's instructions (NuPAGE electrophoresis system, Invitrogen Corp, Calsbad CA) to measure degradation of myosin in tuna pieces during previously described timed isothermal incubation. Treated meat samples were solubilized in SDS- urea buffer (pH 8.0) as described by Nishimoto and others (1990).

The solution was heated in boiling water for 2 min followed by overnight holding with gentle agitation in a rotary shaker (KS 125 BS1, Kika Labortechnik) to assure complete solubilization. The protein concentration of each sample (diluted 1:20) was determined from the difference in absorbance at 280 nm and 260 nm (Segel 1976). This method was preferred over common protein assay methods such as Biuret and Lowry methods due to interference by urea in those assays.

Solubilized samples were diluted 1:10 with distilled water, then mixed 1:1 (v/v) with LDS sample buffer (Invitrogen Inc., Carlsbad, CA), vortexed to mix then heated at 100 °C for 5 min. Cooled solutions at equal protein loading (40 μg protein/well) were applied to pre-made 3-8% Tris-Acetate polyacrylamide gels (Invitrogen, Inc., Carlsbad, CA). Electrophoresis was performed at a constant current of 150 V for 1 hour. Gels were subsequently stained with Coomassie Blue (Invitrogen, Inc.) for 1 hour at room temperature with gentle shaking. Gels were destained for 1 hour in 100 ml of potable water with moderate shaking. Gels were photographed and individual lanes were density-scanned to determine the extent of myosin degradation (Alpha Innotech Corp., San Leandro, CA). Density of the myosin heavy chain (MHC) band was compared to that of the total protein density of the entire lane. Percent MHC remaining was calculated from the ratio of MHC content of each sample to that of the same tuna sample heated only at 90 °C for 5 min (control, or 0 time heat treatment). First order rate constants (k) at

each isothermal incubation temperature were calculated from the percent myosin reaming data using the normalized equation

$$y = 100e^{-kt},$$

where t= time.

Measurement of meat texture and water holding capacity

Samples subjected to varying precooking conditions as described previously were evaluated for texture and water holding capacity. Instrumental texture testing was performed using an Instron Universal Testing Machine (Model 5565, Canton, MA) with a 509.86 kg load cell and a Kramer shear attachment. Crosshead speed was 100 mm/min. For each treatment combination, cooked samples (described previously; n=4) were placed on the plate so the muscle fibers were oriented perpendicular to the blades; this sample orientation has given reproducible results in the past (Stagg 1999). Each sample was sheared once.

Water-holding capacity (moisture loss). Moisture loss of precooked albacore tuna samples was measured by using the standard method (AOAC 1995).

Sensory analysis. A 12 member trained panel used descriptive analysis to assess textural attributes of cooked dorsal and belly samples from tuna lot A. Samples were first precooked at either 50 or 70°C for 150 min followed by 90°C for 20 minutes to ensure that the product was safe for consumption. Controls were cooked only at 90°C for 20

min. The terms used on the ballot are defined in Table 1. Each panelist was presented with 3 samples (5 ×2 × 0.8 cm) of each treatment coded with 3 digit random numbers. Samples were equilibrated to room temperature for 30 min before testing. Two replications were presented on 2 separate days.

Statistical analysis. For the myosin degradation study at precooking temperatures, analysis of variance (ANOVA) was performed on the first order rate constants (calculated from the percent myosin remaining data with the NLIN procedure) using the MIXED Procedure (SAS version 9.1; SAS Institute, Cary, NC) with fixed factorial effects for fish lot (2), position (3), and temperature (4); and random effects for fish (2) nested within fish lot. Initial myosin content was used as a covariate. Pre-planned pairwise means were compared with the diff option.

For the myosin degradation study at cooling temperatures, ANOVA was performed on the first order rate constants (calculated from the percent myosin remaining data with the NLIN procedure) using the GLM procedure for an unbalanced design with fixed factorial effects for fish lot (2), position (3), heating temperature (4) and cooling temperature (7). Pre-planned pairwise means were compared with the pdiff option.

For the myosin degradation study with pH of fish adjusted to 5.6, 6.2, or 6.7 and treated at precooking temperatures, ANOVA was performed on the first order rate constants (calculated from the percent myosin remaining data with the NLIN procedure)

with the MIXED procedure with fixed factorial effects for fish (3 from lot A), pH (3) and temperature (4). Pre-planned pairwise means were compared with the diff option.
For Kramer texture data, only 1 fish from each lot was used so ANOVA was performed on a balanced factorial design with fixed effects for fish lot (2), position (3), and heating temperature (4), while time was treated as a continuous variable.

For sensory analysis data, Pearson correlation coefficients (r) were determined for all possible combinations between sensory attributes, Kramer force values and moisture content. Also, correlation within individual sensory attributes was measured.

All significant differences noted in the text are reported at a 5% level.

RESULTS AND DISCUSSION

Precooking study

ANOVA comparing myosin degradation rates across all treatments showed no significant difference ($p > 0.05$) between the two lots of fish sampled (lots A, B; Figs 1, 2), despite prior experience that fish from these two sources typically varied dramatically in quality and performance (product quality, can yield). There was actually greater difference between individual fish ($p<0.05$) of each lot than overall between the two lots. While differences in myosin degradation rate between individual fish could possibly be attributed to variation in the initial amount of myosin present, due to prior proteolysis immediately post-harvest (Ishiura 1981) the ANOVA showed that the initial myosin

contents of individual fish did not significantly differ ($p>0.05$). Such differences might also occur due to fish physiological conditions, such as spawning, as proteolytic activity can vary between males and females of a single lot (Sikorski and others 2000; Yamashita 1991; Mommsen 2004).

The rate of MHC autolysis was however significantly affected by heating time, temperature and meat sampling location (Figs 1, 2). The rate of MHC degradation in meat of the belly portion ($K= - 0.00559$) was generally greater ($p<0.05$) than that for meat from the tail ($K = - 0.00214$) and dorsal portions ($K = -0.00209$) (Figs 1, 2), which were equivalent. Siebert (1973) had reported that acid protease activity in cod muscle taken from the tail location was almost twice that of meat from the abdominal location. However, Makinod and others (1984) reported no significant differences in acid, neutral or alkaline protease activity between different body locations of carp.

Perez-Villareal and others (1990) and Price and others (1991) both surmised that proteases responsible for MHC degradation in albacore tuna have a slightly acidic optimum pH. However, other types of proteases may also be present in muscle via leakage from the belly cavity (trypsin, chymotrypsin; serine proteases) or from residual kidney tissue (Haard 1994; Gildberg 1982); these are known to degrade belly tissues during fish stowage (Sikorski and others 2000) or subsequent cooking (Cao and others 2006).

MHC of meat samples from all three sampling locations degraded at the greatest rate ($p<0.05$) when precooked at 50-60°C (Fig 1 and 2). At 70°C MHC degraded at a

much reduced rate, this slow rate being equivalent ($p>0.05$) amongst the meat samples taken from the different body locations.

Heu and others (1997) reported that a cathepsin L-like protease in anchovy exhibited maximum activity at 50 °C. Cathepsin L typically exhibits very little or no activity below 50 °C but considerable activity near 60 °C, and is inactivated at temperatures above 70 °C. Cathepsin L is thought to be the major protease responsible for heating-induced softening of the cooked meat of many fish species (An and others 1994, 1995, 1996; Makinodan and others 1987; Porter and others 1995; Visessanguan and others 2001, 2003). A similar temperature optimum for autolysis has been observed for meat of skipjack tuna, mackerel and some other fish species (Stagg 1999; Haard 1994; Muramoto and others 1989; Jiang and others 1997; Ho and others 2000; Makinodan and others 1987). While autolysis in these species was also attributed to acidic (cathepsin) protease activity, heat-stable serine proteases (trypsin-like myofibril-bound serine protease) in several fish species have also been reported to have a similar optimum temperature range of activity (Toyohara and Shimizu 1988; Boye and Lanier 1988; Lin and Lanier 1980; Chio and others 1999; Yongsawatdigul and others 2000; Cao and others 1999, 2005, 2006).

It is interesting to note that tuna myosin denatures at temperatures (Tm) of about 50- 52°C, as reported for skipjack tuna (Bell 2000; Webb 2003). Denaturation of the myosin likely provides more active sites for the protease to cleave, which could additionally contribute to the high rate of myosin degradation noted in this temperature

range and above (until the protease becomes increasingly inactivated at 70°C and higher). A similar effect of protein denaturation on enzymic-induced crosslinking of myosin was seen by Joseph and others (1994).

Cooling study

As had previously been noted in the precooking study, the MHC degradation rate during cooling also differed significantly between individual fish ($p<0.05$). Once again, this variation could not be accounted for by variation in the initial myosin content ($p>0.05$).

MHC autolysis was observed to continue in the dorsal portion of albacore tuna of meat heated to endpoint temperatures up to 70°C when subsequently held at temperatures above 30°C (Fig 3). The highest rate of MHC degradation ($p<0.05$) occurred in samples precooked to end temperatures of 55 or 60°C and subsequently held at 50°C for up to 150 min (Fig. 3a and 3b).

Thus, even precooking albacore tuna to an end temperature of 70°C (Fig. 3d) did not completely inactivate autolysis. Yongsawatdigul and others (1997) reported degradation of MHC in Pacific whiting surimi by endogenous proteinase even at 75°C. These data suggest that autolysis, initiated in albacore meat during precooking, likely continues during the early cooling period in those portions of the meat heated only to

70°C or below. The heat stability of this autolytic activity suggests that certain cathepsins and serine proteases are likely the enzymes responsible (Heu and others 1997; Makinodan and others 1987; An and others 1994; Visessanguan and others 2001, 2003; Toyohara and Shimizu 1988; Yongsawatdigul and others 2000; Simpson 2000; Sikorski and others 2000; Cao and others 1999, 2005, 2006).

Effect of initial meat pH on autolysis

In the precooking study (Table 2) meat from Lot B which had a lower initial meat pH (~pH 5.4-5.8) showed higher autolytic activity than meat from the same lot having a pH nearer 7.0. Because the autolytic activity measured also seemed to coincide with that reported for cathepsin L (Kirschke and others 1981), this seemed to suggest that acid proteases might be the primary causative agent of autolysis in albacore.

However, when the initial meat pH was adjusted over the range of 5.6 to 6.7, no significant effect of meat pH was observed on the rate of myosin degradation ($p>0.05$) during incubation at 50°C (Fig 4). Stagg (1999) similarly reported that meat pH had no effect on myosin degradation rate of skipjack tuna incubated at 55°C.

Effect of autolysis on texture and water-holding ability of albacore meat

The presumed quality differences between lots A and B showed no effect on meat texture or water-holding ability of precooked meat. However, heating temperature and sampling location significantly ($p<0.05$) affected the texture (Kramer press

measurements) and moisture content of albacore tuna after precooking (Table 3). Meat from the belly location was less strong in texture and of lower moisture content ($p<0.05$) while meat from the tail region was strongest ($p<0.05$) in texture and highest in moisture content ($p<0.05$). Meat from either dorsal or belly locations, when precooked at 50°C, was less strong in texture ($p<0.05$) as measured by both the Kramer press and sensory analysis. This weakness in texture could not be attributed to higher moisture content; indeed the moisture content was also minimal for this treatment ($p>0.05$) (Tables 3, 4 and Fig 5).

The observed significantly stronger ($p<0.05$) texture of tail meat as compared to dorsal/belly meat may be in part due to greater proteolytic degradation in the latter two meat samples (Fig 1c, 2c). However, the structure of the tail meat may also be a factor. The muscle fiber diameter varies according to location in the fish; thinner muscle fibers are associated with firmer texture (Hatae and others 1990, Hurling and others 1996; Kanoh and others 1988). Albacore tail meat cooked more rapidly (5 min at 90°C) was stronger than dorsal and belly meat similarly cooked (Table 5).

The higher moisture content of tail meat may be related to the presence of heat-coagulated sarcoplasmic proteins between muscle fibers which can contribute to water retention (Offstad and others 1993). This can also possibly account for the stronger texture of the cooked tail meat, since Hatae and others (1990) observed that coagulated sarcoplasmic proteins seem to glue the muscle fibers to each another in a way that

obstructs the displacement of the fibers when they are compressed, resulting in a stronger texture.

MHC almost disappeared in most albacore samples heated at 50°C for 2 to 3 hr (Fig 6). In these samples, proteolytic degradation of the meat is likely responsible for the greater texture degradation and moisture loss upon cooking of these samples ($p<0.05$). Moisture loss during cooking is largely caused by lateral shrinkage of denatured myosin (Bell and others 2001; Offstad and others 1993), additional moisture loss could result from proteolytic breakdown of the myofibrillar proteins and thus the meat microstructure. Cooking at higher temperature (70 °C) not only likely resulted in less breakdown of the myofibril structure, but also formation of aggregates by denatured sarcoplasmic proteins and collagen in the intercellular space of the muscle fibers likely occurred, which can additionally contribute to water holding ability (Offstad and others 1993).

Sensory testing of cooked samples confirmed that all meat portions cooked at 50°C were less firm, required the fewest chews to completely masticate, and produced an excessive amount of grittiness and particulate residual in the mouth ($p<0.05$) as compared to samples cooked at 70°C (Table 4). These are generally considered to be negative textural attributes with respect to desirable canned albacore texture. Good correlations were noted between sensory firmness, minimum number of chews for mastication, grittiness, and particulate residual and the Kramer shear-maximum force values ($r = 0.76$, $r = 0.839$, $r = 0.899$, $r = 0.820$, respectively) (Fig 7).

The Kramer Shear test predicted very well the mechanical sensory attributes of cooked albacore tuna, as has been reported for cooked meat of other fish species, (Borderias and others 1983; Howgate 1977). Correlation between individual sensory attributes of both dorsal and belly samples showed that they were independent of each other, except for residual and grittiness, which tended to be related to each other ($r = 0.763$) (Table 5).

The Kramer press has similarly proved useful for assessing effects of cooking processes on texture of skipjack tuna (Stagg 1999) and mullet (Deng 1981). This instrument is able to make reproducible measurements of such cooked fish meat because it overcomes some problems of sample heterogeneity (Dunajski 1979; Sigurgisladottir and others 1999; Barroso and others 1998; Borderias and others 1983).

CONCLUSIONS

Albacore tuna displays autolysis of myosin and a corresponding weakening of texture and water loss during precooking at temperatures from 50 to 70 °C. The texture weakening is manifest primarily by a grainy mouthfeel and propensity of the precooked meat to easily fragment into smaller particles or flakes. Autolysis was confirmed to continue during the cooling phase after precooking, even at temperatures as low as 30°C. Initial meat quality and postmortem pH of fresh muscle albacore did not affect heating-induced autolysis. The highest rate of MHC degradation ($p<0.05$) and associated textural/water loss change occurred in belly meat precooked at 50 - 60°C.

These results would suggest that when precooked the albacore meat should be rapidly raised to a backbone (coolest point) temperature higher than 70°C to inactivate proteases which can adversely affect piece integrity, texture and cook yield during the precooking/cooling step of canning. However, the tuna canning industry also is concerned about overcooking of meat near the surface, which could be exacerbated by attempts to raise backbone temperature at a greater rate to a higher endpoint temperature. Previous (unpublished) attempts to commercially precook smaller pieces of meat (rather than whole or split large fish) have shown reduced cook yields, attributed to greater exposure of cut meat surfaces and a higher surface/mass ratio. Thus minimizing heat-induced autolysis of albacore tuna is not a simple task to achieve.

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Table 1.1: Definitions of tuna texture

Firmness: Force required compressing a 2 x 2 x 0.8 cm sample between the molar teeth.
Number of Chews: Using a 2 x 2 x 0.8 cm, count the number of chews until your first swallow (or impulse to swallow if you expectorate).
Cohesiveness of Mass: Degree to which the sample holds together in a mass.
Grittiness/Graininess of Mass: Degree to which the sample has small, hard, gritty/grainy/sandy pieces
Moistness: degree to which sample is wet/moist during chewing.
Moisture Release: degree to which sample releases moisture during chewing.
Residual: Amount of particles left in mouth after swallowing or expectorating.

Table 1.2: Initial meat pH of raw albacore tuna from two lots and three different fish portions (dorsal, belly and tail)

Fish (No/Lot)	Dorsal	Belly	Tail
Fish 1 (Lot A)	6.3	6.3	6.3
Fish 2 (Lot A)	6.2	6.2	6.2
Fish 1 (Lot B)	5.4	5.8	5.6
Fish 2 (Lot B)	6.4	6.6	6.5

Table 1.3: LS mean values of Kramer texture measurements and moisture contents as affected by fish portion location and precook temperature

	Kramer shear press (Force/wt)	Moisture content (%)
Belly	**2.4** ^{A}	**66.47** ^{A}
Control *	3.22 ^{a}	71.13 ^{a}
50	2.12 ^{b}	66.33 ^{b}
70	2.87 ^{a}	67.24 ^{c}
Dorsal	**2.63** ^{B}	**66.89** ^{B}
Control *	3.70 ^{ac}	70.56 ^{a}
50	2.04 ^{d}	66.52 ^{b}
70	3.47 ^{c}	67.97 ^{d}
Tail	**3.64** ^{C}	**68.57** ^{C}
Control *	4.60 ^{e}	70.52 ^{a}
50	2.70 ^{f}	66.80 ^{b}
70	4.27 ^{e}	68.52 ^{d}

Different capital letters in the same column mean that the effect of fish location in texture (Kramer press) and moisture content were significantly ($p<0.05$) different.
Different lower case letters in the same column mean that the effect of heat treatments in texture (Kramer press) and moisture content were significantly ($p<0.05$) different.
* Albacore tuna samples heated 5 minutes at 90 °C.

Table 1.4: LS mean values of sensory textural scores as affected by fish portion location and precook temperature

Variables	Belly [A]		Dorsal [B]	
	50 °C	70 °C	50 °C	70 °C
Sensory*				
Firmness	4.73[a]	5.97[b]	6.35[a]	8.68[b]
Chews	11.90[a]	14.25[b]	14.63[a]	18.87[b]
Cohesiveness	4.80[a]	5.58[a]	5.15[a]	4.95[a]
Grittiness	10.32[a]	8.77[b]	7.97[a]	6.56[b]
Moistness	4.87[a]	4.88[a]	4.97[a]	3.78[b]
Moisture release	6.10[a]	5.91[a]	5.74[a]	4.39[b]
Residual	11.14[a]	8.60[b]	9.26[a]	7.78[b]

Different capital letters within a row mean that fish location were significantly ($p<0.05$) different
Different lower case letters within a row mean that those heat treatments were significantly ($p<0.05$) different in each fish location.
*Texture profile panel

Table 1.5: r and p values from Pearson correlation analysis results to examine the association between individual sensory textural attributes of precooked albacore tuna

	Firmness (r/p)	Chews (r/p)	Cohesiveness (r/p)	Grittiness (r/p)	Moistness (r/p)	Moist. Release (r/p)	Residual (r/p)
Firmness	1	-0.2729 0.197	0.1209 0.5734	0.4179 0.0421	0.2465 0.2455	0.2874 0.1733	0.3577 0.086
Chews	--	1	0.3406 0.1034	-0.3106 0.1396	-0.1903 0.373	-0.2162 0.310	-0.2399 0.2588
Cohesiveness	--	--	1	-0.3484 0.0952	0.3326 0.1123	0.11429 0.595	0.3631 0.0812
Grittiness	--	--	--	1	0.0.2879 0.894	0.4031 0.0508	0.7625 <.0001*
Moistness	--	--	--	--	1	0.1432 0.504	-0.2074 0.331
Moisture release	--	--	--	--	--	1	0.1977 0.354
Residual	--	--	--	--	--	--	1

Values of marked with * indicate statistical significant influence of measured variable on sensory attributes (p=0.05, or 95 % confidence)

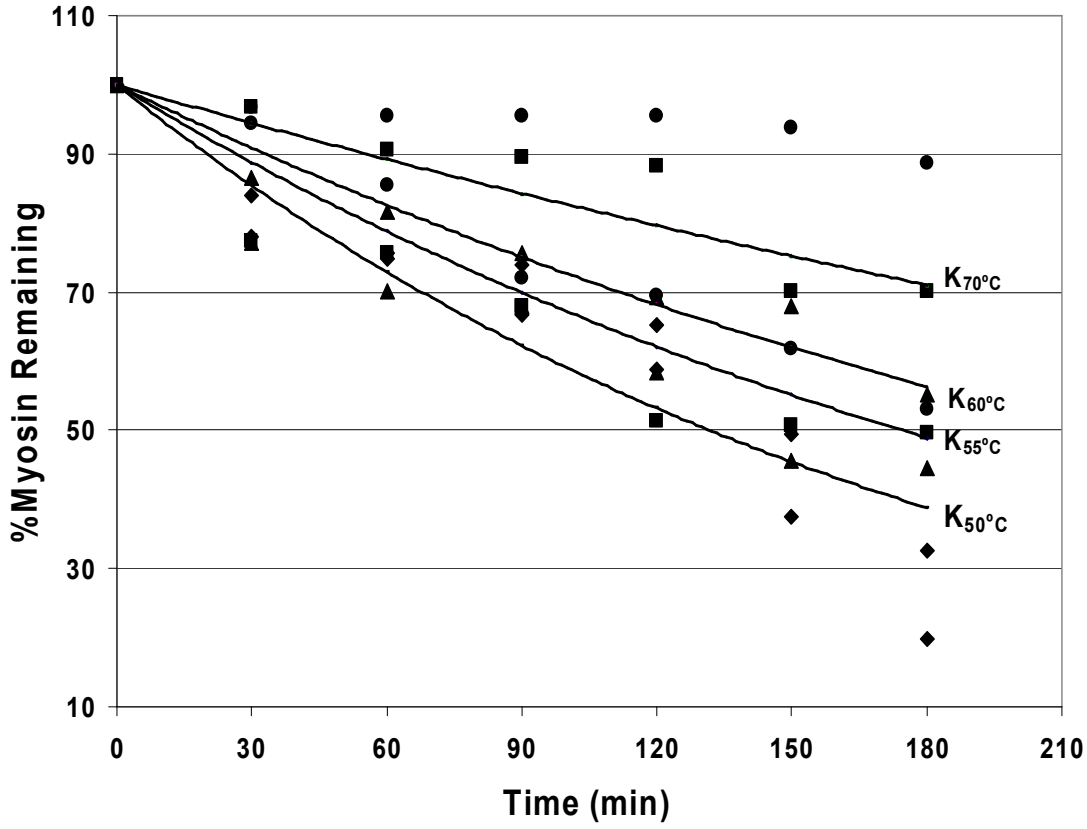

Figure 1.1a: Degradation rate of MHC (K-value) in albacore dorsal portions (Fish lot A) when precooked at 50-70 °C. $K_{50°C}$ = -0.00526 ♦, $K_{55°C}$ = - 0.00397▲, $K_{60°C}$ = -0.00319 ■, $K_{70°C}$= - 0.00189●.

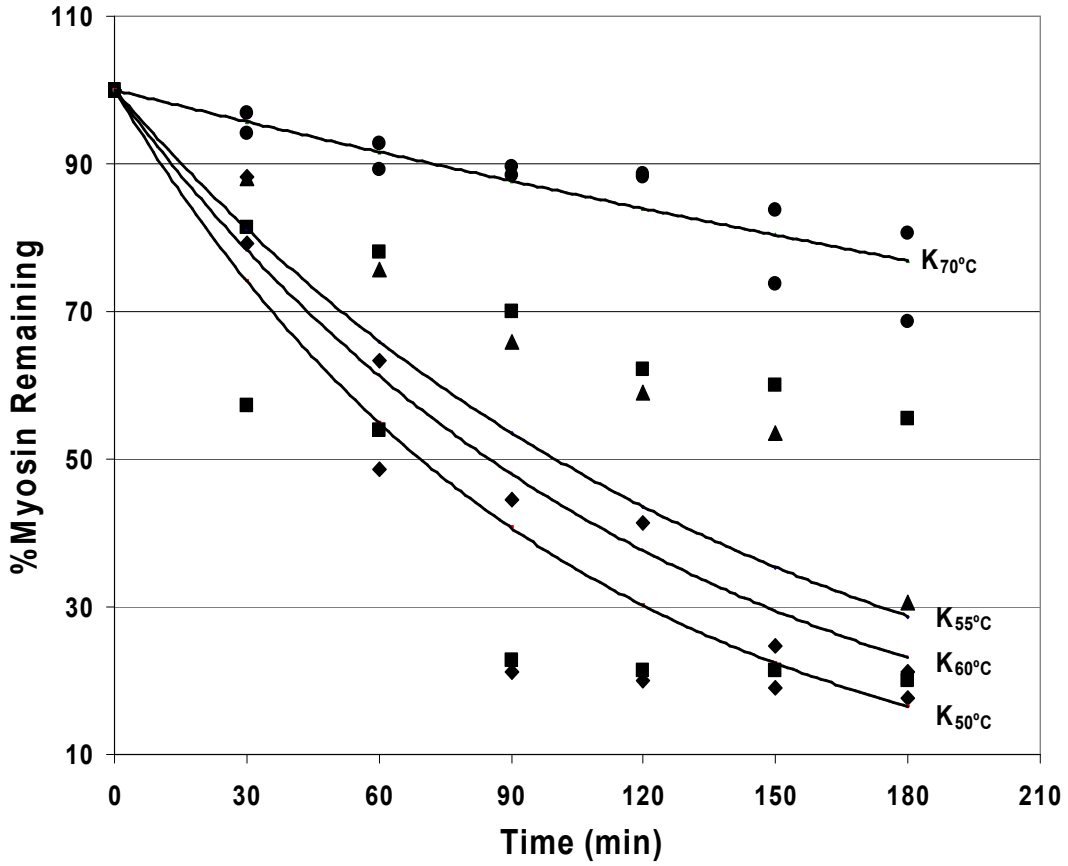

Figure 1.1b: Degradation rate of MHC (K-value) in albacore belly portions (Fish lot A) when precooked at 50-70 °C. $K_{50°C}$ = -0.00998 ♦, $K_{55°C}$ = - 0.00693 ▲, $K_{60°C}$ = - 0.00815 ■, $K_{70°C}$= - 0.00146 ●.

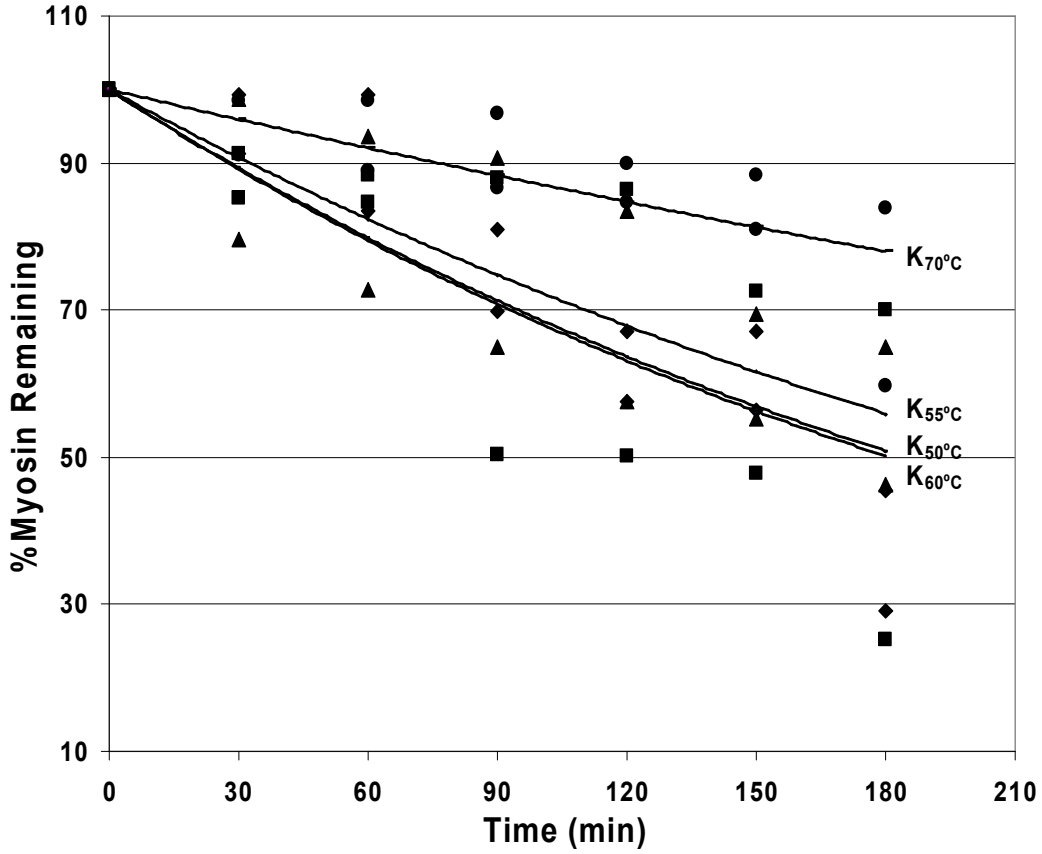

Figure 1.1c: Degradation rate of MHC (K-value) in albacore tail portions (Fish lot A) when precooked at 50-70 °C. $K_{50°C}$ = -0.00376 ♦, $K_{55°C}$ = - 0.00323 ▲, $K_{60°C}$ = - 0.00383 ■, $K_{70°C}$= - 0.00138 ●.

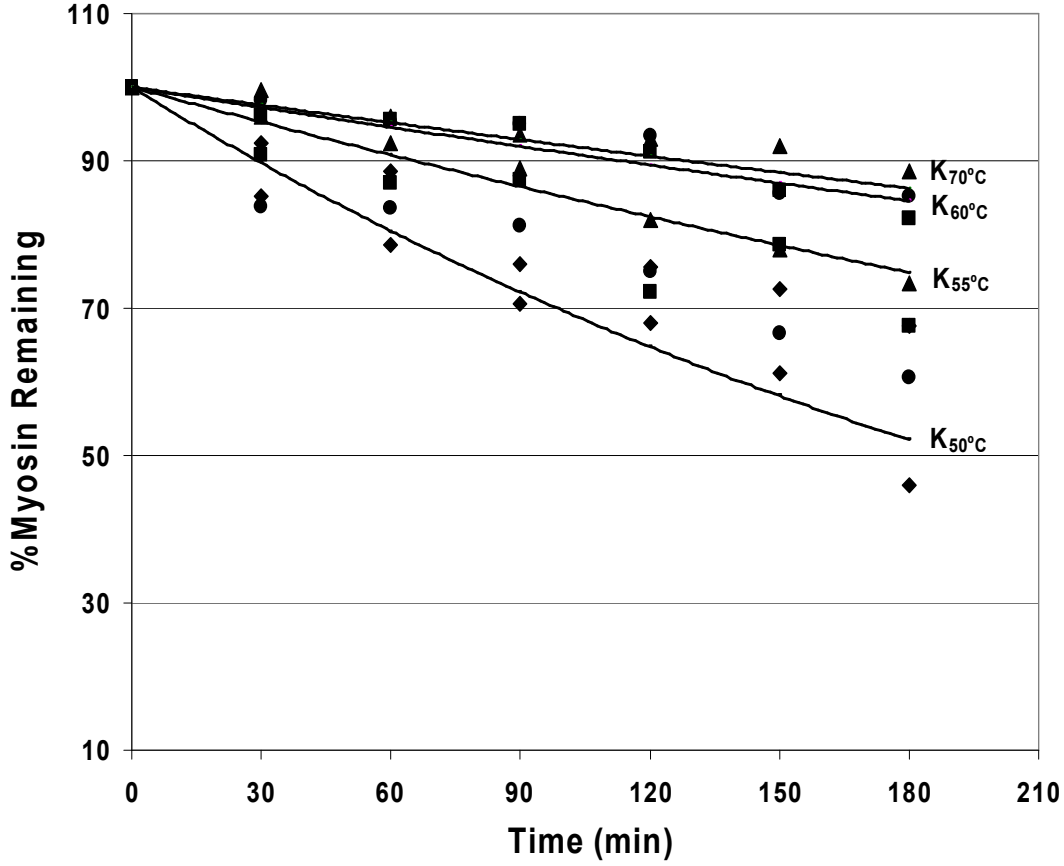

Figure 1.2a: Degradation rate of MHC (K-value) in albacore dorsal portions (Fish lot B) when precooked at 50-70 °C. $K_{50°C}$ = -0.00362 ♦, $K_{55°C}$ = - 0.00161▲, $K_{60°C}$ = - 0.00093 ■, $K_{70°C}$= - 0.000822●.

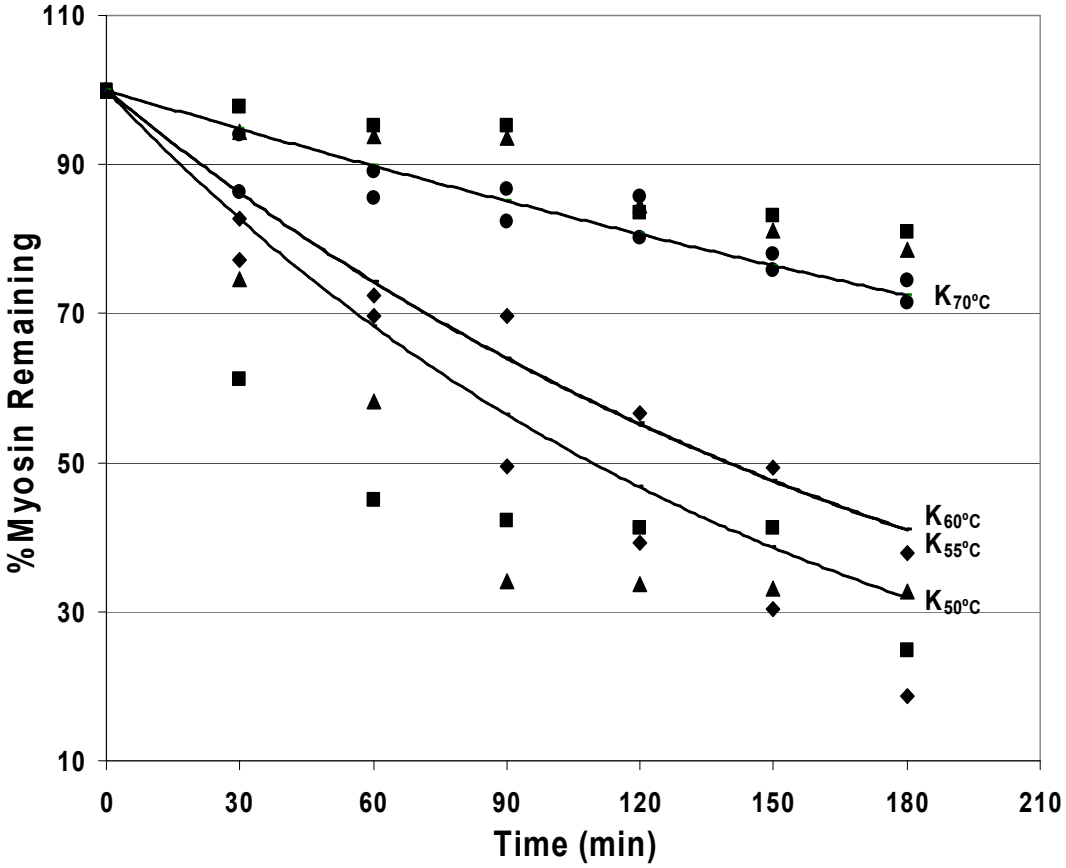

Figure 1.2b: Degradation rate of MHC (K-value) in albacore belly portions (Fish lot B) when precooked at 50-70 °C. $K_{50°C}$ = -0.00635 ♦, $K_{55°C}$ = - 0.00496▲, $K_{60°C}$ = - 0.00495 ■, $K_{70°C}$= - 0.00179●.

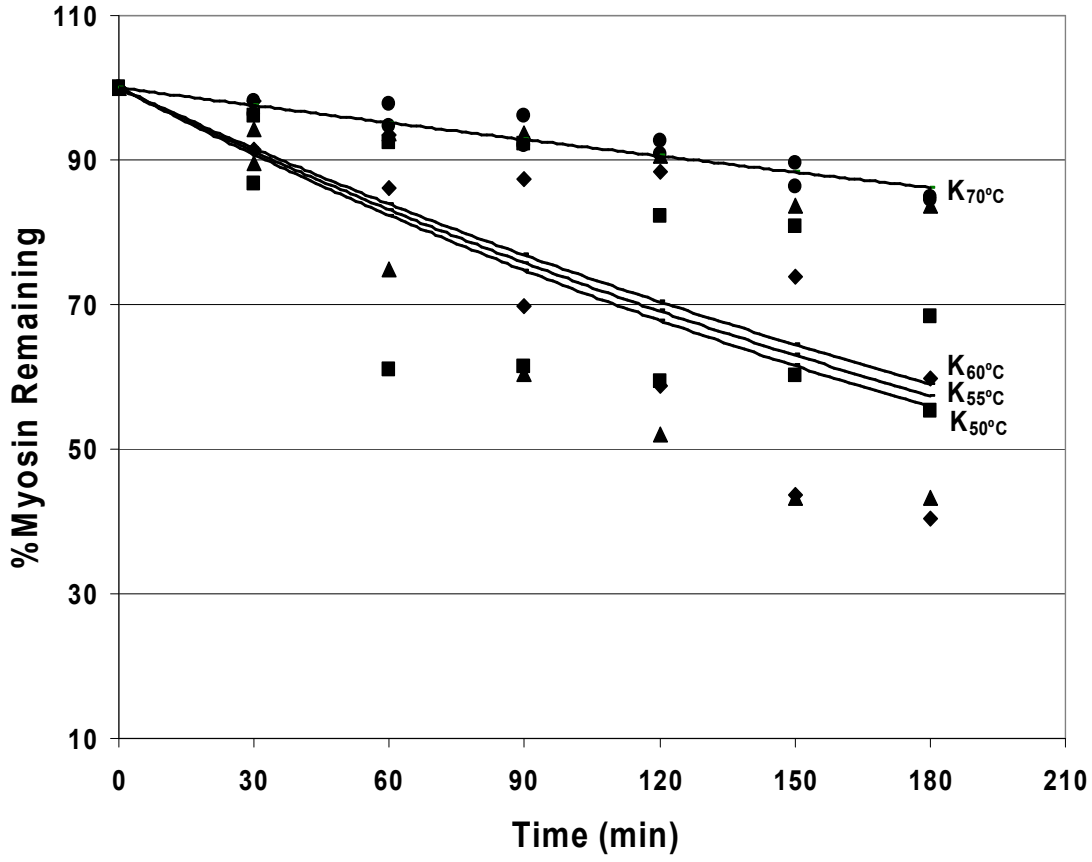

Figure 1.2c: Degradation rate of MHC (K-value) in albacore tail portions (Fish lot B) when precooked at 50-70 °C. $K_{50°C}$ = -0.00323 ♦, $K_{55°C}$ = - 0.00308▲, $K_{60°C}$ = - 0.00293 ■, $K_{70°C}$= - 0.000827●.

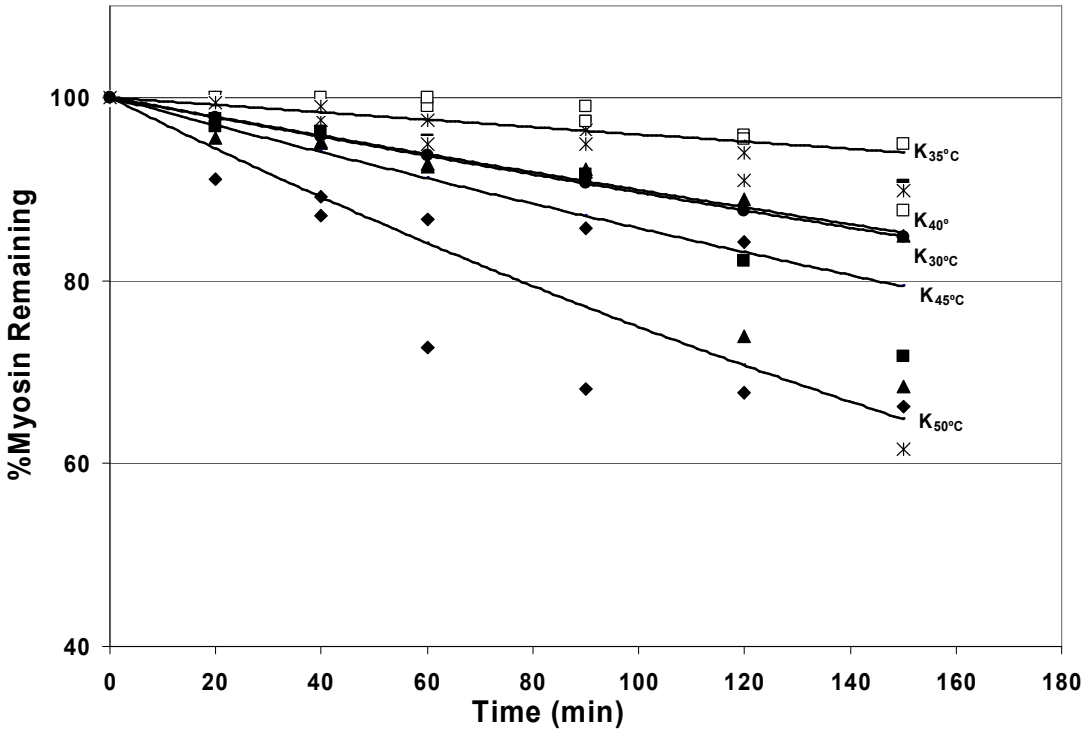

Figure 1.3a: Tuna dorsal portions heated to 55°C followed by cooling in water baths at lower temperatures. $K_{50°C}$ = -0.002888 ♦, $K_{45°C}$ = - 0.00154▲, $K_{40°C}$ = -0.00107 ■, $K_{35°C}$= - 0.000412 □, $K_{30°C}$ = -0.00109 ✳.

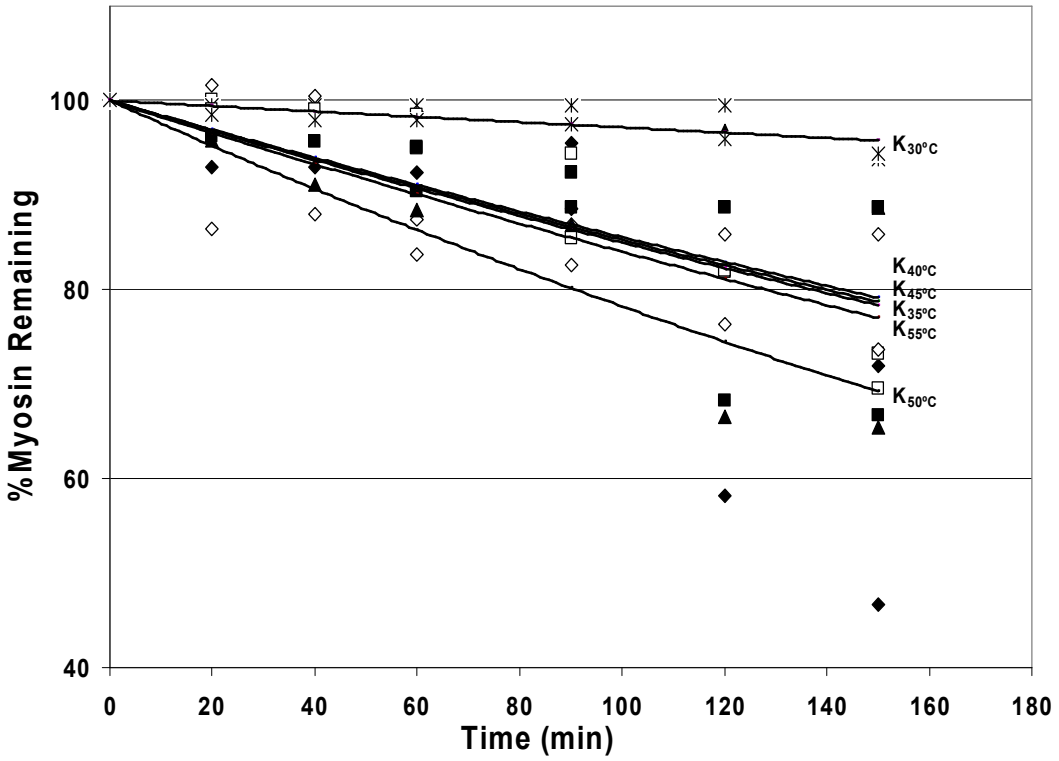

Figure 1.3b: Tuna dorsal portions heated to 60°C followed by cooling in water baths at lower temperatures. $K_{55°C}$ = -0.001742◊, $K_{50°C}$ = -0.002456 ♦, $K_{45°C}$ = - 0.001559▲, $K_{40°C}$ = -0.001595 ■, $K_{35°C}$= - 0.001627 □, $K_{30°C}$ = -0.000288 *.

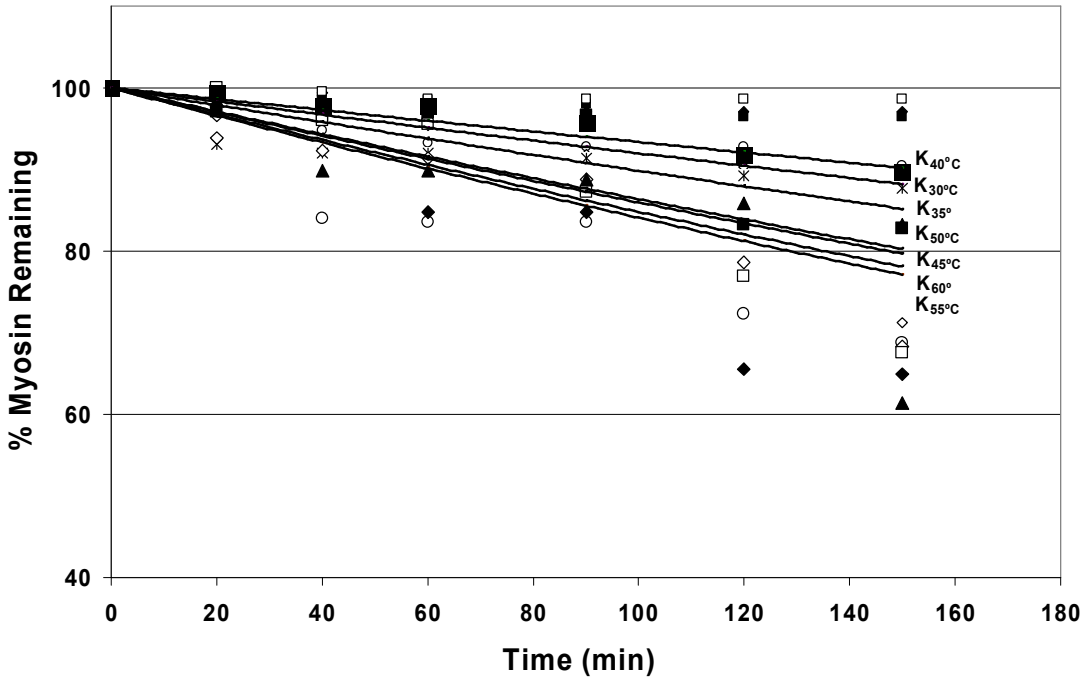

Figure 1.3c: Tuna dorsal portions heated to 65°C followed by cooling in water baths at lower temperatures. $K_{60°C}$ = -0.001649 ○, $K_{55°C}$ = -0.001734◊, $K_{50°C}$ = -0.001466 ♦, $K_{45°C}$ = - 0.001513▲, $K_{40°C}$ = -0.0006877 ■, $K_{35°C}$= - 0.00107 □, $K_{30°C}$ = -0.000836 *.

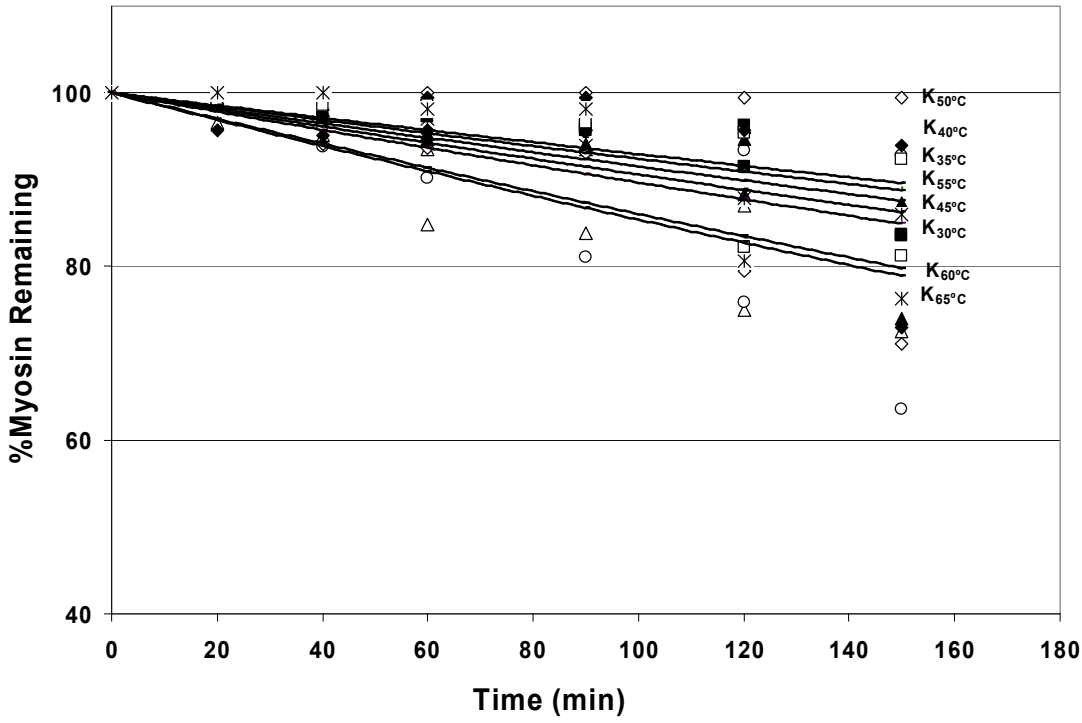

Figure 1.3d: Tuna dorsal portions heated to 70°C followed by cooling in water baths at lower temperatures. $K_{65°C}$ = -0.001578 ∆, $K_{60°C}$ = -0.001502 ○, $K_{55°C}$ = -0.000885◊, $K_{50°C}$ = -0.000731 ♦, $K_{45°C}$ = - 0.000987▲, $K_{40°C}$ = -0.000774 ■, $K_{35°C}$= - 0.000791 □, $K_{30°C}$ = -0.00109 *.

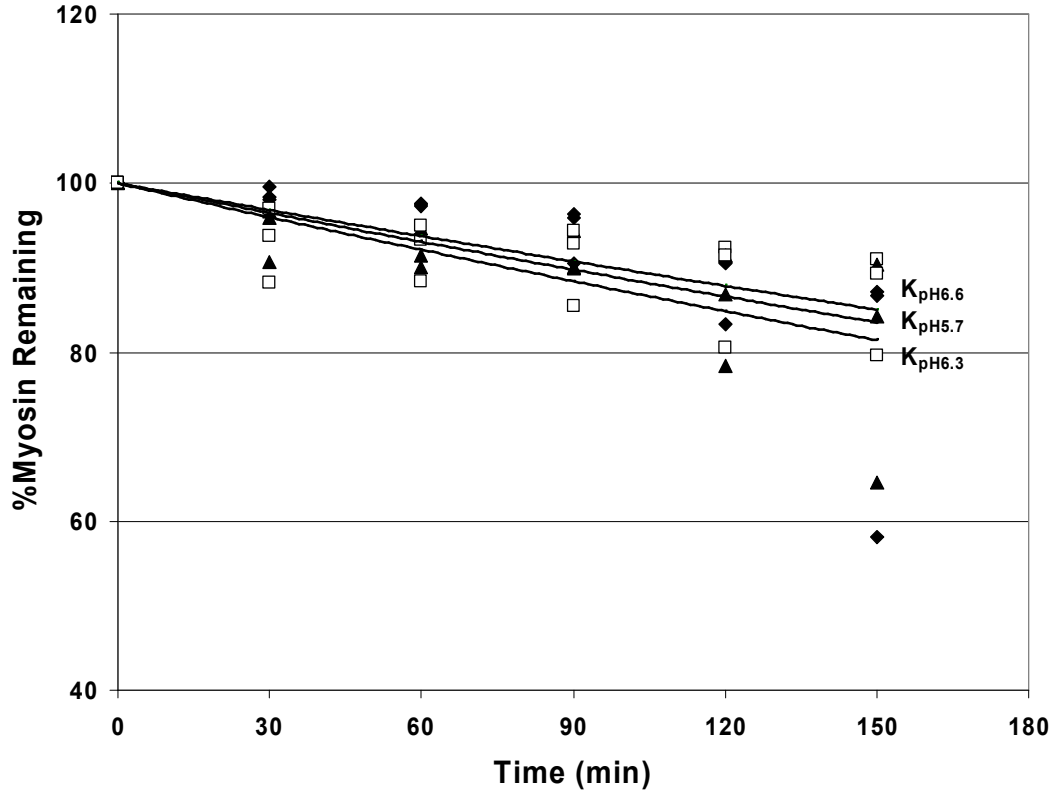

Figure 1.4: Effect of initial meat pH on rate of myosin autolysis during cooking at 50°C. $K_{pH\ 5.7}$= -0.0012 ♦, $K_{pH\ 6.3}$= -0.00137 ▲, $K_{pH6.6}$= -0.00108 ■.

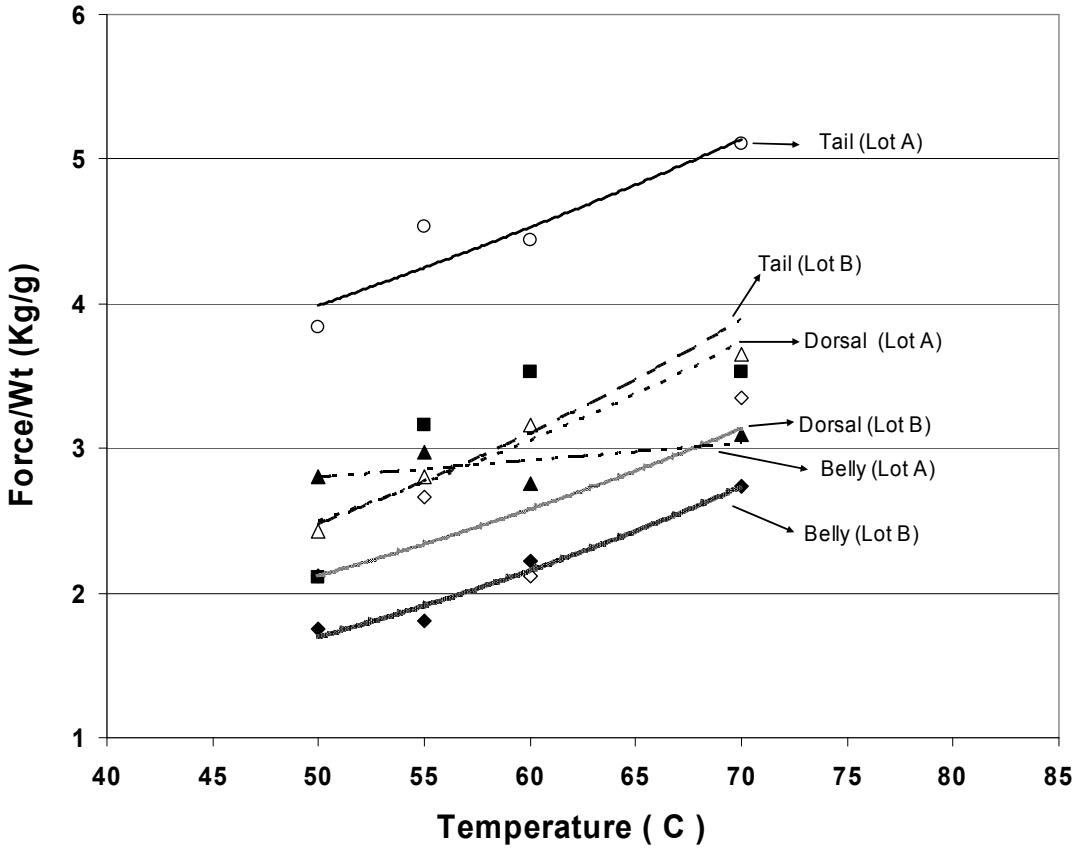

Figure 1. 5: Effect of endpoint cook temperatures, fish portion, and fish lot on meat texture (measured by Kramer shear). Tail (Lot A) ○, Tail (Lot B) ■, Dorsal (Lot A) ∆, Dorsal (Lot B) ◊, Belly (Lot A) ▲, Belly (Lot B) ♦.

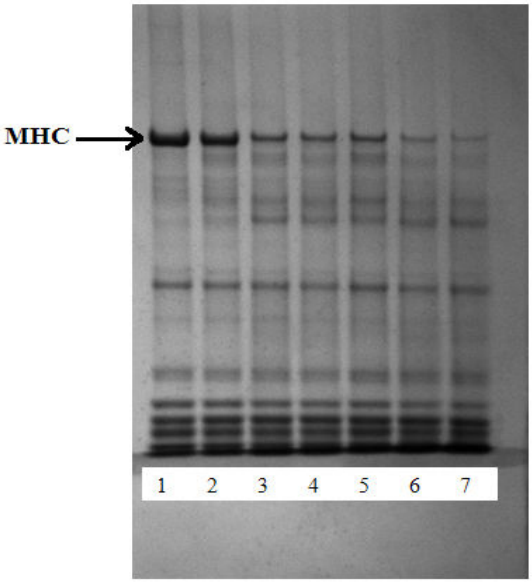

Figure 1.6: SDS-PAGE gel of albacore tuna meat precooked for varying times at 50°C: 0 time (Lane1), 30 min (Lane2), 60 min (Lane 3), 90min (Lane 4), 120 min (Lane 5), 150 min (Lane 6), and 180 min (Lane 7).

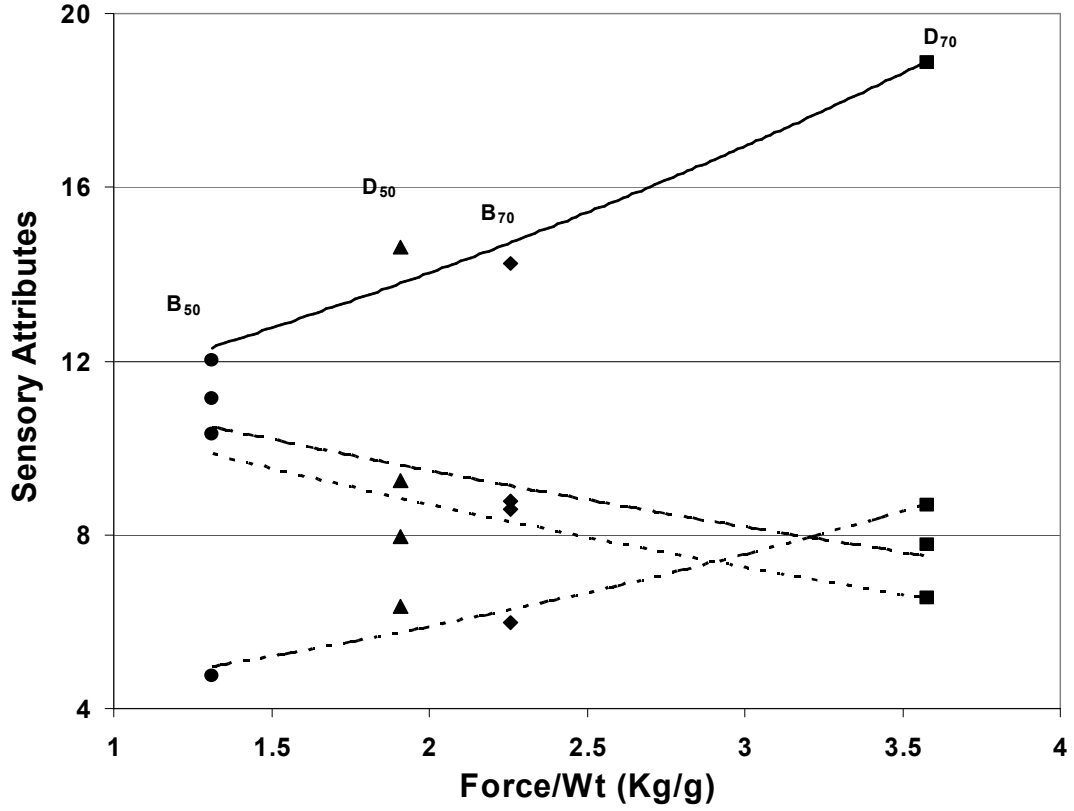

Figure 1.7: Plot of Kramer shear values vs. sensory attributes. Belly at 50 ºC ●, Dorsal at 50 ºC ▲, Belly at 70 ºC ■, Dorsal at 70 ºC ♦. —— Number of chews (r=0.839), - - - Residual (r=0.820), ⁻ ··· ⁻ Grittiness (r=0.899), ··· Firmness (r=0.760)

Paper 2

Effect of Autolysis During Precooking on Albacore Tuna Piece Integrity and Can Yield

Ruilova-Duval M.E., Lanier T.C., and Amato P.M.

Department of Food, Bioprocessing and Nutrition Sciences

N.C. State University

Raleigh, NC 27695-7624

ABSTRACT

Autolysis, induced by heat-stable endogenous proteases during precooking of albacore tuna, was shown previously to occur during conditions similar to those imposed by commercial precooking of albacore tuna. It seemed likely that such proteolysis could lead to a weakening of the meat texture such that piece integrity might be compromised during the manual deboning and packing operations that follow precooking in the normal tuna canning process. Water holding properties could also be affected, possibly affecting can yield. A standardized tumbling treatment was applied to induce flaking of tuna precooked by the most damaging conditions identified in prior work, and piece integrity was quantified by passing pieces of meat through a series of screens of decreasing mesh size. The meat was subsequently canned, and can yields were measured by the mandated press test and drain weights; yields following precooking were also measured. Meat taken from the belly portion of the fish, when precooked at 50 °C, evidenced the highest rate of autolysis. A greater percentage of small flakes, plus lower yield after both precooking and canning, were associated with higher autolytic activity during precooking.

Keywords: Albacore tuna, autolysis, piece integrity, moisture loss, can yield.

INTRODUCTION

A continuing goal of the tuna canning industry is to increase case yield (cases of canned product per ton of whole tuna processed) while maintaining an acceptable level of canned meat quality (in particular, good texture, which is regarded as a high percentage of the meat in large, firm chunks) (Bell 2000). Canned tuna yield and textural quality can be affected by both the initial quality of the meat being canned, and by the parameters chosen for the processing steps to which it is subjected during canning.

Earlier work from our laboratory reported that skipjack tuna precooked at temperatures which promote autolytic activity (near 55°C) evidenced soft texture as determined by Kramer shear press and sensory analysis. This soft texture correlated to myosin heavy chain (MHC) degradation as revealed by SDS-PAGE (Stagg 1999). Bell and others (2000, 2001) found that canned skipjack tuna previously precooked to a backbone temperature no greater than 55°C evidenced lower press weight yields (US standard method for assessing canned yield of tuna; 21CFR161.145).

Our previous report (Ruilova and others 2008) showed that the meat protein (MHC in particular) of albacore tuna could similarly exhibit heating-induced autolysis during the precooking and cooling process, prior to filling of cans. The greatest rate of autolysis occurred at precook temperatures lower than 70°C (especially near 50°C), and if precooking did not achieve higher temperatures for sufficient time, autolysis could

continue during subsequent cooling, even at temperatures as low as 30°C. Based upon the previous work by our laboratory on skipjack tuna (Stagg 1999), we suspected that such autolytic activity occurring during the precooking and cooling process of albacore prior to canning might adversely affect the canned meat quality (texture) and canned/case yield of albacore tuna. Thus the objective of this study was to determine if autolytic activity during precooking and cooling, prior to canning, might negatively affect the meat quality and yield of canned albacore tuna.

MATERIALS AND METHODS

Frozen albacore tuna loins (*Thunnus alalunga*) were obtained from a primary processing plant in Trinidad which offloaded whole fish frozen at sea. Loins taken from the anterior dorsal and belly portions of a single harvest lot of fish were vacuumed sealed separately and shipped frozen to the NCSU Food Science Department, and stored in a -35°C freezer.

Pre*cooking treatment*

Tuna portions (body sampling locations) and heat treatments used in this part of the study represented the extremes of precooking process conditions identified in a prior study which induced the greatest amount of autolysis (50°C/150 min, belly portion) vs. that which produced the least amount of autolysis (70 °C/10 min, dorsal portion)

(Ruilova and others 2008). A 50°C / 150 min treatment approximates the typical time that some portion of large, intact albacore tuna might be exposed to a temperature range of 45-60°C (ideal for autolysis) during the precooking step of an industrial canning process.

Steaks (2.54 cm thickness) were cut from thawed dorsal and belly anterior loins and sealed in plastic bags. These were either (a) precooked at 50°C / 150 min in a 50°C water bath, then transferred to a 90°C water bath until the center of the meat reached 70°C (additional 4 min), or (b) directly cooked at 90°C until the coldest point reached 70°C, which took ca. 10 min. Following cooking, all bagged steaks were immersed in ice water and cooled to 30°C.

Measurement of autolysis by SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted according to the manufacturer's instruction (NuPAGE electrophoresis system, Invitrogen Corp, Calsbad CA) to determine the extent of degradation of myosin. Treated meat samples were solubilized in a SDS- urea buffer (pH 8.0) as described by Nishimoto and others (1990). The solution was heated in boiling water for 2 min followed by overnight holding with gentle agitation in a rotary shaker (KS 125 BS1, Kika Labortechnik) to assure complete solubilization. The protein concentration of each sample (diluted 1:20) was determined from the difference in absorbance at 280 nm and 260 nm; values that allowed determination of the protein concentration of each sample

(Segel 1976). This method was used instead of common proteins assays (Biuret and Lowry methods) because the SDS-urea buffer in which the treated tuna sample was solubilized develops color in the blank solutions of those methods due to its high urea content.

Solubilized samples were diluted 1:10 with distilled water, then mixed 1:1 (v/v) with LDS sample buffer (Invitrogen Inc.), vortexed and heated at 100°C for 5 min, then cooled. Cold solutions at equal protein concentration (40 µg protein/well) were loaded onto pre-made 3-8% Tris-acetate polyacrylamide gels (Invitrogen, Inc.). Electrophoresis was performed at a constant current of 150 V for 1 hour. Gels were subsequently stained using Coomassie Blue staining reagent (Invitrogen, Inc.) for 1 hour at room temperature with gentle shaking. Gels were destained for 1 hour in 100 ml of potable water with moderate shaking. The gels were photographed and densitometry used (Alpha Innotech Corp., San Leandro, CA) to determine the degree of myosin degradation. The density of the myosin heavy chain (MHC) bands was expressed as a percentage of the density of all bands in the entire lane. The % MHC remaining is indicative of the level of endogenous protease activity that occurred as a result of that treatment. Percent remaining MHC was calculated from the ratio of MHC content of each treated sample to the original amount of myosin in the control (tuna sample heated for 5 min. at 90°C; which count as 0 time), which was not exposed to the treatment conditions (Fig 2a).

Measurement of meat piece integrity

A method previously developed for standardizing flake separation of precooked skipjack tuna was used (Stagg 1999). Cooked (by the two previous processes, 50°C/150 min and 70°C/ 10 min) dorsal and belly portions were flaked by rotation in a large tumbler (Model No. 350 FG-V; serial No. 001550; Key Laboratories, Inc. Largo, Florida) for 5 minutes (24 rpm). Figure 3 is a representative photo of albacore flakes produced after the tumbling treatment.

The tumbled meat was then sorted by flake size using a Ro-Tap machine (ASC Scientific, Canada) equipped with 5 stacked sieves (Fig. 1) of progressively smaller-sized sieve openings: 5.08, 3.8, 2.54, 1.27, and 0.95 cm, plus a final collection pan. Piece size distribution was determined as the percent of original weight of the original meat sample that was retained on each sieve/pan after shaking for 5 min.

Measurement of can yield

Canned meat originating from both dorsal and belly body locations were prepared by filling each can with 93.55 g of precooked solid albacore meat, 11.34 g of flakes (2.54 cm or less in size) plus 62.37 g of potable water containing 1.2 % NaCl. Fifteen cans from each fish portion and each heat treatment were generated. The cans were sealed and retorted using a process schedule meeting industry standards (111.6°C for 98 min).

Following canning and cooling, drained weight and pressed weight data was measured, following industry standard procedures (21CFR 161.145). Drained weight was

determined by draining the canned tuna for 2 min on a # 8 sieve and weighing the screen retentate. Pressed weight was calculated as the weight of tuna remaining after hydraulic pressing at 384 pounds per square inch for 1 min using a standardized press cylinder and plunger (21CFR 161.145). Drained and pressed weight data were used to calculate the percent mass loss of canned albacore tuna after retorting, as follows:

% mass loss (by draining) = [(filled weight-drained weight)/filled weight]×100

% mass loss (by pressing) = [(filled weight-pressed weight)/filled weight]×100

The percent moisture loss of precooked albacore tuna meat before canning and retorting was calculated as follows:

% moisture loss = [(raw meat moisture content– cooked meat moisture content)÷ raw meat moisture content]×100

Statistical analysis

Analysis of variance (ANOVA) was performed with the general linear models (GLM) program (SAS version 9.1; SAS Institute, Cary, NC) with fixed factorial effects for tuna position, temperatures and fish pieces size (identified as sieve) to measure the dependent variable (percent of flake). Main and interaction effects of sieve×position, sieve×temperature and sieve×temperature×position were measured at a 5% level of significance. The same model was applied for the canning study, with the difference that the factorial included only tuna position and temperature effect and the dependent variable was percent mass loss.

RESULTS AND DISCUSION

Autolysis, piece integrity, and moisture loss during precooking

SDS-PAGE results confirmed that greater MHC degradation ($p<0.05$), for meat from both sampling locations, occurred when albacore samples were heated at 50°C/150 min as compared to 70°C/10 min (Figs. 2a, 2b). We had previously found that 22 % of MHC was degraded in meat (taken from both body locations) heated at 70C/150 min, while > 60 % of MHC was degraded in meat heated at 50ºC/150min (Ruilova and others, 2008). This marked effect of precook temperature on MHC degradation was seen even during the initial 10 minutes of cooking, wherein 2 and 8 % of myosin degraded at 70°C and 50°C, respectively. A similar trend in myosin loss was also seen in this study for tuna pieces rapidly heated to 70 C/10min (in a 90°C water bath), as well as for those tuna pieces incubated at 50 ºC/150min prior to canning and retorting (Fig. 2b).

The size distribution of flakes produced by tumbling (Figs. 3, 4) reveals that meat from the dorsal location produced a greater percentage of larger sized flakes, regardless of the heating process used, and that the 70°C/10 min heating process resulted in a greater percentage of larger sized flakes, regardless of the fish location from which the meat was sampled. The greatest percentage of larger sized flakes therefore was produced from dorsal meat processed at 70°C/10 min whereas the lowest percentage of larger flakes was produced from belly meat processed at 50°C/150 min. Since the latter combination of belly meat, processed at 50°C, corresponds to the combination which

exhibited the greatest degree of MHC autolysis (Fig. 2a, 2b), the greater propensity of this treatment combination to flaking upon tumbling of the cooked meat can likely be attributed to autolytic breakdown of the meat structure during the heating process. A similar effect of meat autolysis during precooking on meat piece integrity was observed earlier in our laboratory by Stagg (1999) in analogous studies with skipjack tuna.

By regulation (21CFR161.190) canned albacore 'solid' packs cannot contain >18% of meat 'flakes', defined as pieces that will pass though a 1.27 cm mesh screen, and 'chunk' packs cannot contain >50% of such flakes. While this regulation applies to the canned (twice cooked; precooked then canned and retorted), not to meat that is precooked only (once cooked), processors must be careful in packing cans with precooked meat prior to retorting to avoid unacceptable levels of flake occurrence in the final pack. Combining the meat fractions which were retained on or which passed through the 0.95 cm sieve (that is, all flakes which passed through a 1.27 cm sieve per the regulatory definition of 'flakes'), the measured percentages of 'flakes' for each treatment combination are: belly meat, 50°C/150 min: 37.9%; belly meat, 70°C/10 min: 23.3%; dorsal meat, 50°C/150 min: 31.1%; dorsal meat: 70°C/10 min: 15.1%. Because of the higher selling price/value of solid pack tuna, the goal is to produce as much solid pack as possible. It is thus apparent that the time/temperature of the precooking stage of processing can have an economic impact on the canning operation, as meat from both body locations is more prone to produce flakes (that are limited in quantity to 18% of the

total pack) when the precooking conditions become favorable for autolysis to occur for any extended period of time.

Measurements of moisture loss in dorsal and belly meat, after cooking at 50 °C (isothermal for 150 minutes, then taken just to 70°C in a 90°C water bath) vs. direct cooking to 70 °C (in a 90°C water bath) (Fig. 5), revealed that both meat location and heat process affected precook yields, in agreement with our previous findings (Ruilova and others 2007). These data seem to correlate well with effects of meat location and heat process on myosin degradation (Fig. 2b), implying that autolytic degradation of meat contributes to lower meat yield after precooking. This lower precook yield could in turn contribute to a lower canned yield (less cans produced per ton of fish processed).

Canned yield

Both dorsal and belly meat samples, when previously precooked at 50 °C/150 min, trended higher in mass loss after canning as measured by draining than did the same meats precooked to 70°C (Fig 6). There was little apparent effect of meat location, with a slight but significant temperature effect ($p<0.5$), on mass loss, but only in dorsal meat.

However, by regulation tuna canning yield is measured by the standard press test, not drained weights. Similar results were obtained when mass loss during canning was measured after pressing (Fig. 7), and a significantly lower yield ($p<0.5$) was obtained for belly or dorsal meat processed at the lower temperature that accentuated heating-induced autolysis (50°C/150 min).

Bell (2000) similarly reported a lower pressed yield in canned skipjack tuna (canned in water) previously precooked at 55°C as compared to having been precooked at 70°C. However, Stagg (1999) reported that skipjack tuna precooked at 55 °C exhibited a higher pressed yield than did similar samples precooked at 70°C, when both were canned with added vegetable broth (Stagg 1999). The cause for these contradictory data, obtained with skipjack is not clear; however, it may relate to the ability of the vegetable broth to enhance the water uptake, and also the viscous nature of partially degraded skipjack meat resulting from the measured autolysis induced by precooking at 55°C. The more viscous nature (mushy textured) of this degraded skipjack meat could effectively plug the narrow channel between the inner and outer cylinders of the press test instrument, blocking moisture flow out during the timed pressing test.

The texture of albacore meat tends however more toward brittle flakiness upon autolysis, rather than to softness and mushiness as with skipjack. Thus the present data support the conclusion that autolysis during precooking can affect canned yield in two ways: decreasing weight of meat available after precooking (Fig. 5), so that more meat would be needed at the initiation of processing to provide sufficient precooked meat to fill the same number of cans, plus greater mass loss from cans after retorting and press testing (Fig. 7).

Mass loss of pressed retorted albacore tuna was probably a consequence of the degraded meat structure which facilitated the release of liquid from the tuna cake when high pressure was exerted upon it. Such a weak meat structure would be expected as a

consequence of myosin degradation induced by the endogenous heat-stable proteases: we observed that the highest percentage of myosin loss (Fig. 2), and mass loss (Fig. 7), occurred when the tuna meat was precooked at 50°C/150 min.

CONCLUSIONS

The fragility (tendency to flake) of the precooked meat and canned yield of albacore tuna were affected by the thermal conditions of the precooking step, prior to filling of the can with the cooked meat for retorting. Meat from the dorsal and belly regions of the anterior loin region when precooked at 50°C generated a higher percentage of small flakes (smaller than 2.54 cm in size) than did the same meats heated to 70°C. These meats that were precooked at 50°C also exhibited higher mass loss, during precooking and canning, than meat precooked to 70°C. These deleterious effects of the 50°C precook may be attributed to degradation of meat proteins by endogenous proteases, as evidenced by greater MHC degradation during this precook process. It is thus clear that the process parameters of the precooking process can affect both process yield and canned meat quality in albacore tuna.

ACKNOWLEDGMENTS

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Figure 2.1: Ro-Tap machine equipped with 5-stacked sieves

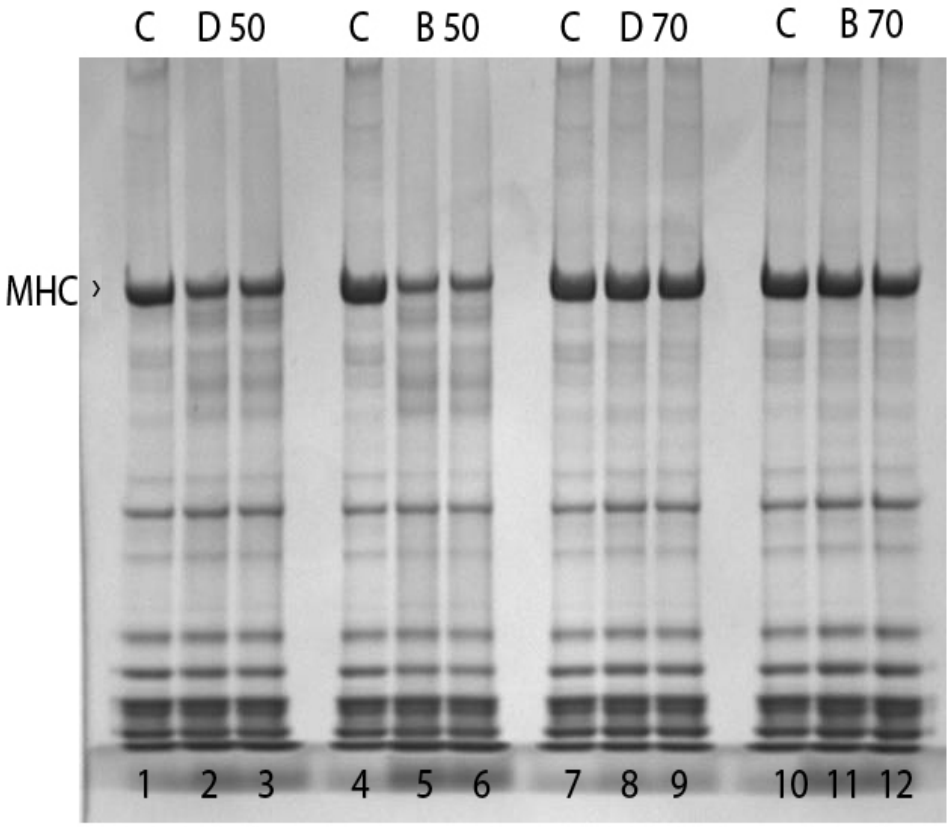

Figure 2.2a: SDS-PAGE gel of albacore tuna meat: Control; dorsal tuna cooked for 5 min in 90°C water bath (Lane 1, and 7) and belly control (Lane 4 and 10); Dorsal at 50°C (Lane 2,3); Belly at 50°C (lane 5,6); Dorsal to 70°C (Lane 8,9); Belly to 70°C (Lane 11,12).

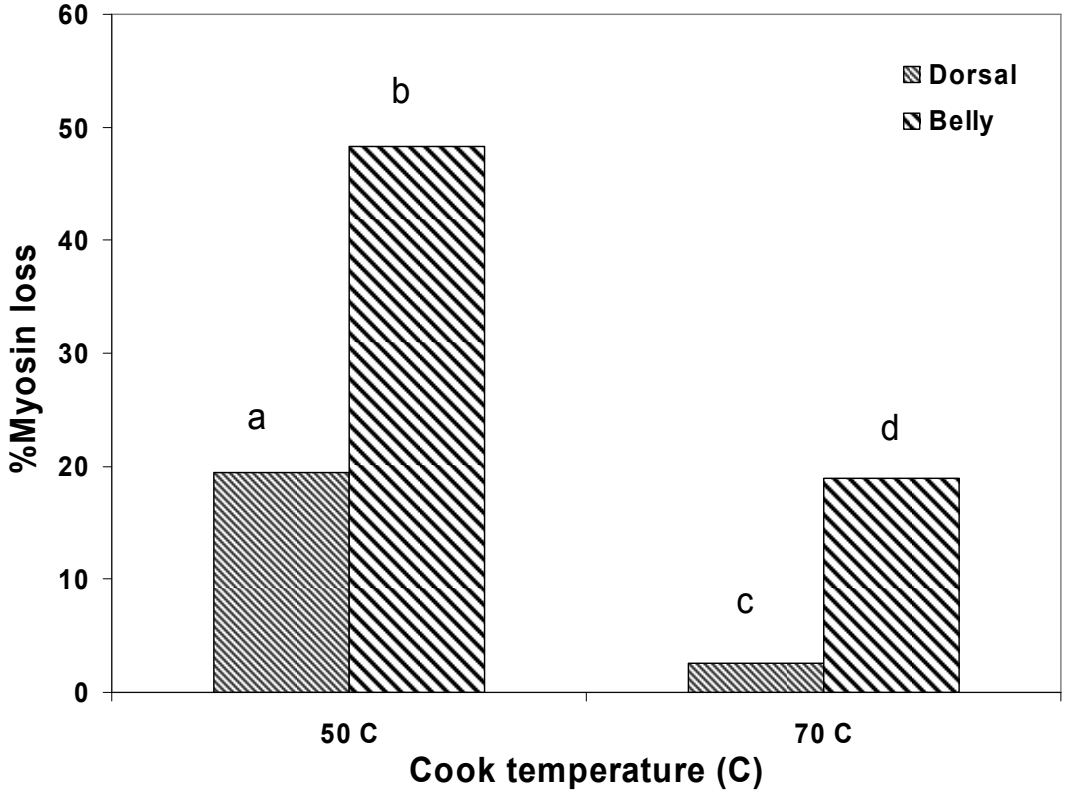

Figure 2.2b: Myosin loss of albacore tuna precooked to 70ºC/10min or precooked at 50ºC/150min (before canning and retorting). Different lowercase letters within and between treatments indicate significant differences ($p<0.05$)

Figure 2.3: Representative albacore flakes produced after tumbling treatment

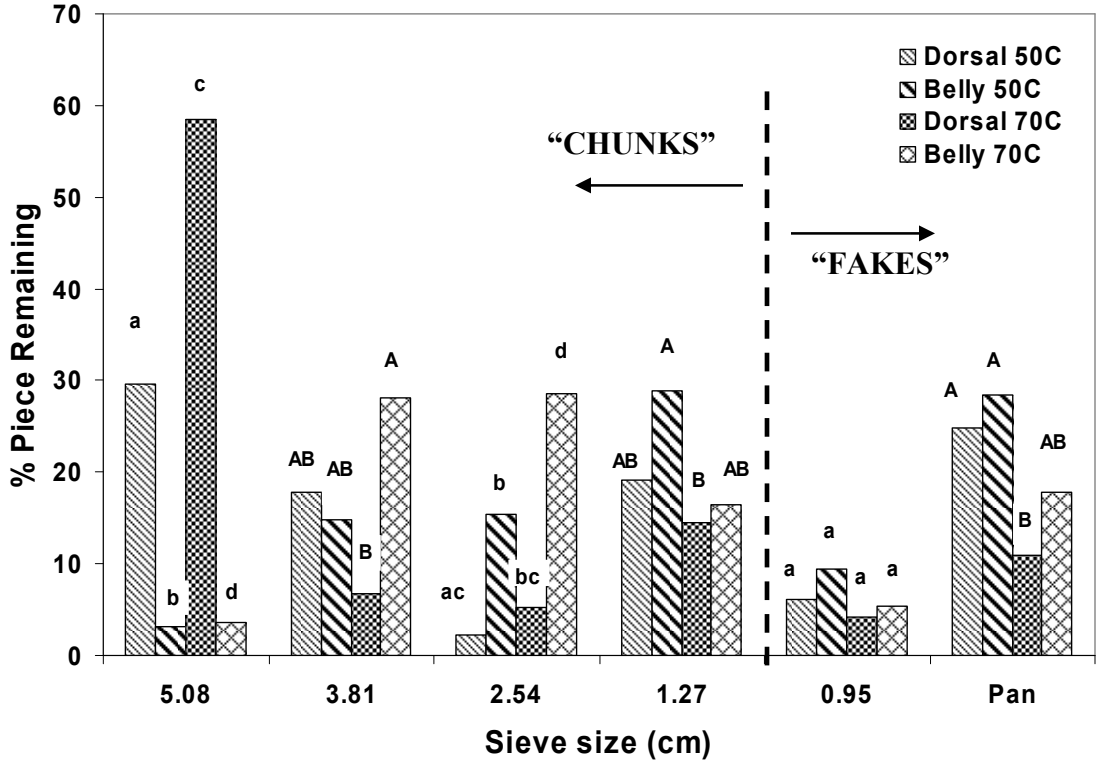

Figure 2.4: Piece size distribution of dorsal and belly meat of albacore tuna precooked to 70°C/10min or precooked at 50°C/150 min and then heated to 70°C. Different letters within a sieve size indicate significant differences ($p<0.05$)

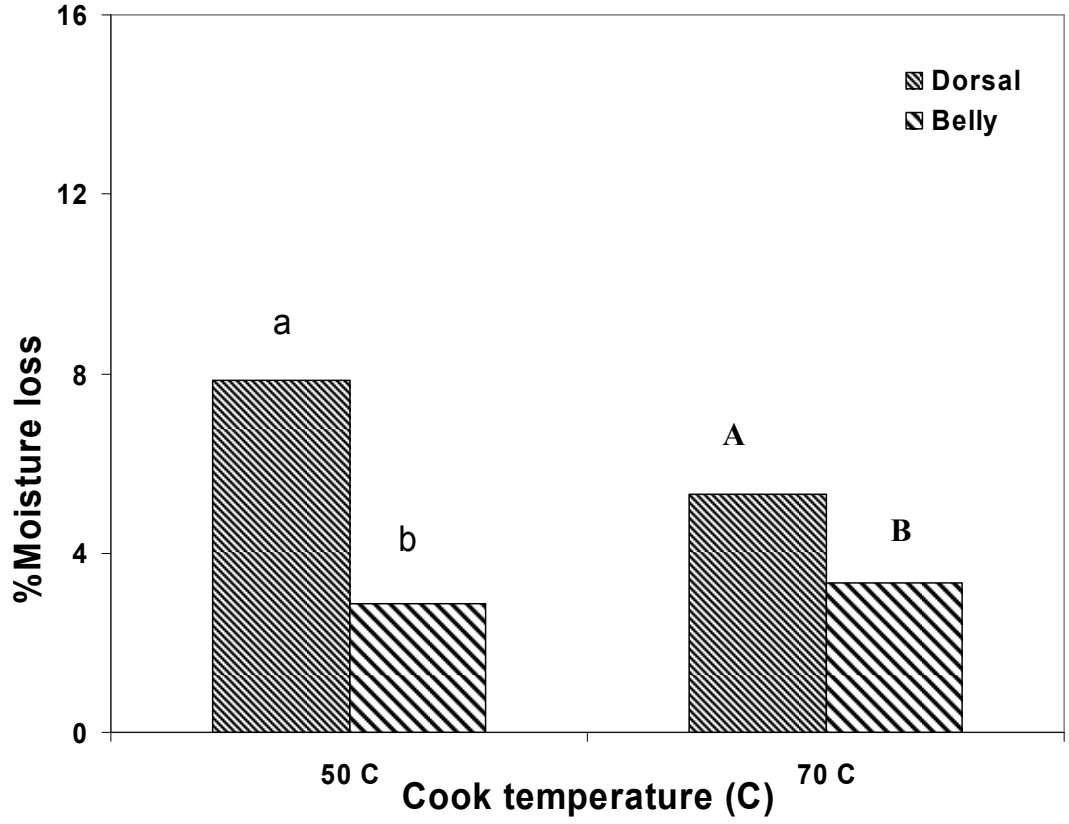

Figure 2.5: **Effect of fish sampling location and precooking process on moisture loss during precooking. 50°C: 50°C for 150 minutes then heated to 70°C in a 90°C water bath. 70°C: to center temperature of 70°C in a 90°C water bath. Different lowercase letters within and between treatments indicate significant differences ($p<0.05$)**

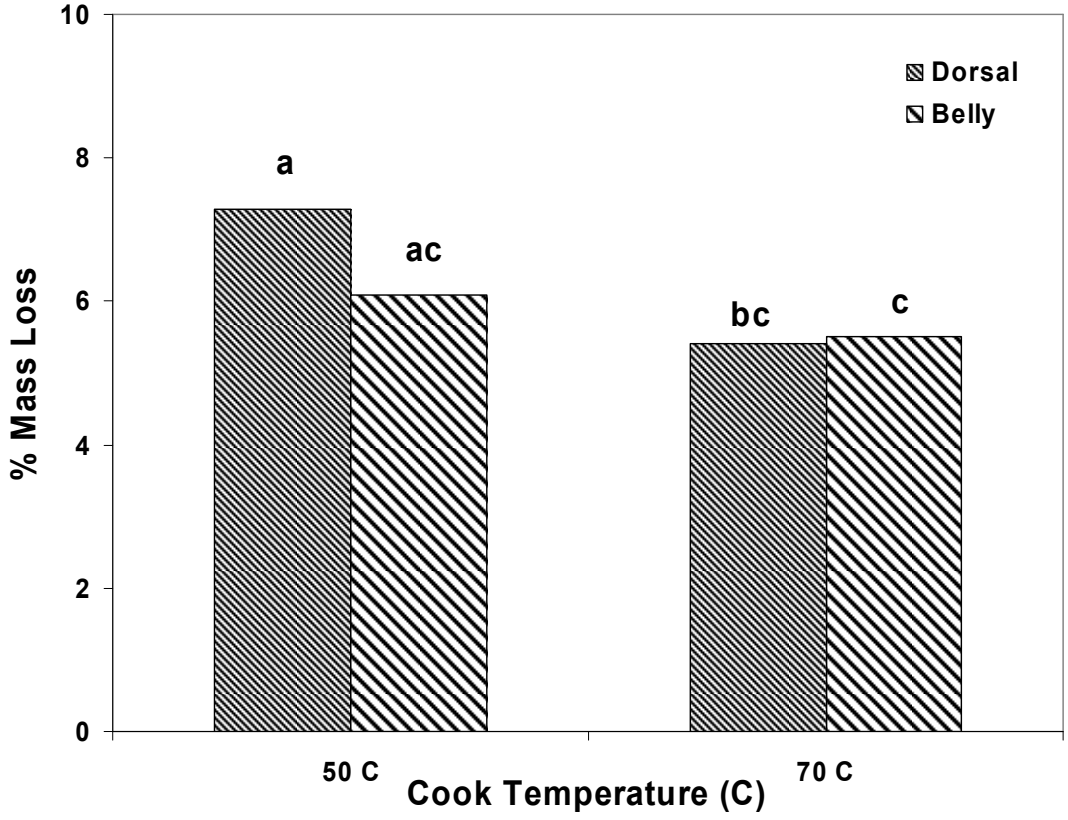

Figure 2.6: **Mass loss from <u>drained retorted</u> albacore muscle cakes previously precooked at 50 °C or to 70 °C. For precooked samples at 50 °C: dorsal and belly samples were held at 50°C for 150 minutes, then heated to 70 °C in a 90 °C water bath. For treatment to 70 °C: muscle samples were heated to center temperature of 70 °C in a 90 ºC water bath. Different lowercase letters within and between treatments indicate significant differences ($p<0.05$).**

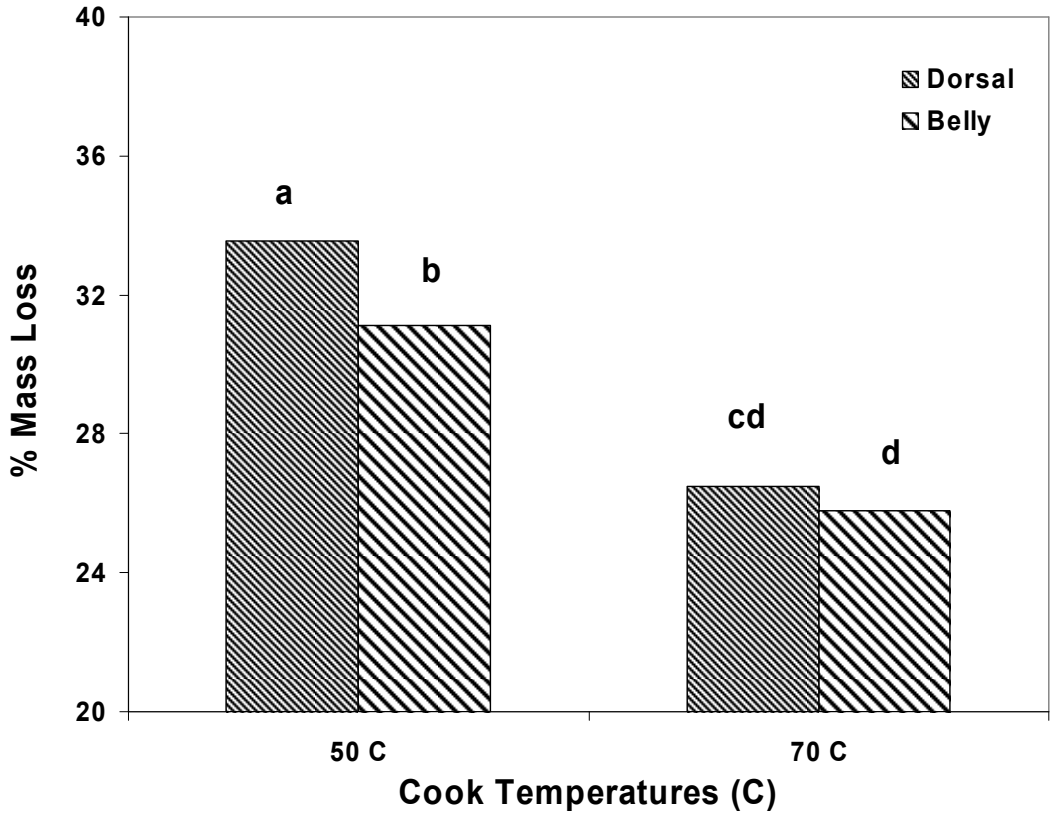

Figure 2.7: **Mass loss from <u>pressed retorted</u> albacore muscle cakes previously precooked at 50ºC or to 70ºC. For precooked samples at 50°C: dorsal and belly samples were held at 50°C for 150 minutes, then heated to 70°C in a 90°C water bath. For treatment to 70°C: muscle samples were heated to center temperature of 70°C in a 90ºC water bath. Different lowercase letters within and between treatments indicate significant differences ($p<0.05$).**