ABSTRACT

MENSAH, MARGARET EDEM. Desalination of Tuna after Excessive Salt Uptake from Brine Freezing. (Under the direction of Brian Farkas).

Brine freezing is a technique used extensively on board fishing vessels to preserve the quality of harvested tuna due to the preservative effect of fast freezing from salt mixtures.

The process occasionally results in excessive salt uptake by fish and thus presents the need to find techniques to desalt fish so affected. This involves an evaluation of variables such as temperature, fish piece size and fish to water ratio on the desalination process. The aim of this research was to analyze the influence of these process variables on the desalination kinetics of tuna and the effect of the process on the final product quality (protein loss, water uptake, yield and overall acceptability). A solid liquid extraction (single stage dispersed contact) technique was used to desalt fish previously soaked with excess salt in a custom-made laboratory brine freezer. The influence of the desalination variables on the process was determined with a 2x2x3 factorial design (temperature: 10^{o}C and 25^{o}C; fish to water ratio 1:1.5, 1:3 and 1:4.5; fish piece size: cut loins and flakes). Total nitrogen content (crude protein) of desalted fish was determined by Kjeldahl method, water uptake determined by mass balance and yield determined by drain weight analysis, and the overall acceptability of canned desalted fish determined by a consumer acceptance test (n=99).

Among the process variables studied, fish piece size influenced salt loss, protein loss and water uptake the most; smaller piece sizes favored each phenomenon. Temperature (at the levels studied) did not result in a significant difference in the extent of salt and protein loss ($p<0.05$). The lower temperature however appeared to favor water uptake ($p=0.015$).

Fish to water ratio did not seem to affect salt loss, protein loss and water uptake during desalination ($p<0.05$). However, when considered with fish piece size, there was an interaction effect on salt loss ($p=0.027$). Canned desalted tuna products had lower drain weights than control tuna products. Among the twelve treatments studied, cut loins desalted in 1:1.5 fish to water ratio at 25^{o}C and canned in brine recorded the highest preference for aroma, taste and texture among consumers.

Desalination of Tuna after Excessive Salt Uptake from Brine Freezing

by
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DEDICATION

To my father, Charles Yao Mensah and all the people who believed in me and supported me throughout the program.

BIOGRAPHY

Margaret Edem Mensah was born in Accra, Ghana. With a career objective of becoming a food safety auditor and a food industrialist, she pursued a bachelor's degree in Food Science and Nutrition at the University of Ghana, legon-Accra. Her career goals were motivated by her strong desire to contribute to improving food safety in Africa (especially Ghana).

Following her graduation from the University of Ghana in 2010, she went on to work for one of Ghana's major tuna canneries (MYROC Food Processing Co. Ltd) where she gained experience in quality assurance, production supervision and internal auditing. In 2011, she was sponsored by the company to pursue a Master's degree in Food Science at North Carolina State University, where she conducted research in the area of tuna desalination technology, under the supervision of Dr. Brian Farkas.

After graduating with her Master's degree, she is planning to return to her country to become a highly productive member in any food industry/organization she may become associated with or establish in the near future.

ACKNOWLEDGMENTS

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To the rest of my family and friends, especially Kennedy Bomfeh, Emmanuel Smend Joshua Smend, Claudio Nini and Sheriff Brown I say a heartfelt thank you for all of your supports.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1.0 Introduction

Tuna is a globally valuable commercial commodity. It is an important source of healthy animal protein that is frequently recommended for consumption. This is particularly so because of its good amino acid profile, high content of long-chain polyunsaturated omega-3 fatty acids, relatively greater availability and access (cheaper cost) (Gebauer et al., 2006; Mozaffarian et al., 2003; Nesheim & Yaktine, 2007; Usydus et al., 2008;).

Over the years there has been a growing world demand for tuna products (fresh, frozen, smoked, canned). This increased total global production volume from less than 0.6 million tons in 1950 to above 6 million tons in 2010 (FAO, 2010), which is more than a 900% increase in output. For example, in 2004 the European Union (EU) and United States alone consumed 734,444 and 445,847 tons of canned tuna respectively, the total of which was higher than the global output five decades before (UNDP, 2010). With such a high demand for tuna products, a constant supply of good quality raw tuna from the fisheries to the tuna industries became necessary.

In tandem with the increase in global industrial demand for large volumes of raw tuna was a remarkable transformation in the methods of fishing. Longline and pole and line methods that resulted in low fish catches (approximately less than 600 metric tons) have given way to purse seines for example, which can pack onboard an average of 1500 metric tons of frozen tuna (FAO, 2003). Such vessels can take fishing trips lasting up to several months before

returning to port (FAO, 2003), presenting a challenge of maintaining the quality of raw fish on those vessels. A common technique used to address this challenge is brine freezing.

Brine freezing involves immersing live or dead fish in a chilled (-12^{o}C to -18^{o}C) salt mixture (brine, typically sea water and salt) at 19 to 21% salt concentration thus reducing the fish temperature until they are frozen (Burns, 1985). Behind this practice is the principle that the freezing point of a solution decreases by a known predictable degree at a given salt concentration (Hilderbrand, 1999; Burns, 1985). Using 19 to 21% NaCl brine therefore results in a freezing point between -15 to -18^{o}C (Hilderbrand, 1999). At this temperature, the fish is frozen while the brine solution is still in liquid state. The temperature of the fish is reduced to levels unfavorable for microbial growth, thereby satisfactorily preserving onboard fish quality until they are delivered for sale and processing.

Brine freezing is however not without short falls. A potential problem associated with this technique is excessive uptake of salt by the fish (Burns, 1985). Different factors such as an increase in brine temperature, slow freezing, extended storage time of fish in brine, increase in brine salinity, and abrading or splitting of fish are known to increase the occurrence of this problem (Burns, 1985). High brine temperature (>-12^{o}C) is the biggest contributor to this problem (Burns, 1985; D. Sullivan, personal communication, November 12, 2012). If the refrigerated seawater (RSW) or brine is not cold enough when the fish is added or the vessel does not have enough refrigeration capacity then the salt will penetrate into the fish before the fish is frozen. Many fishing companies have increased the refrigeration capacity of their

vessels over the years to combat this problem, but others have not (D. Sullivan, personal communication, November 12, 2012). Also mechanical failure may occur during the fishing trips that may cause high brine temperature and lead to high salt content fish.

1.1 Rationale

With regulatory standards and customer specifications on salt limits and an increasing awareness of consumers to the negative health impact associated with excessive intake of salt, buyers and processors tend to reject fish with excessive salt content. Most canneries reject tuna when the salt content of the meat exceeds 2% . Depending on the market, this may or may not constitute a total loss to the vessel, as fish considered to be salty per this criterion can be channeled into other uses, albeit at a lower price.

With the scarcity of tuna during the fishing lean seasons, and the constant demand for tuna by tuna processors, losing any quantity of tuna as a result of high salt content can be frustrating to processors. This has presented a challenge to the tuna industry and some processors are interested in finding ways of reversing this problem. If desalting is successful, processors will be able to buy the high salt tuna at a discounted price and successfully process it to add value.

Quite a number of studies have been conducted on salt uptake by fish (during brine freezing and other salting techniques such as pickling and brining) but only a small number of papers

analyze the desalting process. Almy and Field (1921) studied salt penetration in fish frozen in chilled brine. Barat et al. (2002) also studied cod salting and Andres et al. (2001) applied vacuum impregnation technology to salting and desalting cod. In the studies conducted, it was found that the major mass transfer mechanism responsible for sodium chloride transport is diffusion, in both the salting and desalting process.

Barat and Rodriguez-Barona (2004) studied process variables, controlling the desalting of cod. Their studies indicated that the efficacy of the desalting process is affected by different processing variables such as water changes, quality of water, process temperature, vacuum, fish muscle zone, sample size and the additives used in the desalting water. Currently, there is no research that explores how to efficiently desalt tuna while maintaining its safety and quality for market and industrial use.

1.2 Project Goal

To study the desalination kinetics of tuna and the effect of the process on the final canned product quality.

1.3 Objectives

The specific objectives of the study are:

1. To determine the distribution of salt in brine frozen whole fish within the fish muscle.

2. To evaluate the influence of temperature, fish piece size and fish to water ratio on the desalination kinetics of tuna to enable the design of an efficient process for desalting.
3. To determine the effect of the desalting treatment on protein content, texture and overall acceptability of the canned end product.

CHAPTER 2

2.0 Literature Review

2.1 Geographical Distribution of Tuna

Related to mackerels and bonitos, tunas are epipelagic marine fishes belonging to the family Scombridae (Collette and Nauen, 1983). They are distributed in temperate and tropical oceans, and occur at different depths within those habitats. Pacific, Atlantic and Indian Oceans are the main fishing sites for commercial species. Data from the 2010 Year Book of the Western and Central Pacific Fisheries Committee (WCPFC) indicates that of the three, the Pacific Ocean has the highest tuna catch. For example, between 1960 and 2010 period, 70% of fish catch was from the Pacific Ocean (fig 2.1).

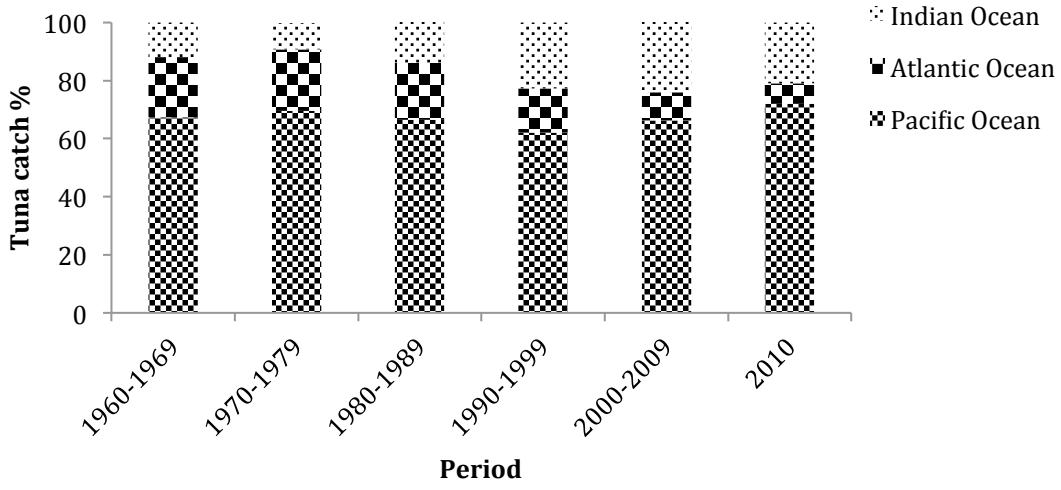

Figure 2.1.World tuna catch by ocean. Adapted from WCPFC Tuna Fishery Year Book 2010. Pp. 108

2.2 Economic Importance of Tuna

Tuna is an important source of animal protein and is consumed all over the world. It is marketed in fresh, frozen, canned and dried form. Japan, United States and Europe have the largest tuna market. In Japan, tuna is mostly consumed as a fresh product (including *sashimi*) while in Europe and the United States, it is consumed as a canned product. According to Gilman and Lundin (2008), in 2004 Europe and United States consumed an estimated 734,444 and 445,847 tons of canned tuna respectively representing 83% of the total global consumption of the product. In 2005, Japan consumed 78% of the world's fresh tuna products. With such high global consumption of tuna, there is a high commercial demand for the various species including skipjack (*Katsuwonus pelamis*), albacore or longfin (*Thunnus alalunga*), yellowfin (*Thunnus albacares*), Southern bluefin (*Thunnus maccoyii*), bigeye (*Thunnus obesus*), Pacific bluefin (*Thunnus orientalis*), and Atlantic bluefin (*Thunnus thynnus*) (Globefish Research Program, 2004) (fig 2.2).

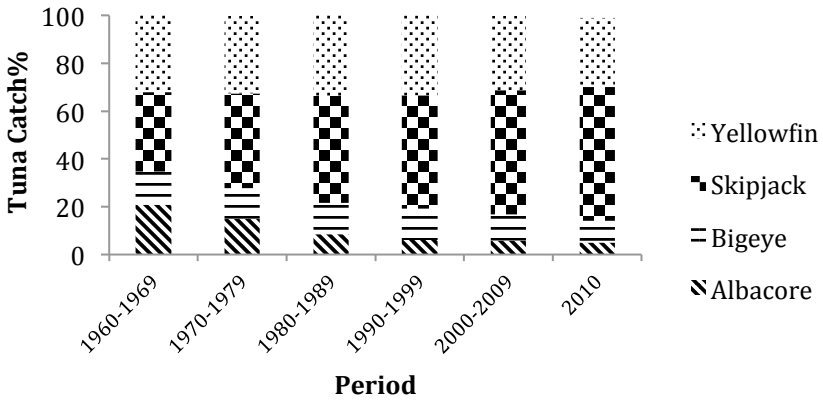

Figure 2.2. Global tuna catch by species. Adapted from WCPFC Tuna Fishery Year Book 2010. Pp. 106

The demand for tuna products has been rapidly and steadily increasing. The reported catches of the principal market species of tunas increased from less than 700,000 tons in the early 1960s to more than 4 million tons in 2010 (WCPFC Year book, 2010) (fig. 2.3). The value-at-landing of the 2010 catch of the principal species was estimated to be more than US$10 billion (FAO, 2013). Tuna is therefore a valuable commercial commodity.

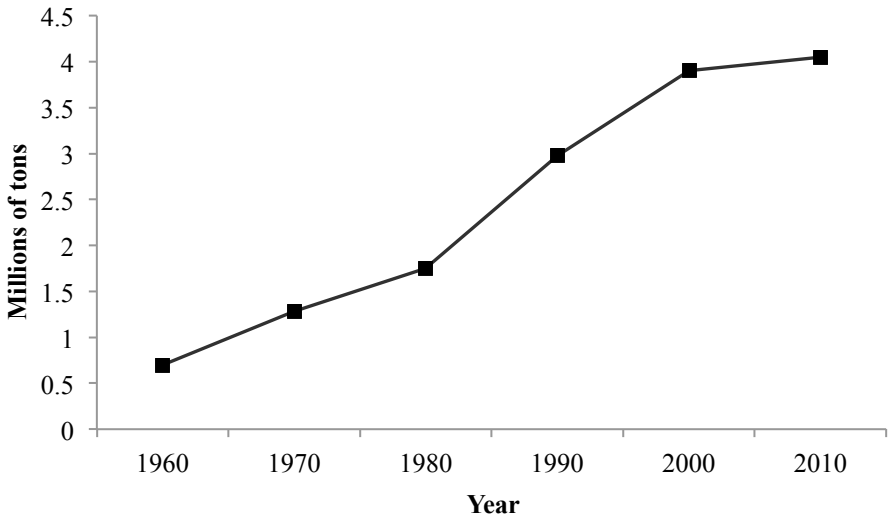

Figure 2.3. Total world tuna catch. Adapted from WCPFC Tuna Fishery Year Book 2010. Pp. 108

2.3 Physiology and Chemical Composition of Tuna

In comparison to other fish, tunas are physiologically characterized as having a higher proportion of red to white muscle and vascular countercurrent heat exchangers between the red muscles and blood vessels (Graham & Dickson, 2004; Bushnell & Jones, 1994). The red muscles have a high aerobic metabolism and are rich in myoglobin (a red pigment with a

strong chemical affinity for O_2) to provide tunas with sufficient power for continuous and fast swimming. The vascular countercurrent heat exchangers act as a thermal barrier allowing tunas an ability to maintain their body temperature significantly warmer than the surrounding water, allowing them to survive in cooler environments (Graham & Dickson, 2004; Bushnell & Jones, 1994). For example several studies have shown Atlantic bluefin tuna to be able to maintain body temperatures as high as 21 °C while occupying water as cold as
3 °C (Carey and Teal 1966, 1969; Carey and Lawson 1973).

Tuna (whole fish) is composed of about 64% water, 24% protein, 8.6% oil, and 3.1% ash (Vlieg & Murray, 1988). They have a pH between 5.9 to 6.2 (FDA, 2007).

The compositional attributes (high moisture and protein content) of tuna make it a suitable medium for microbial growth. Tuna tissue also contains high levels of free non-protein nitrogen (NPN) compounds, which are readily available to support post-mortem bacterial growth (Lund *et al*., 2000). These reasons make it necessary to apply appropriate post-harvest handling and storage procedures to preserve the quality of the fish.

2.4 Storage Stability of Tuna

Once harvested, the change in natural environment, death, high body temperature, unique chemical composition, handling and exposure of tuna (and fish in general) to atmospheric microflora and physical conditions trigger the onset of spoilage and deterioration. The rates

at which both autolytic and microbial spoilage take place are dependent upon the temperature and how fast the fish is chilled and stored (Amos, 2007). Deteriorative processes are retarded

at reduced temperatures and, when the temperature is low enough, spoilage can almost be stopped (Hall, 1997). On board fishing vessels, harvested tuna are kept in a chain of cold systems, combined with storage in brine, until they reach the processors. This technique, known as brine freezing, provides the rapid heat transfer needed to cool the fish quickly.

2.5 Brine Freezing

Brine freezing involves immersing the fish directly in salt brine (typically NaCl brine in the tuna industry), which has been chilled to a temperature below the freezing point of the fish. The brine freezing technique is based on the principle that the freezing point of water is lowered by a known predictable degree at a given salt concentration (Hilderbrand, 1999; Burns, 1985). The extent to which the brine temperature can be lowered is dependent on the concentration of the brine. For NaCl brine, the freezing point can go as low as -21^{o}C (-6^{o}F) at a salt concentration of 23.3%. This concentration is known as the eutectic point (Hilderbrand, 1999). Above this concentration, the freezing point starts to increase (Fig. 2.4) due the crystallization of salt out of solution.

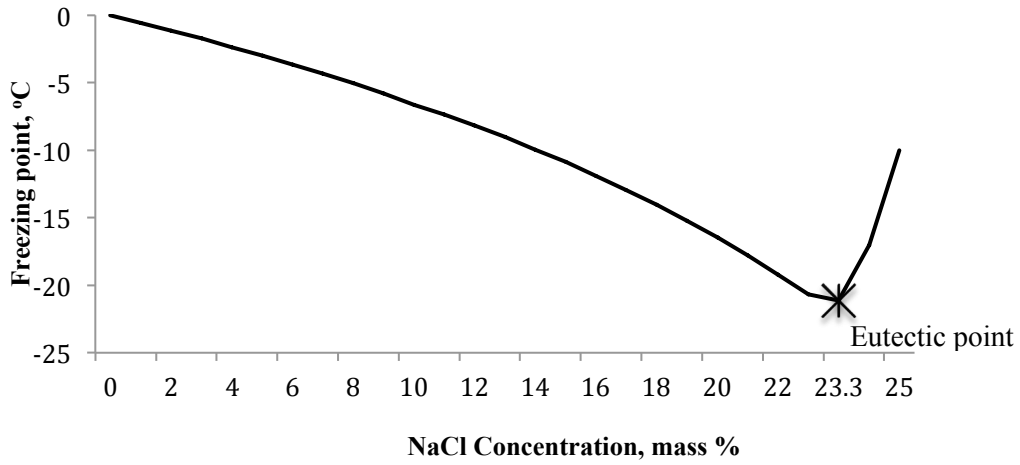

Figure 2.4. Freezing point of NaCl brine. Adapted from K. S. Hilderbrand, 1999.

2.5.1 Brine Freezing Practices

Brine freezing practices vary depending on the type of fishing vessel, catch rate and size of brine tank. A typical brine freezing system is made up of an insulated brine tank with a refrigeration system, and a brine transfer system. Prior to fishing, brine tanks (or hatches) are filled with seawater, which is then cooled to around -1^{o}C (just before the freezing point of seawater which is -2^{o}C). As fish are caught, they are washed and then dropped into the hatches containing chilled seawater, where they are initially chilled and held at the reduced temperature. When the hatch is full, the seawater is pumped out and refrigerated (-15 to -18^{o}C) brine mixture (19 to 21% concentration) is pumped in. The brine mixture is typically seawater and NaCl salt. Once the fish is frozen (-12 to -14^{o}C or lower) they are held in the refrigerated brine during storage on board the vessel until they are offloaded ashore.

2.5.2 Advantages of Brine Freezing

Practical experiments performed both on a laboratory scale and under commercial conditions have shown that brine freezing has many advantages over air convection freezing. The first major advantage is that brine immersion freezing is a more rapid method of cooling/freezing than air freezing because liquids convect heat faster than air. In the food industry, freezing rate affects the quality, texture and nutritional properties of the food. It is known that slow freezing produces ice crystals comparatively larger in size and fewer in numbers, which can as a result puncture the cell membranes, causing fluid loss/drip loss and textural changes upon thawing. In contrast, fast freezing produces smaller and more crystals thereby reducing the possibility of this problem (Garthwaite, 1997).

According to Hall (1997) however, drip loss in fish is not as a result of the formation of large crystals but rather due to denaturation of proteins during freezing. This is explained by the fact that the cell membranes of fish are considered to be sufficiently elastic to withstand excessive damage from the ice crystals. The optimum temperature range for protein denaturation is -1^{o}C to -2^{o}C (Hall 1997). Therefore in order to minimize thaw drip loss in fish, the time spent in this temperature zone during freezing must be as short as possible, hence the need for the fast freezing provided by brine immersion.

2.5.3 Salt Uptake during Brine Freezing

During the course of brine freezing, there is an uptake of salt by the fish due to a difference between the natural salt content of fresh tuna (0.25%) and that of the brine (Borgstrom 1965;

Burns, 1985). The amount of salt uptake varies under different handling practices, but there is always some uptake. Proper fish handling practices will result in just small amounts of salt uptake by fish flesh, usually less than 2% at 16 to 19% brine salinity (D. Sullivan, personal communication, November 12, 2012).

2.5.4 Mechanism of Salt Uptake in Fish

A number of studies have been conducted on salt uptake by fish flesh during brine freezing and other salting techniques (kench salting, pickling and brining). Almy and Field (1921) studied salt penetration in fish frozen in chilled brine. Barat et.al (2002) also studied cod behavior (changes in weight, NaCl content and texture) during salting. In all the studies conducted, it was found that the major mass transfer mechanism responsible for salt transport is diffusion (Andres et.al, 2001; Yao and Le Maguer, 1996; Raoult-Wack 1994).

As soon as fish comes in contact with salt, the salt starts to penetrate into the flesh and the salt penetration ends when all the fish has reached equilibrium with the surrounding brine. Voskresensky (1965) divided the process of salt uptake into three stages. During the first stage, the fish is exposed to a high osmotic gradient causing salt to move from the brine into the fish flesh and consequently water moves from fish to brine. At this stage no profound chemical changes occur and the salt just penetrates as far as the outer layers and not the inner layers. At stage two, there is a decrease in osmotic pressure but salt and water still diffuse in and out of the fish flesh almost at the same rate. Towards the end of this stage, the salt concentration in the surface layer of the fish tissue reaches equilibrium with that of the

surrounding brine which creates a barrier that prevents further movement of water from the fish. At stage three, salt in the outer layers moves into the remaining fish tissues (towards the inner layers) and any decrease in the salt concentration of the outer layer is immediately compensated from the brine.

2.5.5 Factors that Affect the Rate of Salt Uptake

The rate of salt uptake is affected by many factors. These include; brine temperature and concentration, chemical composition of the fish, fineness and composition of the salt used and fish handling practices.

2.5.5.1 Brine Temperature

Brine temperature is the most critical factor affecting the rate of salt penetration in fish (Borgstrom, 1965; Burns 1985). This is because the kinetic energy of the salt molecules increases with an increase in temperature allowing them to diffuse more quickly into the fish. Thus any practice (such as poor refrigeration system in the vessel and loading hatches at once with a large catch) that slow the heat removal from the fish may lead to fish becoming too salty. Also, salt does not readily diffuse through ice so slower freezing as a result of an increase in brine temperature will allow more time for salt to diffuse through fish.

Almy and Field (1921) reported in their study of preservation of fish in chilled brine that in a few instances, salt uptake by fish frozen in brine at its freezing point was slightly less than that which occurred when the brine temperature was several degrees above this point.

Bohdan et al. (1987) also noticed the same effect of brine temperature when they studied the effect of brining on salt uptake and retention by herring. They recorded that salt uptake was slightly higher in brining with 10°C as compared to 0°C.

Almy and Field (1921) also reported in the same study that there were many instances however that no such temperature influence could be observed. Aitken and Baines (1969) also reported the same effect of brine temperature on salt uptake when they studied the uptake of salt in the kippering of herring. Brine temperature had little effect on salt uptake. The reason why temperature seemed to have little or no significant effect on salt uptake in these studies could be attributed to the short length of time (1 – 2 hours) fish were stored in the brine. Onboard fishing vessels however, the fish are stored in brine for several weeks, giving a considerable amount of time for salt uptake to occur.

2.5.5.2 Brine Concentration

In general the higher the salt concentration of the surrounding brine, the faster and deeper the salt penetration (Bellagha et al. 2007) as a result of higher osmotic gradient. In the study conducted by Almy and Field (1921), an attempt was made to determine the effect of variations in brine concentration on the salt uptake of fish. Salt penetration was higher in flounder fish frozen in 20% brine than fish frozen in 15% brine, all at the same temperature. The amount of salt penetration was also greater in whiting fish frozen in 18.9% brine than it was in those frozen in 15% bine. However no consistent differences in the amount of salt absorbed by the fish could be noted.

2.5.5.3 Chemical Composition of Fish

The chemical composition of fish including the fat and protein content also influences to a lesser extent the rate of salt penetration (Aitkin and Baines, 1969; Almy and Field, 1921; Czerner and Yeannes 2012; Deng, 1977). As the percentage of fat and protein increases, the rate of salt penetration into fish decreases (Zugarranmurdi and Lupin 1980). This is explained by the fact that salt only diffuses through aqueous mediums and the higher the fat and protein content of fish the lower the water content and hence the lower the salt penetration (Bohdan et al., 1987).

2.5.5.4 Composition of Salt Used

Commercial salts used for brining or brine freezing vary widely in their composition. For best results, it is recommended that high quality salt, which contains about 99.9 per cent sodium chloride is used. Lower quality salt usually contains chemical impurities such as calcium and magnesium chlorides and sulphates, sodium sulphate and carbonate, and traces of copper and iron apart from the physical contaminants such as dust and sand (Rahman et al., 2010; Clucas, 1990). Calcium and magnesium chlorides, even when present in small quantities, tend to slow down the penetration of salt into the fish flesh and also imparts a bitter taste (Clucas, 1990).

Brine used for brine freezing onboard fishing vessels is typically prepared from seawater and rock salt. Seawater is mainly comprised of various salts and water. The proportion of water

to salts is approximately 96.5% to 3.5% respectively (Watson, 1988) (fig. 2.5). The main ions that make up the sea salts are chlorine, sodium, sulphate, magnesium, calcium, potassium and bicarbonate (Open University, 1995; Sverdrup, Fleming, & Johnson, 1942) (fig. 2.6).

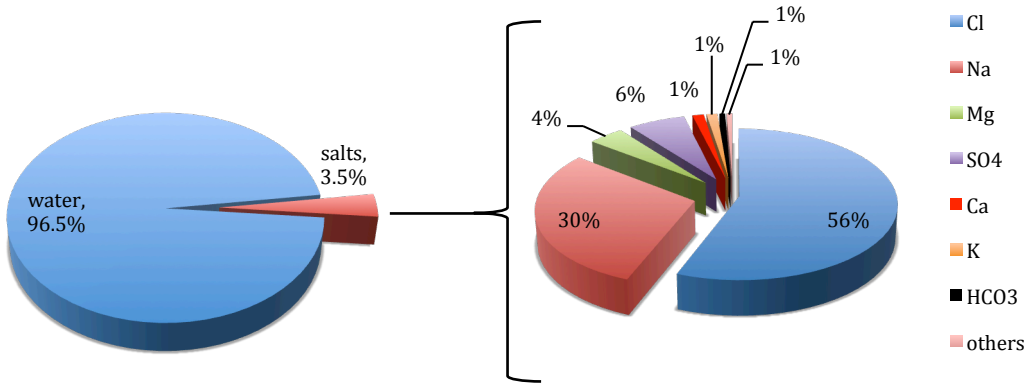

Figure 2.5. Composition of sea water and the average amounts of major ions therein. Adapted from The Oceans: their physics, chemistry, and general biology by Sverdrup, H. U., Fleming, R. H., & Johnson, M. W. (1942).

2.5.5.5 Fish Handling Practices

Abrading or splitting of the skin of fish may occur during brine freezing when the brine tanks are densely packed. This directly exposes fish muscles to salt and therefore enhances uptake.

2.6 Desalination of Tuna

Desalination becomes necessary when excessive uptake of salt by fish flesh occurs. The desalination process requires that excess salt solutes be extracted from the fish to yield a tuna product with an acceptable salt concentration. In order for this to happen, the pores of the fish

have to be filled with some liquid medium so that the solute molecules (sodium and chloride ions) can migrate through the pores into the surrounding liquid. From an engineering point of view, this process can be described as a mass transfer mechanism in a solid- liquid phase.

2.6.1 Mass Transfer of Solutes in Food Systems

Mass transfer is defined as the migration of a component in a mixture in the same phase or from phase to phase because of a difference in concentration between two points (Geankoplis, 2003). Mass transfer may occur by either of two mechanisms: molecular diffusion or convective mass transfer (Sun, 2012). When the migration of a component occurs as a result of concentration gradient between two points of the system, then mass transfer is caused by molecular diffusion. On the other hand when there is transport of material between a boundary surface (such as solid or liquid surface) and a moving fluid or between two relatively immiscible, moving fluids, then the mass transfer is caused by convection.

Molecular diffusion is generally accepted as the basic mass transfer process for small solutes in food systems (Saravacos & Maroulis, 2001) and it is described or analyzed using the Fick model (Lebowitz and Spohn, 1982; Saravacos & Maroulis, 2001; Geankoplis, 2003). The Fick model for diffusion comprises two laws; the first law applies to steady state systems, where concentration differential remains constant. It states that the mass flux per unit area of

a component is proportional to its concentration gradient (Singh and Heldman, 2009; Geankoplis, 2003). Thus for a given substance,

$$J = -D\frac{\partial c}{\partial x} \qquad [2.1]$$

where:

- J is the diffusion flux per unit area per unit time (mol/m^{2}s)
- D is the diffusion coefficient or diffusivity (m^2/s)
- c is the concentration (mol/m^3)
- x is the position/length (m).

However in many cases of diffusion, such as when dealing with biological materials like food, concentration changes with time, demanding Fick's second Law. This law postulates that the movement of a solute is directly proportional to the change of the concentration gradient with position (Aguilera & Stanley, 1999). Thus the diffusion of the food component would be described by:

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2}\right) \qquad [2.2]$$

Where:

- c is the concentration of the component diffusing within the solid food structure, as a function of time, t.
- D is the diffusion coefficient
- x is the length.

With the above equations (2.1 and 2.2), it is possible to quantify an effective diffusivity,

which describes the transfer of solutes within a solid porous food, provided experimental data as well as initial and boundary conditions are known (Sun, 2012). The initial condition in this case is the initial concentration of the solutes in the samples and the boundary conditions predict what happens at the boundaries of the phase to be investigated such as a flux.

2.6.2 Mass Transfer Mechanism During Desalting (Solid-Liquid Extraction)

Solid-liquid extraction (leaching) is the process of removing solutes from an insoluble, permeable solid phase. The solute may exist mechanically in the pore structure of the solid material or chemically combined with that material. This solute is removed through dissolution in a solvent. During the process of extraction/leaching, a series of steps occur between the solid phase and the solvent that leads to the separation (Aguilera & Stanley, 1999; Fellows, 2009):

(i) Diffusion of the solvent through the pores of the solid matrix;

(ii) Solubilization and/or breakdown of the solutes;

(iii) Transport of the solute to the exterior of the solid matrix;

(iv) And lastly, migration of the solute from the exterior of the solid to the main bulk of the solution.

In this review, no publications were found on desalination of tuna, however a few studies have been done on the desalting of cod. In one conducted by Barat et al., (2003) to analyze the cod desalting process from the mass transfer phenomena point of view, it was found that the major transported components in the studied systems were water and salt as well as proteins. The extraction of proteins from the fish muscles is enhanced by the presence of salt

which increases the solubility of some proteins and also because high NaCl concentrations cause protein precipitation (Duerr & Dyer, 1952; Cheftel, Cuq, & Lorient, 1989; Dunajski, 1979).

In the same study (cod desalting process), they reported that the driving forces present in the system and the mass transfer mechanisms associated with them were:

(i) Component activity gradients, which promote the transport of water, salt and soluble proteins in: the cod tissue by diffusion or osmotic mechanisms; desalting solution by diffusion, and turbulent mechanism promoted in case of stirring conditions or the existence of differences in densities associated with temperature or NaCl concentration gradients.

(ii) Pressure gradients, which promote the bulk transport of the solution (water plus solutes) by hydrodynamic mechanisms (HDM) in the intercellular spaces/pores. This occurs as a result of the fish matrix rehydration, which includes among others the capillary forces and volume generation as a consequence of salt–protein interactions.

During food processing, the rate of desalting (which is in turn driven by all of the above processes and mechanisms) affects the safety (microbiological) and quality of the end product. The shortest possible time is important to obtain safe desalted product with high nutritional and sensory quality, while also maintaining a high production output and low energy cost.

2.6.3 Factors that Affect Mass Transfer Kinetics/Rate during Desalting

Many factors have been reported to affect the rate of extraction or leaching of a solute from a solid substance. They include the following:

2.6.3.1 Physical Characteristics (Microstructure) of the solid

The solid's microstructure impacts the desalting rate through its effect on the diffusion coefficient (Aguilera & Stanley, 1999). For example the diffusion coefficient is affected by the solid's porosity, the pore size distribution and the tortuosity of the pores (Liu & Nie, 2001). Effective diffusion coefficient is used to describe diffusion through the pore space of porous solids (Tzia & Liadakis, 2003; Liu & Nie, 2001; Aguilera & Stanley, 1999). The effective diffusion coefficient for transport through the pores, D_{eff}, is estimated as follows:

$$D_{eff} = D\frac{\varepsilon}{\tau} \qquad [2.3]$$

where:

- D is the diffusion coefficient of the liquid filling the pores (m^2s^{-1})
- ε is the void fraction or porosity of the solid (-)
- τ is the tortuosity (which accounts for the longer distance traversed by the solute along a sinuous path) (-)

For example a typical value of apparent diffusivity for untreated meat, vegetables, fruits and food gels (for a given temperature, 25°C), is found to be: D_{eff} = 2× 10^{-10} m^2/s. Hard structure foods such as nuts and cereals, especially rice, show, under the conditions

mentioned above (untreated, ambient temperature), typically one order of magnitude less: $D_{eff} = 10^{-11}$ m^2/s (Doulia, Tzia & Gekas, 2000).

2.6.3.2 Process Temperature

Diffusivity of the solute in the solid as well as its solubility in the solvent increases with temperature. The diffusivity in solids at different temperatures is often predicted by the Arrhenius equation (Mehrer, 2007)

$$D = D_o \times e^{-\frac{E_A}{RT}} \qquad [2.4]$$

where:

- D is diffusivity (m^2/s)
- D_o is proportionality constant (m^2/s) independent of T
- E_A is activation energy for diffusing species (J/mol)
- R is molar gas constant and R = 8.314 Jmol-1 K-1; or 1.987cal mol-1K-1
- T is absolute temperature (K)

Also, the viscosity of the solvent becomes less at an elevated temperature making it easier to penetrate into the pores of the solid (Dutta, 2007). The viscosity of liquid water at different temperatures is listed in table 2.1.

Table 2.1. *Viscosity of liquid water at different temperatures*. Adapted from "Viscosity of water at various temperatures," by Korson, L., Drost-Hansen, W., & Millero, F. J. (1969). *The Journal of Physical Chemistry*, *73*(1), 34-39.

Temperature (^{o}C)	Viscosity ((Pa s) x 10^{-3}
0	1.7916
10	1.3069
20	1.0020
30	0.7975
40	0.6532
50	0.5471
60	0.4666
70	0.4039
80	0.3538
90	0.3128
100	0.2783

2.6.3.3 Water Management

Rate of salt removal decreases with increasing solute concentration in desalting water (causing a decrease in concentration gradient) so the water management technique employed for the desalination procedure is important. There are different techniques employed for water management. Depending on the solid liquid contacting strategy, it may be a fixed bed contact (percolation) or a dispersed contact in an agitated tank (Dutta, 2007) technique. It may also be categorized by the operating cycle, as a batch or continuous system; and by the direction of the streams as concurrent or countercurrent.

Fixed Bed Contact

In fixed bed contacting, the solid material is stacked as a fixed bed on a perforated support plate placed a little above the bottom of an open or closed vessel. The size of the perforations are such that the liquid can leave through it without much hindrance but the solid cannot. The liquid is introduced into the system by one of two ways; spray percolation or full immersion (Dutta 2007). In spray percolation, the liquid is sprayed at the top of the bed while it trickles down through the bed of the solid and the liquid is recirculated until the desired concentration is reached. With full immersion, the solid bed is fully dipped in the liquid for a predetermined time after which the liquid portion is drained.

Dispersed Contact

With the dispersed contact in an agitated tank, the solid material is not in a fixed position like the fixed bed contact. Instead, it is suspended/dispersed in the liquid with some form of agitation to enhance mass transfer. The solid and the liquid are therefore in motion with each other.

The optimum water management technique for desalting would be the combination of techniques from the various categories that is capable of yielding the desired salt concentration in the shortest time, with minimum waste and cost.

2.6.3.4 Pressure/Vacuum

Mass transfer kinetics in solid-liquid systems can also be affected by pressure gradients due the action of the hydrodynamic mechanism (Chiralt & Fito, 1997; Chiralt et al., 2001; Fito and Pastor, 1994). According to Andrés, Rodríguez-Barona, & Barat (2005), pressure gradients resulting from the application of vacuum pulse resulted in a faster desalting in codfish. The effectiveness of the vacuum pulse depends on the porosity and the viscoelastic properties of the solid as it has to resist a pressure gradient (Andrés, Rodríguez-Barona, & Barat, 2005).

2.6.3.5 Surface Area of Solids Exposed to the Solvent

The rate of mass transfer is directly proportional to the surface area, so reductions in particle size (resulting in an increase in surface area per unit volume) increase the rate of desalting to a certain degree. The reductions in particle size also reduces the distance (x) that the solute travels thus, J increases ($J = -D\frac{\partial c}{\partial x}$).

2.6.3.6 Flow Rate of the Solvent

Higher flow rates reduce the boundary layer of concentrated solute at the surface of particles and thus increase the rate of desalting (Fellows, 2009).

2.6.3.7 Agitation

Intense agitation increases the solid-liquid mass transfer coefficient and thereby increases the rate of desalination but the disadvantage is that it may disintegrate the solid as well (Dutta,

2007). However in a study conducted by Andrés et al. (2005) to analyze the influence of some cod-desalting variables on the mass transfer kinetics, no significant differences in salt loss were found between setups that were agitated and those that were not.

2.6.4 Effects of Desalting Process on the Safety and Quality Attributes of Canned Tuna

During the desalination process, certain important changes to the safety and quality of the fish may occur that will affect the acceptability of the final canned product. Some of these changes may include histamine formation, nutrient loss and textural changes.

2.6.4.1 Histamine Formation

Histamine (also referred to as Scombrotoxin) is a toxin produced in fish tissue when certain spoilage microorganisms containing the enzyme histidine decarboxylase cause the breakdown (decarboxylation) of the amino acids histidine to amines (histamine) (Frank & Yoshinaga, 1984; Shifrine et al., 1959; Kimata, 1961: Ferencik, 1970; Edmunds and Eitenmiller, 1975; Arnold and Brown, 1978). Tuna and other members of the Scombridae family are especially associated with the formation of histamine due to the fairly high levels of histidine in their muscles (exceeding 1 g per 100 g of skipjack tuna tissue) (Lukton and Olcott, 1958). The microorganisms that cause histamine formation naturally occur in the environment of tuna and are thus present on the surface of the fish and gills. The microorganisms therefore grow as soon as the fish is harvested and the conditions are favorable.

The main conditions that favor the growth of histamine forming bacteria are time and temperature. Histamine-forming bacteria are capable of growing and producing histamine over a wide temperature range, with the optimum being around 90°F (32.2°C) (FDA, 2001a). Also the growth of bacteria is more rapid at high-abuse temperatures (e.g. 70°F [21.1°C]) than at moderate-abuse temperatures (e.g. 45°F [7.2°C]) (FDA, 2001a). The development of histamine is usually the result of high temperature spoilage rather than long term, relatively low temperature spoilage.

Histamine, once formed cannot be eliminated by heat treatment (including retorting) or freezing. The heat treatment will destroy the bacteria and inactivate the enzyme histidine decarboxylase, but the toxin will still remain. After cooking, recontamination of the fish with the enzyme-forming bacteria is necessary for additional histamine to form. Also, once the enzyme histidine decarboxylase has been formed, it can continue to produce histamine in the fish even if the bacteria are not active (for example when fish is frozen). The enzyme may remain stable while in the frozen state and may be reactivated very rapidly after thawing.
For these reasons, histamine development is more likely in raw, unfrozen fish.

2.6.4.2 Nutrient Loss

The nutrient composition of tuna includes approximately 85% protein, 3.7% lipid, 1.85% carbohydrate and 9.25% ash (Hall, 1997). During desalination, some soluble proteins in the fish may likely be lost to the liquid phase. Proteins in fish muscles can be separated into three groups according to their solubility properties (Hall, 1997):

1. Sarcoplasmic proteins- these are water-soluble proteins and constitute 18 to 20% of total muscle protein. They are found in the cell plasma where they act as enzymes and oxygen carriers.
2. Myofibrillar proteins- these are soluble in salt solutions of increasing concentration up to 0.3 M. They constitute 65 to 80% of total proteins and are responsible for the textural structure of the muscles. This group includes proteins such as actin, myosin, tropomyosin and troponin.
3. Stroma proteins- these are resistant to solublization except in strong salt solutions and by cooking. These proteins (includes collagen and elastin) make up the connective tissues and form 3 to 5% of total proteins.

2.6.4.3 Texture Change

Texture is one of the important attributes of fish products. The mouth feel during chewing and swallowing is important for the consumer. The International Standards Organization (ISO, 1992) defines texture as all the mechanical, geometrical and surface attributes of a food product perceptible by means of mechanical, tactile, and, where appropriate, visual and auditory receptors. Textural characteristics of fish depend on the chemical composition and the structural properties, in particular the myofibrillar and connective tissue proteins (Dunajski, 1979). Since the process used for desalination could affect those characteristics, it can in turn affect the texture of fish.

CHAPTER 3

3.0 Materials and Methods

3.1 Raw material

Fresh whole skipjack tuna (2.5±0.2kg), harvested in May 2013 were acquired from artisanal fishermen at the shores at the Gulf of Guinea, Ghana, and transported to the laboratory on ice under sanitary conditions were used.

3.2 Brine Preparation and Salting of Fish

3.2.1 Brine Preparation

An 883L brine freezer was designed and custom-made for the study (Fig. 3.1). A brine solution (22% by mass) was prepared by weighing 540 pounds of rock salt (NaCl) into the empty brine freezer. The amount of salt added was determined from Appendix 1.

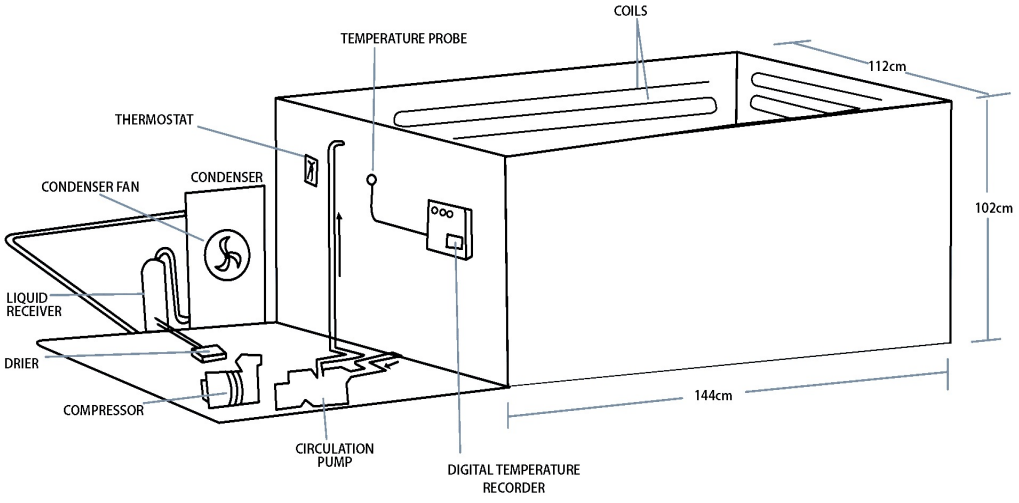

Figure 3.1. Scheme of equipment used for brine freezing

The freezer was then filled with clean water and the brine stirred until all the salt had dissolved. The concentration of brine was measured using a hydrometer (Durac, EW-08298-45).

3.2.2 Achieving Excessive Salt Uptake by Fish

Immediately after receipt at the laboratory, all the fresh fish (N=80) were transferred into the brine freezer containing circulated chilled brine at 5^{o}C and kept at that temperature for 18 days, within which period they attained an average salt concentration of 8$\pm$1%. During this period, the salt content of randomly selected fish was determined every 72 hours to monitor salt uptake. The fish samples were kept at 5^{o}C to prevent freezing and thus enhance salt uptake. A metal mesh was used to hold fish beneath the surface of the brine to prevent floatation and ensure a more uniform salt absorption.

After the 18 days of brine chilling all the fish were transferred to an air freezer (-21^{o}C) and stored for laboratory analyses.

Figure 3.2. Fish sections sampled

3.3 Sampling of Fish to Test for Salt Distribution

Fish was sampled while hard frozen. The fish were first cut into three sections; head, middle and tail (Fig. 3.2). The skins of these sections (while still frozen) were carefully separated from the muscular tissue. Samples were taken from the edible portions of the head and tail regions. To enable determination of the degree of salt penetration, two successive slices (labeled as the first and second muscular layers, Fig. 3.3) were cut and sampled from the middle section. The first muscular layer was the part closest to the skin and was about 10mm in depth . Following this layer was the muscular region leading to the backbone (second muscular layer) and was about 25mm in depth (Fig 3.3). The samples therefore consisted of the head, tail, first and second muscular layers. Salt content in the skin was not measured because that part is removed during processing.

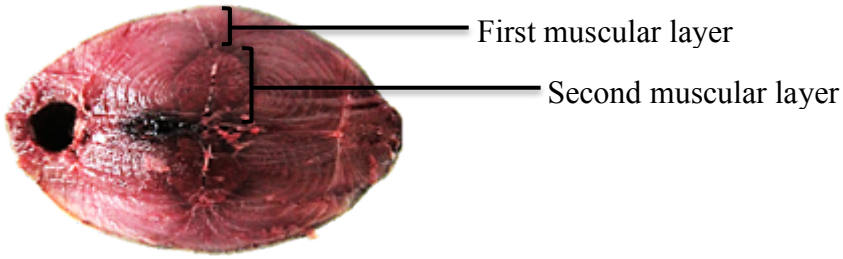

Figure 3.3. Cross section of tuna showing muscular layers that were sampled

3.4 Salt Analysis of Fish

Salt content of fish samples was determined using a salt meter (Horiba C-121) (Fig. 3.4). The instrument employs a selective sodium ion electrode that is unaffected by pH and the

presence of other ions, enabling the accurate measurement of sodium ion concentration. This method of salt analysis involved two main procedures: calibration of the salt meter and preparation of sample for analysis.

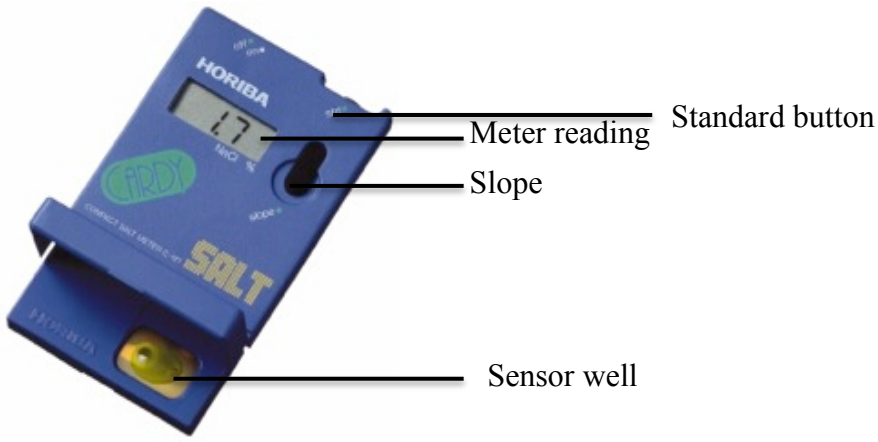

Figure 3.4. Salt meter (Horiba, model c-121)

3.4.1 Calibration

The salt meter was calibrated at the start of each day before readings were taken. The salt meter was set to zero by filling/covering the sensor with deionized water. The deionized water was wiped off with a sterile tissue paper after which the surface of sensor was filled with 0.5% NaCl solution and the standard button adjusted until the reading on the salt meter corresponded to 0.5%. The 0.5% NaCl solution was wiped off and the sensor well filled with 5.0% NaCl solution. The slope was adjusted with the screw supplied until the reading on the salt meter was 5%.

3.4.2 Sample Preparation

Fish tissue (15g) was homogenized with 100ml of distilled water and the mixture filtered after homogenization. With the aid of a micropipette, the filtrate was dispensed unto the sensor of the salt meter and reading recorded. Per cent salt content was calculated using equation 3.1 contained in the Horiba C-121 user guide manual (Horiba, 2012):

$$\% \text{ Salt} = \frac{(A+B)}{A} \times MR \qquad [3.1]$$

Where,

MR= Meter Reading

A= Mass of sample taken (in grams)

B= Volume of distilled water used (ml)

% Salt= Percentage of salt in the sample

3.5 Desalination

3.5.1 Experimental Design

A 2×2×3 full factorial design was performed to evaluate the influence of different desalting process variables (temperature, fish piece size and fish to water ratio) on product changes (salt loss kinetics, mass change, protein loss, and overall consumer acceptability). The experimental conditions for each process variable were as follows:

Water temperature: 25^{o}C and 10^{o}C

Sample piece size: cut loins and flakes (Fig. 3.5)

Fish: water ratio: 1:1.5, 1:3 and 1:4.5

Experiments on each treatment were conducted in triplicates yielding a total of 36 experimental units.

3.5.2 Preparing Samples for Desalting

Tuna loins were used for the desalting experiment (Fig. 3.5). They were obtained by thawing whole frozen fish in air at room temperature (25^{o}C) until the backbone temperature reached -2^{o}C to 0^{o}C. For each batch of fish, the thawing time was approximately six hours. Water thawing (which is the standard thawing practice in industry) was not used in order to prevent any salt loss at this stage. This was to help maintain a fairly constant initial salt concentration for all the fish samples before desalination. After thawing, the samples were precooked (viscera in) with steam at atmospheric pressure for one and a half hours, until the backbone temperature reached 55^{o}C - 57^{o}C. Following this, fish were immediately cooled in a refrigerator for approximately two hours until the backbone temperature reached $\leq$ 25^{o}C. Cooled samples were then cleaned to remove the skin and separate loins from red meat and viscera.

Whole fish were not used for the desalination process because of their size (large and would thus require more time for the process) and the associated cost implications (may be more expensive to adopt in industry).

Figure 3.5. Tuna loins in the three forms used for the experiment

3.5.3 Desalting Process

Batch processing with dispersed contact was the desalting technique used. This requires lower investment costs and is easier to apply in industry. For each treatment (3.5.2), 100g of fish was immersed in the appropriate amount of water (at a set temperature) in a closed container with manual agitation. The closed container with the fish and water was then placed in a refrigerated water bath that maintained the set temperature by means of a thermostat and a water circulation system. After desalting, the liquid phase was drained with the aid of a sieve (perforation size 2.5mm) leaving only fish portion solids.

3.5.4 Analytical Determinations

3.5.4.1 Desalting Kinetics

The desalting kinetics for the individual treatments were determined by measuring the salt content of the liquid phase until there was no more change in the concentration of salt in the liquid phase. For the treatments involving flakes, measurements were made every two

minutes for twenty minutes. However for the cut loins, measurements were taken every ten minutes for sixty minutes because of their slower rate of salt loss. A salt meter (Horiba B-721 LAQUAtwin) was placed in contact with the liquid phase to measure the salt content. The salt meter readings were converted to % salt content with equation 3.1. The salt meter was calibrated at the start of each day before measurements were taken. Calibration was carried out as instructed by the Horiba LAQUAtwin user manual (Horiba, 2013). The determined concentration in the samples was then plotted against extraction time.

3.5.4.2 Moisture Content

Moisture content of fish samples (after precooking and after desalting treatment) was determined using AOAC air oven drying method 950.46B (AOAC, 1995).

3.5.4.3 Protein Content

Total nitrogen content was determined by the Kjeldahl method (ISO, 1871:1975) for the fish samples before and after each desalting treatment. Total nitrogen was converted to crude protein by multiplying by 6.25 (AOAC, 1995).

3.5.4.4 Water Uptake

Water uptake (ΔM_t^w) by fish samples after each desalting treatment was calculated using equation 3.2 (Andres et al., 2005):

$$\Delta M_t^w = \frac{(M_t^o . x_t^w) - (M_0^o . x_0^w)}{M_0^o} \qquad [3.2]$$

where ΔM_t^w: water weight changes at time *t* (after desalination treatment); M_t^o: mass of sample after time *t* of desalination treatment (g); (M_0^o): initial mass of sample (after precook) (g); x_t^w: weight fraction of water in the sample at time t (after desalination treatment); x_0^w: weight fraction of water in the sample at time 0 (after precook).

3.5.5 Statistical Methodology

Data analysis was conducted using SPSS (version 20). Descriptive statistics were generated for all variables and presented either in graphical or tabular form. The mean, standard deviation, minimum and maximum values and the skewness were displayed for salt loss, protein loss and water uptake during desalination.

Three 3-way univariate analysis of variance (ANOVA) tests were conducted. For each test, fish piece size, temperature and fish to water ratio were used as the independent variables. Salt loss, water uptake and protein loss, were the dependent variables. An alpha level of 0.05 was used to conclude whether results were statistically significant.

3.6 Canning Tuna

Following desalination, two of the best treatments with flakes and two with cut loins were canned. The best treatments were selected on the following criteria: ≥70% salt loss; and ≤ 30% protein loss. Canning involved filling 400g net tinplate/steel cans with 260g of tuna and

140g of media (brine or vegetable oil), seaming the cans and subjecting them to a steam heat treatment (retorting) until commercial sterility was attained. Tuna from each of the selected treatments were packed in both water and vegetable oil. Tuna products in water were retorted at 242^{o}F(117^{o}C) for 72 minutes while tuna products in vegetable oil were retorted at 242^{o}F(117^{o}C) for 92 minutes, in accordance to the thermal process schedule of MYROC Food Processing Company (a HACCP certified tuna processing company located in Ghana).

To ensure the safety of the end products, histamine levels of randomly sampled tuna were measured before (unprocessed) and after canning. Histamine levels of samples were determined quantitatively using Enzyme-linked Immunosorbent assay (ELISA) method. The procedure for this method was carried out as described in the Enzyme Immunoassay (EIA) kit Ref: 1M2369 instruction manual (Manufacturer: Beckman Coulter).

3.7 Mass (yield)

Yield of the desalted tuna after canning were determined by drained weight analysis. The drain weight analysis was carried out 24 hours after retorting as described by the Codex Standard for Canned Tuna and Bonito (CODEX STAN 70-1981). The tuna can contents were emptied into a pre-weighed sieve (made with square openings of 2.8mm× 2.8mm) and allowed to drain for two minutes. The sieve containing the drained fish was weighed and the drained weight of the fish was obtained by subtracting the weight of the sieve from the weight of the sieve and drained product. Three tuna cans per treatment were tested for drained weight.

3.8 Sensory Analysis (Consumer Acceptance Testing)

3.8.1 Experimental Design

A balanced incomplete block design (Appendix 7) was used to determine differences in acceptability for each of the sensory attributes (aroma, taste and texture) among 12 (n=12) different tuna samples/treatments (Table 3.1).

Table 3.1. Control and test samples used for sensory analysis

Sample	***Treatment***	***Symbol***
1	Cut loins/brine*	(Cbr)*
2	Cut loins/oil*	(Co)*
3	Flakes/oil*	(Fo)*
4	Flakes/brine*	(Fbr)*
5	10^{o}C/cutloins/f:w=1:3.0/oil	(10/C/1:3/)o
6	25^{o}C/cutloins/f:w=1:1.5/brine	(25/C/1:1.5)br
7	10^{o}C/cut loins/f:w=1:3.0/brine	(10/C/1:3/)br
8	10^{o}C/flakes/f:w=1:4.5/oil	(10/F/1:4.5)o
9	10^{o}C/flakes/f:w=1:3/oil	(10/F/1:3)o
10	25^{o}C/cut loins/f:w=1:1.5/brine	(25/C/1:1.5)br
11	10^{o}C/flakes/f:w=1:4.5/brine	(10/F/1:4.5)br
12	10^{o}C/flakes/f:w=1:3/brine	(10/F/1:3)br

Samples with * are the controls

3.8.2 Consumer Recruitment

Consumers (n = 99) were randomly recruited from in and around the University of Ghana Campus, Legon, Accra. Subjects were recruited by phone calls and emails. Prospective consumers were screened on the following criteria: 1) at least 18 years old; 2) do not have any known food allergies; 3) willing and available to participate in a particular testing date and time.

3.8.3 Sensory Methodology

Four evaluation sessions were carried out from 10:00 am – 2:00 pm over three days. Consumers were asked to sign a consent form (Appendix 2) as required by North Carolina State University. Consumers were seated in a testing room (a maximum of four people at a time) with good lighting and positive airflow, free of distracting odors. Each consumer was presented with four coded samples of tuna. Consumer acceptance testing was used to determine how much each sample was liked based on a 9-point hedonic scale for a set of attributes: overall liking, aroma, taste and texture where 9=dislike extremely and 1=like extremely (Appendix 3). Room temperature drinking water and unsalted crackers were provided to consumers to cleanse their palate in between samples evaluation, in order to minimize sensory carry over and/or fatigue effects. No incentives were given to the consumers after participation.

3.8.4 Statistical Methodology

Analysis of variance was conducted on the sample means for overall liking, aroma, taste and texture using SPSS. An alpha level of 0.05 was used to conclude whether results were statistically significant.

CHAPTER 4

4.0 Results and Discussion

4.1 Distribution of Salt in Brine Frozen Whole Fish

The average distribution of salt in different sections (head, tail, outer layer and inner layer) of fish kept in chilled brine for 18 days as described in Section 3.2.1 can be observed in figure 4.1.

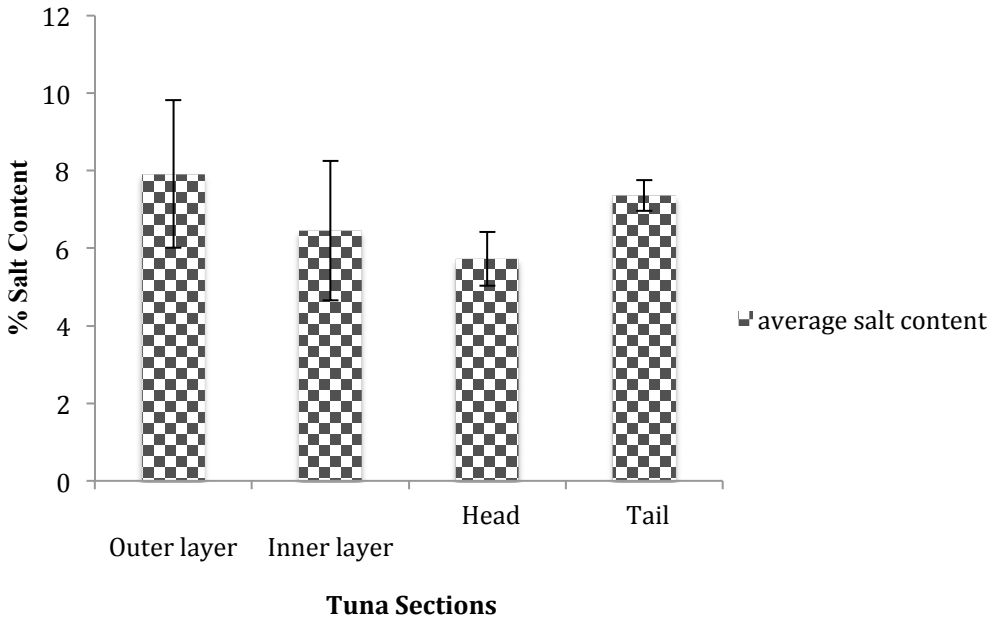

Figure 4.1. Average Salt Content in Various Sections of Tuna

Salt distribution differed in all of the fish samples analyzed (N=9) and this is evident in the large standard deviations on the graphs (Fig. 4.1). This variation is due to the complex nature and heterogeneous structure of fish tissues between different parts of a fish (Doulia, Tzia & Gekas, 2000; Fito & Chiralt, 2003). However, on an average, salt content was highest in the

outer layer (7.9%), followed by the tail region (7.4%), inner layer (6.5%) and head region (5.7%). These differences point to structural variations in the fish sections that influence mass transfer kinetics during desalination.

The observed differences could be explained by the mechanism of salt penetration as described by Almy & Field (1921) and Voskrensensky (1965). In the first stage of salt uptake by fish flesh, salt penetrates as far as the outer muscular layers until the salt concentration in that region reaches equilibrium with the surrounding brine. After this occurs, the salt in the outer muscular layer starts to distribute itself evenly throughout the entire fish muscles by penetrating into the inner muscular layers (hence the higher salt content in the outer layer than inner layer).

The salt content in the tail region was almost as high as the outer layers because it has a smaller circumference (hence less flesh) compared to the mid section of the fish. From this premise, it could be expected that the edible portion of the head region would have the highest salt content. However, the lowest salt content was observed at that region. A plausible explanation for this is that the region generally has the highest fat content (Wheeler & Morrissey, 2003) and hence retards salt penetration.

4.2 Influence of Temperature, Fish Piece Size and Fish to Water ration on Salt Loss Kinetics

Salt loss kinetics for cut loins and flakes at different temperatures and fish to water ratios can be observed in figures 4.2 and 4.3.

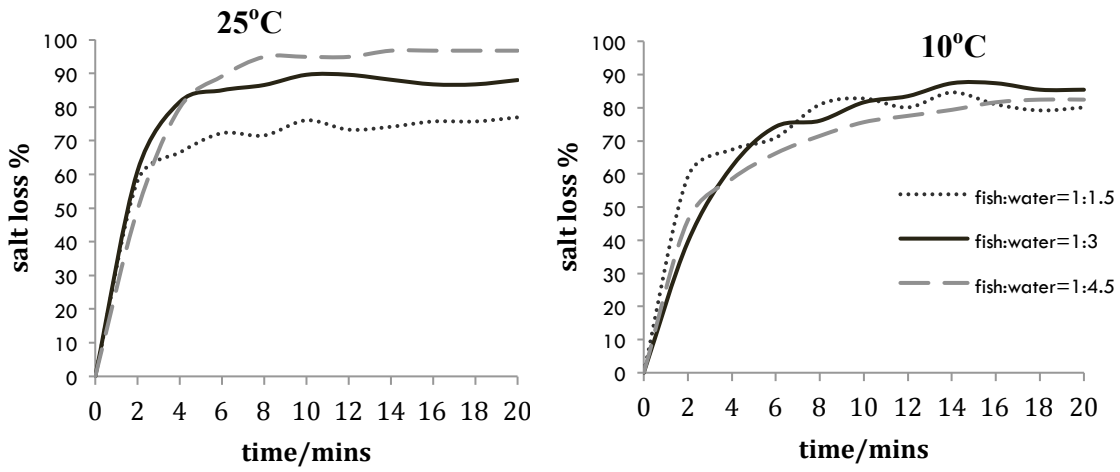

Figure 4.2. Desalination kinetics of flakes

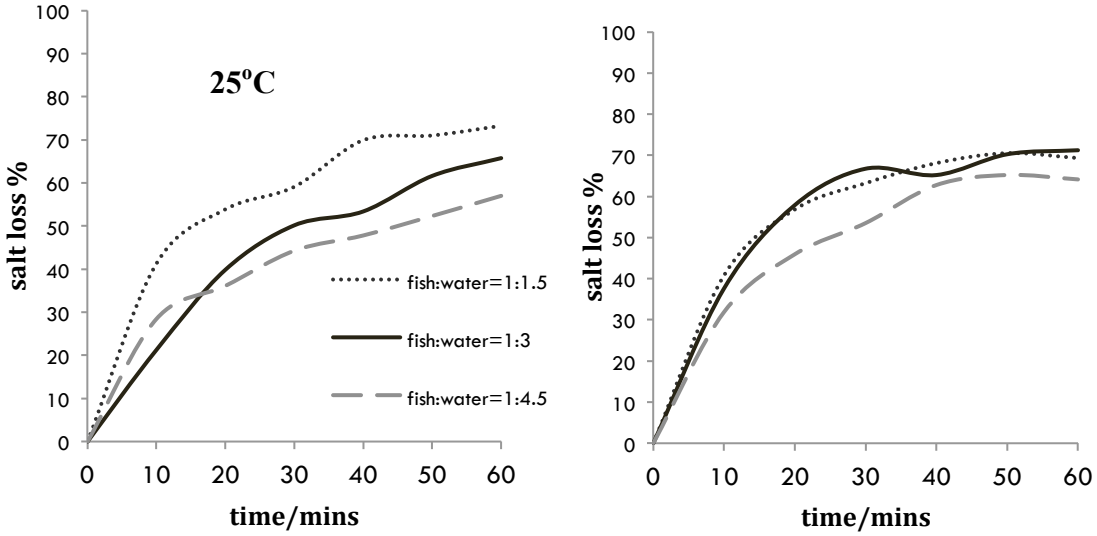

Figure 4.2. Desalination kinetics of cut loins

Taken alone, those two factors seemed not to significantly affect the rate of salt loss from the fish tissues. However, at smaller fish to water ratios (1: 1.5) for flake samples, there was a continuous fluctuation in salt content after about 6 minutes of desalination, suggesting that a state of equilibrium in salt concentration between the solid and liquid phase had been reached (fig. 4.2). This was not observed for cut loins under the same conditions (fig. 4.3). The greater distance (x) that the solute travels in cut loins as compared to flakes causes a decrease in diffusion flux (J), thus delaying equilibrium.

4.3 Influence of Temperature, Fish Piece Size and Fish to Water Ratio on Salt Loss, Protein loss and Water Uptake

In the desalination system studied, salt and proteins were lost from the solid phase (fish) to the liquid phase while there was water uptake by the solid phase. The means, standard deviations, maximum and minimum values for the transported components are shown in table 4.1. Results of the 3-way ANOVA conducted to examine whether temperature, fish piece size and fish to water ratio affects salt loss, protein loss and water uptake are presented in Table 4.2.

Table 4.1. Descriptive Statistics for Salt Loss, Protein Loss and Water Uptake

	Mean (%)	***SD (%)***	***Max (%)***	***Min (%)***
Salt loss	76.10	14.21	99.86	50.22
Protein loss	37.18	15.65	55.10	9.00
Water Uptake	26.23	24.70	76.24	2.96

Table 4.2. ANOVA Results for Salt loss, Water Uptake and Protein loss

	Associated P- values		
Source	**Salt loss**	**Protein loss**	**Water uptake**
Temperature	0.443	0.991	0.015*
Fish piece size	0.000*	0.001*	0.000*
Fish to water ratio	0.841	0.109	0.138
Temperature×fish piece size	0.051	0.246	0.951
Temperature×fish to water ratio	0.527	0.190	0.764
Fish piece size×fish to water ratio	0.027*	0.483	0.036*
Temperature×fish piece size×fish to water ratio	0.344	0.163	0.252

Significance is indicated by *

4.3.1 Influence of Temperature

Amongst the three dependent variables considered, the main effect of temperature only showed significant difference in water uptake ($F_{(1)}$=6.882, p<0,05). In general, higher increases in mass (as a result of higher water uptake) were observed at lower temperatures ($10^oC>25^oC$) (fig.4.4). Similar results were observed by Andres et al. (2005) when they studied the influence of temperature on water uptake by cod. A plausible explanation for this outcome is the fact that temperature influences water uptake rate but usually does not play a

significant role in water uptake/hydration capacity of the fish (Oliveira & Oliveira, 2010). Fish to fish variations (from compositional and structural differences, degree of protein denaturation etc.) could have affected the water uptake capacity of the fish samples (Damodaran & Paraf, 1997; Fito & Chiralt, 2003), contributing to the observed results.

No significant difference from the main effect of temperature was found in salt loss ($F_{(1)}$=0.609, $p>0.05$) and protein loss ($F_{(1)}$=0.0001, $p>0.05$), demonstrating the important role played fish structure on mass transfer phenomena.

4.3.2 Influence of Fish Piece Size

ANOVA results showed significant differences in salt loss ($p<0.0001$), protein loss ($p=0.001$) and water uptake ($p<0.0001$) as a result of the influence of fish piece size alone. Generally salt loss was faster and higher for flakes than for cut loins (Figs 4.2, 4.3 & 4.6). Protein loss and water uptake were also higher for flakes than cut loins (Figs 4.4 & 4.5). This is as a result of the their larger surface area per unit volume and hence greater contact with the liquid phase.

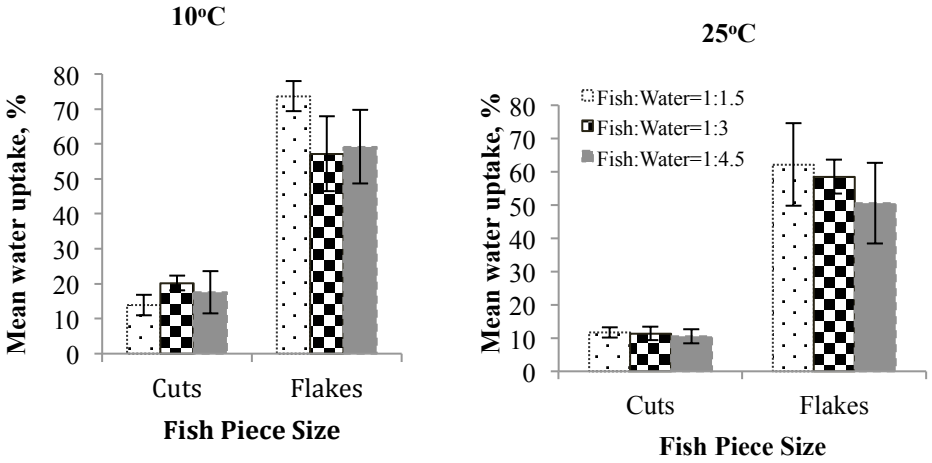

Figure 4.4. Estimated means for water uptake

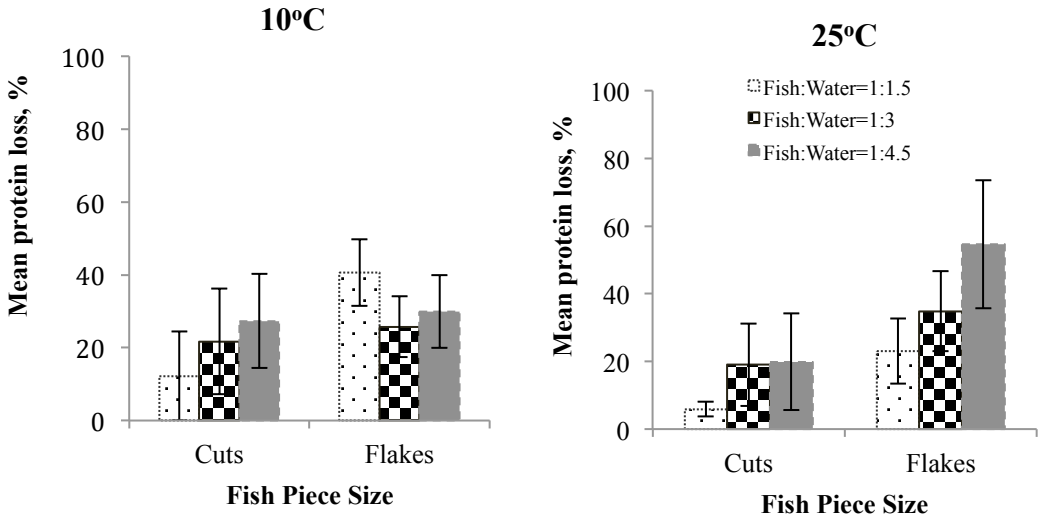

Figure 4.5. Estimated Means for protein loss

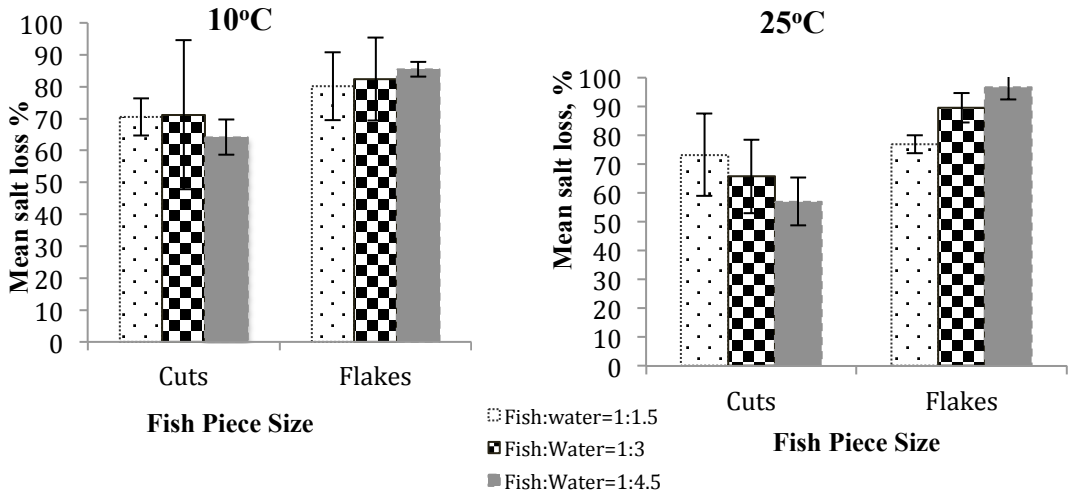

Figure 4.6. Estimated means for salt loss

4.3.3 Influence of Fish to Water Ratio

Fish to water ratio alone at the levels studied did not significantly affect any of the parameters studied (Table 4.2). This finding was unexpected because generally, salt removal decreases with increasing solute concentration in desalting water (causing a decrease in concentration gradient), implying that salt loss decreases as water volume decreases. However, the results suggest that, when fish/ water ratio is considered alone, 1:1.5 fish/water ratio can desalt tuna effectively as 1:3 and 1:4.5 fish/water ratios, which is an advantage from an industrial point of view. The disadvantage however associated with using 1:1.5 fish/water ratio is that flakes tend to absorb a lot of water (more than half of their weight) (fig. 4.4), resulting in a pasty product.

4.4.4 Interaction Effects of Temperature, Fish to Water Ratio and Fish Piece Size

ANOVA results suggest there was no significant interaction effect of fish piece size, temperature, and fish to water ratio ($p>0.05$) on salt loss, protein loss and water uptake. However, a significant difference in salt loss ($p=0.027$) and water uptake ($p=0.036$) were observed for the two-way interaction effect of fish to water ratio and fish piece size. Higher salt loss was observed for flakes as fish to water ratio decreased (larger water volumes) (Fig. 4.6). This observation is coherent with the already established observation that larger volumes of water provide a higher concentration gradient, which facilitates more movement of solutes from the solid phase. However, the opposite effect was observed for cut loins (Fig. 4.6); Higher fish to water ratios resulted in higher salt loss for cut loins. This unexpected observation may have been caused by fish to fish variations.

The average degree of salt loss, protein loss and water uptake for various desalination treatments can be observed and compared in figure 4.7.

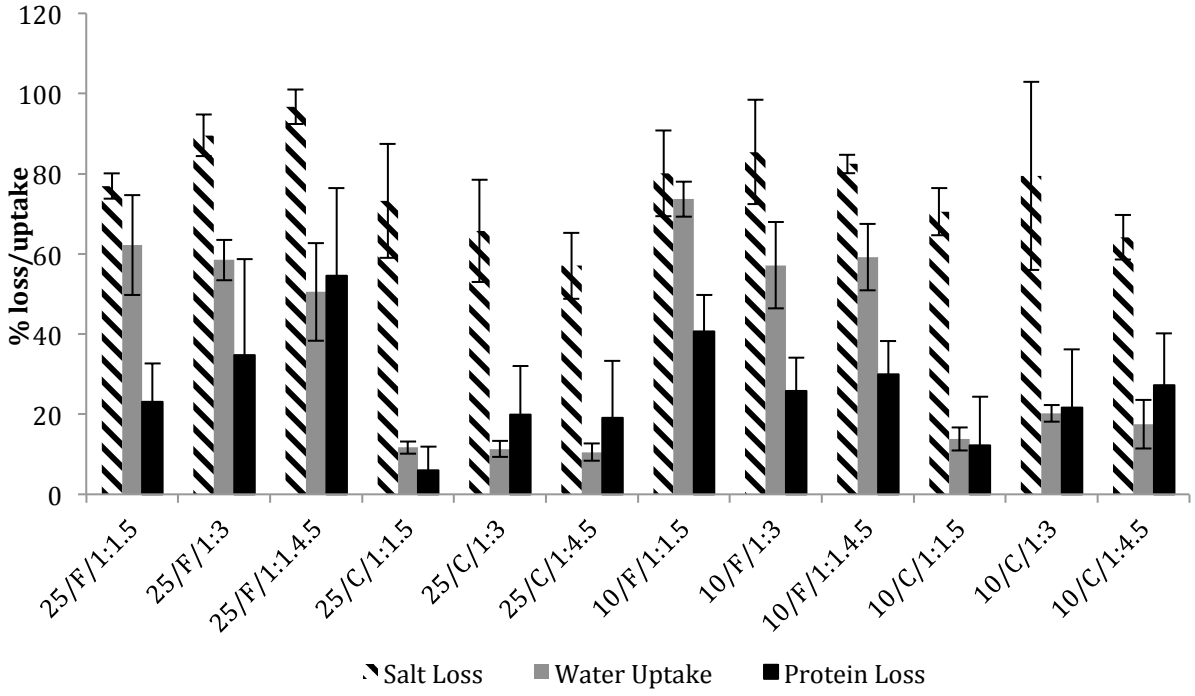

Figure 4.7. Salt Loss, Protein Loss and Water Uptake for Desalination treatments

Comparing the results of the twelve different desalination treatments (Fig. 4.7), those combining 10^{o}C water, cut loin, 1:3 fish/water ratio (10/C/1:3) and 25^{o}C water, cut loins, 1:1.5 fish/water ratio (25/C/1:1.5) were considered to be the best two treatments. This is because their salt loss was considerably high (≥ 70%), while protein uptake and water uptake were also fairly minimal (≤30%). Desalination treatments combining 10^{o}C water, flakes, 1:4.5 fish/water ratio(10/F/1:4.5) and 10^{o}C water, flakes, 1:3 fish/water ratio also showed

high salt loss (≤70%) and low protein loss (≤30%), except water uptake was high (≥40%).

These results suggest that lower temperatures with lower fish to water ratios (large volumes of water) are better for desalting tuna flakes while smaller volumes of water at room temperature (25^{o}C) can be used to desalt cut tuna loins satisfactorily. This demonstrates an important advantage from a practical point of view since a desalination process of cut loins using smaller water volumes at room temperature will be more economical (cheaper and easier) to adopt in industry.

4.5 Effect of desalination on drain weights of canned tuna

The drained weights of desalted tuna were noticeably lower than control tuna products (Table 4.3). This suggests that higher fill weights should be applied when canning desalted tuna in order to achieve the desired drain weight. Also it was observed that for desalted products, those canned in oil had higher drained weights than those canned in brine.

Table 4.3. Mean Drain Weights of Canned Tuna Samples (n=3)

Sample	*Drain weight (g)*	
	Mean	**SD**
Cbr*	281.4	3.21
Co*	268.6	1.03
Fo*	290.1	1.93
Fbr*	317.4	4.60
(10/C/1:3)o	248.3	3.72
(25/C/1:1.5)br	227.3	0.42
(10/C/1:3/)br	222.1	1.96
(10/F/1:4.5)o	229.2	3.78
(10/F/1:3)o	239.8	2.47
(25/C/1:1.5)o	238.2	4.21
(10/F/1:4.5)br	229.0	4.00
(10/F/1:3)br	229.0	3.53

Symbols defined in table 3.1

4.6 Consumer Acceptance Test

Analysis of variance (ANOVA) was performed in order to obtain information about potential difference in the liking of treated (desalted) and untreated (normal level salt content without desalting). Table 4.4 shows mean scores, standard deviations and P-values for ANOVA test for taste, aroma, texture and overall liking for the twelve different samples.

Table 4.4. Sample Means, Standard Deviations and Significance for Consumer Acceptance Testing (n=33)

	Attribute liking			
Treatment	**Taste (SD)**	**Aroma (SD)**	**Texture (SD)**	**Overall (SD)**
Cbr*	2.36 **(**2.01)	2.18 **(**1.40)	3.64 **(**1.62)	2.73 **(**1.00)
Co*	3.36 **(**2.24)	3.36 **(**1.96)	2.64 **(**.92)	3.64 **(**2.11)
Fo*	3.45 **(**1.36)	4.00 **(**1.78)	2.91 **(**1.44)	3.00 **(**1.09)
Fbr*	4.27 **(**1.73)	3.18 **(**.40)	4.00 **(**.63)	4.09 **(**1.64)
(10/C/1:3)o	5.27 **(**2.72)	7.45 **(**2.29)	3.09 **(**2.30)	5.27 **(**1.90)
(25/C/1:1.5)o	4.64 **(**1.96)	5.35 **(**1.20)	4.27 **(**1.00)	4.45 **(**1.03)
(10/C/1:3/)br	4.73 **(**2.32)	3.36 **(**1.63)	2.73 **(**1.19)	4.27 **(**1.34)
(10/F/1:4.5)o	5.55 **(**2.16)	6.18 **(**2.04)	3.64 (1.96)	5.45 **(**1.91)
(10/F/1:3)o	6.91 **(**2.16)	6.64 **(**1.96)	5.36 **(**2.50)	6.55 **(**2.46)
(25/C/1:1.5)br	3.91 **(**1.70)	2.64 **(**1.02)	4.00 **(**1.090	3.45 **(**1.29)
(10/F/1:4.5)br	6.73 **(**1.61)	4.73 **(**1.61)	5.91 **(**1.97)	5.64 **(**1.28)
(10/F/1:3)br	6.00 **(**1.94)	4.00 **(**1.78)	5.91 **(**1.64)	5.73 **(**1.55)
P-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Symbols defined in Table 3.1 and sensory scale defined in section 3.8.3

There were significant differences for all the attributes amongst all the twelve samples ($P<0.0001$). Generally, untreated tuna samples were liked better than treated samples.

4.6.1 Aroma

The aroma scores of test and control products canned in brine were smaller (hence more liked) than those canned in vegetable oil (Table 4.4). Among test and control fish canned in oil, the test samples received higher scores (more disliked) than control samples. This suggests that the combination of desalinated fish and oil may not hold gainful promises commercially, since the sensory data suggests that the fish-in-oil aroma was further marred by the desalting process. Between test samples, (10/C/1:1.3)o was the most disliked (7.45± 2.29) and (25/C/1:1.5)br was the most liked (2.64 ±1.02).

4.6.2 Taste

It was hypothesized that because desalted samples had been soaked in water and had absorbed water, they might be better liked for their taste when canned in oil (sunflower) than when canned in brine (which is essentially water). However on an average, brine products scored slightly better than oil products (5.34 and 5.59 respectively) in regards to their taste. Among the treated samples, (25/C/1: 1.5)br was most liked (mean score of 3.91±1.70) and (10/F/1.3)o was least liked (6.91±2.16)when considering taste only. Some consumers commented that while the oil (sunflower) products may have tasted better than the brine products, their dislike for the aroma of the oil products may have influenced the taste scores.

4.6.3 Texture

Generally, Cut loins products scored lower (better liked) for their texture than flakes products. (10/C/1:1.3)br was the most liked (2.73±1.19) for its texture among the test

products. (10/f/1:4.5)br and (10/F/1.3)o were most disliked (5.9±1.97 and 5.9±1.64 respectively) and this may be attributed to the fact that they absorbed the most water during desalination and thus became too soft to be appealing to the respondents. About 33% of the consumers commented that these products had a pasty, undesirable texture.

4.6.4 Overall Acceptance/Liking

The test product (25/C/1:1.5)br was the most liked (3.45 ±1.29) while (10/f/1:1.3)o was the least liked (6.55±2.46). The most liked product, one canned in brine, could be explained by the same reasons for the general preference of fish-in-brine product to fish-in-oil products. Additionally, (25/C/1:1.5)br, as explained in Table 3.1, was cut loins (larger piece sizes) and desalted in 1.5 parts of water to 1 part of water. The larger sizes and moderate water volume used in the desalination meant a considerable maintenance of the firmness of the fish tissue (prevention of the undesirable pasty feel). These may have influenced its selection as the product of choice among the options presented to consumers.

CHAPTER 5

5.0 Conclusions and Recommendations

5.1 Conclusions

Different sections of tuna absorb different amounts of salt during brine freezing. For the sectioning style used in this study, salt content was highest in the outer muscular layer of the mid-section, followed by the tail region, the inner muscular layer of the mid-section, and then the head region.

In the desalination of tuna, proteins are lost along with salt from the fish, while water is absorbed into the fish muscle. Among temperature, fish to water ratio and fish piece size, the last factor has the biggest influence on salt loss, protein loss and water uptake; smaller sizes favor each phenomenon. The extent of salt and protein loss at 10^{o}C does not differ significantly from that at 25^{o}C. The higher temperature however appears to favor water uptake.

Fish to water ratio does not seem to affect salt loss, protein loss and water uptake during desalination. However, when considered with fish piece size, there is an interaction effect on salt loss.

Desalted tuna products have lower drain weights than control tuna products.

The (25/C/1:1.5) desalted treatment subsequently canned in brine yields a product whose aroma, taste and texture could be generally acceptable to consumers. However, the same

attributes in control products are likely to be preferred to those of the test product.

The (10/f/1:1.3)o desalted treatment, although it results in the removal of appreciable amounts of salt, would yield products that may fail on the market.

5.2 Recommendations

- Desalinated tuna samples should not be canned in vegetable oil as that canning medium results in an aroma consumers perceive as unacceptable.
- Experiments should be conducted to determine the effect of combining (blending) desalted tuna with untreated tuna to enhance their sensory attributes after canning.
- Experiments should be conducted to study the extent to which the addition of vegetable broth to canning media could improve the drain weight of desalted tuna products.
- A cost benefit analysis should be performed to determine if the return on investment (ROI) for desalting tuna is acceptable. Tuna with excess salt could be sold for other purposes (such as pet food) albeit at a lower price, instead of going through the trouble of desalination, which yields a lower quality product. Selling this lower quality product may end up costing the processor more in the long term (by tarnishing the brand name) as compared to the loss made by selling it for cat food.

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APPENDICES

Appendix 1. Sodium Chloride Brine Tables. Adapted from *Preparation of salt brines for the fishing industry* by K. S. Hilderbrand, 1999.

Percent Sodium Chloride by water	Pounds Salt Per Gallon of water
.000	.000
.528	.044
1.056	.089
1.586	.134
2.112	.179
2.640	.226
3.167	.273
3.695	.320
4.223	.367
4.751	.415
5.279	.464
5.807	.512
6.335	.563
6.863	.614
7.391	.665
7.919	.716
8.446	.768
8.974	.821
9.502	.875
10.030	.928
10.558	.983
11.0086	1.039
11.614	1.094
12.142	1.151
12.670	1.208
13.198	1.266
13.725	1.325
14.253	1.385
14.781	1.444
15.309	1.505
15.837	1.568
16.365	1.629
16.893	1.692
17.421	1.756
17.949	1.822
18.477	1.888
19.004	1.954
19.532	2.022
20.060	2.091
20.588	2.159
21.116	2.229
21.644	2.300
22.172	2.372
22.700	2.446
23.338	2.520
23.310	2.531

Appendix 2. Subject consent to sensory evaluation

I agree to participate in the sensory evaluation of canned tuna for the Department of Food Science at North Carolina State University. I also agree that I have no known food allergies and I am 18 or older. I understand that participation is voluntary and that I may withdraw my participation at any time. I also understand that information I provide is confidential and that results will not be associated with my name.

_____

Name Printed Name Signed Date

Appendix 3. Ballot sheet for sensory evaluation

DEPARTMENT OF BIOPROCESSING, NUTRITION AND FOOD SCIENCE

NORTH CAROLINA STATE UNIVERSITY

BALLOT SHEET FOR EVALUATION OF CANNED TUNA FLAKES/CHUNKS IN OIL OR BRINE

NUMBER: _____ DATE: _____

INSTRUCTIONS: Read carefully before you begin
You have been provided with 4 coded samples of canned tuna. Please evaluate them from left to right. After tasting a sample, kindly rinse your mouth with the water provided and **wait for about 30s before evaluating the next sample.** *Re-tasting of samples is allowed.*

ACCEPTABILITY: Please rank the intensity of your **liking** of each canned tuna attribute using the scale below:

Scale:

1=like extremely	**6= dislike slightly**
2= like very much	**7= dislike moderately**
3= like moderately	**8= dislike very much**
4= like slightly	**9= dislike extremely**
5= neither like nor dislike	

1. Aroma

Hold the plate of tuna sample close to the nose and smell.
Write the codes of the samples in the boxes below in the order in which you have been presented. Indicate your **LIKING** for tuna aroma by writing the appropriate score from the 9-point hedonic scale in the space below the code.

Sample code	☐	☐	☐	☐
Score	_____	_____	_____	_____

2. Taste

Take a spoonful of the tuna sample and taste.
Write the codes of the samples in the boxes below in the order in which you have been presented. Indicate your **LIKING** for tuna taste by writing the appropriate score from the 9-point hedonic scale in the space below the code.

Sample code	☐	☐	☐	☐
Score	_____	_____	_____	_____

3. Texture

Write the codes of the samples in the boxes below in the order in which you have been presented. Indicate your **LIKING** for mouth feel for the tuna before you by writing the appropriate score from the 9-point hedonic scale in the space below the code.

Sample code	☐	☐	☐	☐
Score	_____	_____	_____	_____

4. Overall acceptability

Overall, how much do you **LIKE** each of the canned tuna provided before you?

Sample code	☐	☐	☐	☐
Score	_____	_____	_____	_____

Comments:
…….....
.....
.....
.....

Thank you for your participation!

Appendix 4. ANOVA Results for salt loss

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	14003.078[a]	12	1166.923	10.552	.000
Intercept	77437.444	1	77437.444	700.241	.000
Temperature	67.348	1	67.348	.609	.443
Fish piece size (FISHPS)	11959.294	2	5979.647	54.072	.000
Fish/water ratio (FW)	38.607	2	19.303	.175	.841
Temperature * FISHPS	467.849	1	467.849	4.231	.051
Temperature * FW	145.869	2	72.934	.660	.527
FISHPS * FW	938.981	2	469.490	4.245	.027
TemperatuRe * FISHPS * FW	247.177	2	123.589	1.118	.344
Error	2543.498	23	110.587		
Total	176587.912	36			
Corrected Total	16546.576	35			

a. R Squared = .846 (Adjusted R Squared = .766)

Appendix 5. ANOVA Results for Water Uptake

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20164.982[a]	11	1833.180	37.126	.000
Intercept	49758.738	1	49758.738	1007.738	.000
Temperature	339.788	1	339.788	6.882	.015
FISHPS	19062.404	1	19062.404	386.061	.000
FW	212.842	2	106.421	2.155	.138
Temperature * FISHPS	.188	1	.188	.004	.951
Temperature * FW	26.942	2	13.471	.273	.764
FISHPS * FW	378.576	2	189.288	3.834	.036
Temperature * FISHPS * FW	144.242	2	72.121	1.461	.252
Error	1185.040	24	49.377		
Total	71108.760	36			
Corrected Total	21350.022	35			

a. R Squared = .944 (Adjusted R Squared = .919)

Appendix 6. ANOVA Results for Protein Loss

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5494.139[a]	11	499.467	2.665	.022
Intercept	24772.597	1	24772.597	132.183	.000
Temperature	.023	1	.023	.000	.991
FISHPS	2632.562	1	2632.562	14.047	.001
FW	913.542	2	456.771	2.437	.109
Temperature * FISHPS	264.984	1	264.984	1.414	.246
Temperature * FW	668.694	2	334.347	1.784	.190
FISHPS * FW	281.313	2	140.656	.751	.483
Temperature * FISHPS * FW	733.021	2	366.511	1.956	.163
Error	4497.887	24	187.412		
Total	34764.624	36			
Corrected Total	9992.027	35			

a. R Squared = .550 (Adjusted R Squared = .344)

Appendix 7. Balanced Incomplete Block Design used for Sensory Analysis

The SAS System 12:58 Thursday, October 7, 2013 1

The OPTEX Procedure

Class Level Information

Class	Levels	Values
Treatment	12	1 2 3 4 5 6 7 8 9 10 11 12
Block	33	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33

The OPTEX Procedure

Design Number	D-Efficiency	A-Efficiency	G-Efficiency	Average Prediction Standard Error
1	95.0666	89.8313	91.8327	0.6092
2	95.0635	89.8186	91.7869	0.6092
3	95.0635	89.8186	91.8940	0.6092
4	95.0501	89.7624	91.5842	0.6094
5	95.0471	89.7507	91.7097	0.6094
6	95.0440	89.7384	91.6814	0.6095
7	95.0437	89.7366	91.6870	0.6095
8	95.0415	89.7297	91.7717	0.6095
9	95.0357	89.7375	90.8743	0.6095
10	95.0314	89.6800	91.4193	0.6097

The OPTEX Procedure

Design Number	Treatment D-Efficiency	Treatment A-Efficiency	Block Design D-Efficiency
1	81.7952	81.7722	99.9719
2	81.7952	81.7722	99.9719
3	81.7952	81.7722	99.9719
4	81.7952	81.7722	99.9719
5	81.7952	81.7722	99.9719
6	81.7952	81.7722	99.9719
7	81.7952	81.7722	99.9719
8	81.7837	81.7492	99.9579
9	81.7837	81.7491	99.9579
10	81.7722	81.7262	99.9438

Obs	BLOCK	Treatment
1	1	9
2	1	3
3	1	10
4	1	2
5	2	6
6	2	5
7	2	8
8	2	7
9	3	8
10	3	10
11	3	12
12	3	4
13	4	11
14	4	7
15	4	3
16	4	10
17	5	12
18	5	2
19	5	6
20	5	5
21	6	2
22	6	1
23	6	11
24	6	4
25	7	6
26	7	1
27	7	7
28	7	11
29	8	4
30	8	6
31	8	7
32	8	2
33	9	12
34	9	3
35	9	8
36	9	5
37	10	7
38	10	9
39	10	8
40	10	1
41	11	7
42	11	5
43	11	10
44	11	4
45	12	12
46	12	5
47	12	11
48	12	10
49	13	5

Obs	BLOCK	Treatment
50	13	3
51	13	4
52	13	8
53	14	11
54	14	2
55	14	9
56	14	5
57	15	1
58	15	7
59	15	12
60	15	3
61	16	3
62	16	6
63	16	8
64	16	11
65	17	1
66	17	2
67	17	5
68	17	3
69	18	6
70	18	12
71	18	4
72	18	9
73	19	9
74	19	4
75	19	11
76	19	12
77	20	6
78	20	9
79	20	8
80	20	10
81	21	2
82	21	8
83	21	7
84	21	9
85	22	10
86	22	1
87	22	6
88	22	9
89	23	4
90	23	9
91	23	7
92	23	3
93	24	11
94	24	10
95	24	8
96	24	1

Obs	BLOCK	Treatment
97	25	5
98	25	4
99	25	10
100	25	1
101	26	2
102	26	12
103	26	11
104	26	8
105	27	6
106	27	2
107	27	10
108	27	3
109	28	3
110	28	4
111	28	11
112	28	6
113	29	7
114	29	12
115	29	10
116	29	2
117	30	12
118	30	1
119	30	3
120	30	9
121	31	7
122	31	11
123	31	5
124	31	9
125	32	1
126	32	6
127	32	12
128	32	5
129	33	4
130	33	1
131	33	2
132	33	8