The United Nations University
FISHERIES TRAINING PROGRAMME

P.O. Box 1390, Skulagata 4
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Final Project 2006

QUALITY CHANGES IN SALTED, REHYDRATED AND DRY SALTED COD (Gadus morhua) PRODUCTS

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ABSTRACT

The quality and yield of traditionally salted fish in Uganda is still unsatisfactory. The purpose of this study was to investigate the changes that occur during various salting methods. This was done through evaluating the effects of salting methods on selected variables used as indicators of quality in salted, rehydrated and dried salted cod (Gadus morhua). Generally salting and drying significantly decreased the yield in all methods, affecting mainly kenching and brining. Rehydration, however, enhanced the yield in all methods, with kenching attaining the highest. Salt content increased in all methods (19.5-21.4%) but the methods showed decreasing moisture and water activity. The kench products had significantly lower water activity than the rest of the methods during salting and drying. The salt content in rehydrated products reduced to low levels (4.6-6.2%) but attained a high water activity (0.95-0.97). There was a general increase in the microbial load in salted and rehydrated products. A general increase in the protein content in all the methods was observed during the first days but reduced in the advanced stages of drying. In the rehydrated products, the highest total protein content (salt free) was noted in the brine and kench salting methods compared to the inject-salt methods. Results also showed a very low activity of the proteolytic enzymes (trypsin and chymotrypsin) in all products. In general, the overall differences between the methods were minimal; indications were that the yields in the modern methods (inject-salted) were higher than in the traditional methods of kenching and brining.

Key words: Kenching, spectrophotometer, inject-salting, enzyme activity, protein, water activity and microbial load.

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1 INTRODUCTION

Most fish species in Ugandan lakes, other than Nile perch, provide one of the most reliable sources of the high quality animal proteins to the average Ugandan population compared to beef, pork, chicken and mutton. Fish accounts for about 50% of the animal protein supply with an estimated per capita consumption of 12.5 kg/year (Mukibi 2001). Fish contributes significantly to the National Gross Domestic Product (GDP) and is the country's second largest foreign exchange earner to coffee. Total export earnings that accrued from fish export in 2005 amounted to $143 million from an estimated quantity of 36,000 Metric Tonnes mainly from the Nile perch fishery which forms 95% of the total fish exports (UFPEA 2005).

Traditional methods of processing fish in Uganda which provide the most affordable way to preserve fish are salting, smoking and sun drying. These methods are widely used along the main water bodies to preserve fish mainly for distant markets within the country that cannot access fresh fish due to long distances and poor infrastructure. The demand for the traditionally processed fish varies entirely between regions with the northern and north eastern regions preferring smoked and sun dried fish and the western regions bordering the Democratic Republic of Congo preferring mainly salted fish products. Fishing activities and those related to processing and preservation provide employment to close to a million people both directly and indirectly (Bahiigwa and Keizire 2003).

Fish salting plays a role in processing and preserving fish particularly along the shores of lakes Albert, Kioga, Edward, George, Katwe, and Albert Nile (Figure 1) (Nyeko, 2004). Salting is mainly done by the artisanal fisher folk comprising mostly women who obtain salt as a salting medium cheaply from Lake Katwe which is within the vicinity. The most commonly salted fish species include Tilapia, Alestes, Hydrocynus, and the small sized, lean Lates niloticus. Small quantities of these salted products are consumed locally within the country leaving unspecified amounts to be exported informally to the neighbouring countries of Democratic Republic of Congo (DRC), Rwanda, Southern Sudan and Burundi. Because of this informal trade, statistical data about the real quantities exported are scanty, unreliable or not available at all (Nyeko 2004).

The salting method is commonly used in the processing and preservation of fish and meat products and has been practised for centuries in many parts of the world including Uganda. Because of its ability to lower the water activity (free water in food materials) and water content, salt has been used as a preservative during processing to control microbial and enzymatic activities. It additionally enhances the flavour of the products and thus can be used as a condiment (Hermes 2006). Salting is widely used to preserve fish in most parts of Uganda whose limited access to electric power and enough capital curtails the use of modern practices of icing and freezing by the artisanal fisher folk.

In the production of the salted fish products in Uganda, the relatively small and lean fish are salted whole while the larger pieces are split open along the dorsal fin through the head. The gut is then removed and the fish is washed before being subjected to salting. The fish is either soaked in a salt solution (brining), dry salted in vats while draining the exudates (kenching), or salted while retaining the exudates (pickling).

Salting is often combined with drying depending on the requirements of the market or the urgency and weather conditions. During wet seasons, brining and pickling are often employed while in the dry season, mainly dry salting is practised. Often, where it requires drying, the salted products are laid on the ground, sand or less commonly on racks.

The kind of handling fish while processing described above exposes the final salted products to spoiling agents. Chemical changes such as those related to total volatile bases-TVB and trimethylamine-TMA that are more often accelerated by enzymatic and microbial activity are influenced by the high tropical temperatures. A combination of these factors, high water activity in the salted product due to the high relative humidity and poor storage facilities usually lead to a poor quality final products which affects their economic value although they have high potential.

The lack of sufficient knowledge about the effects of quality and optimum amounts of salt to apply during the salt processing of fish is also contributory factor to the production of poor quality salted products in Uganda. High salt concentrations such as those experienced in the dry salting and pickling methods may affect the water holding capacity of the fish muscle. Enzyme activity in the fish muscle at a certain level of ionic strength often changes the texture and results in its softening, therefore affecting the aroma and flavours. The tissue breakdown and muscle softening is due to the action of proteolytic degradation of muscle structural proteins such as myosin (Stoknes et al. 2005).

It is with this background in mind that a study, using salted cod (Gadus morhua), was conducted at the Icelandic Fisheries Laboratories. It was aimed at investigating the quality changes that occur during the various salting techniques which seemingly influence the rate of enzyme activity in the salted products. This study was conducted from November 2006 to March 2007. Cod was used as the raw material because of its significance to the economy of the host country, its availability and biological characteristic of leanness, similar to most species used for salt curing in Uganda. This study was also aimed at arming the researcher with the necessary tools in the form of skills and knowledge to help bridge the information gap particularly existing between the artisanal processors and consumers in Uganda. It is anticipated that a reduction in the information gap will enhance the quality of the salted products from Uganda and boost the economic value that accrues from this fishery.

1.1 Specific objectives

To evaluate the effects of different salting methods on the variables analysed, i.e. after salting, rehydration and drying of salted products.

To determine the protein contents of the variously salted cod products.

To assess the water activity levels and microbial load of the salted products.

2 LITERATURE REVIEW

2.1 An overview of the fishing industries of Uganda and Iceland

2.1.1 Fish production and utilisation in Uganda

Uganda's fisheries industry is currently based on capture fisheries from lakes, rivers and swamps which cover an area of about 18% of the total surface area estimated at 241,038 km2 (Kaelin and Cowx 2002). The fisheries resources are obtained from five major lakes, namely Victoria, Kyoga, Albert, Edward and George (Figure 1)). There are also nearly 160 minor lakes but whose productivity is relatively low (Kaelin and Cowx 2002). Lake Victoria is the most important for Uganda's fisheries by far and supplies about 50% of the national catch (Table 1).

Exploitation of the fisheries in 2000 was conducted mainly by an estimated 250,000 artisanal fisher folk, of whom 136,000 were along Lake Victoria.
About 750,000 people benefit from the fisheries related-activities such as fish processing, fish trading, boat building, industry and administration (Kaelin and Cowx 2002).Sixty per cent of the total catch landed from the Ugandan water bodies is marketed fresh and industrially processed targeting Nile perch and to a lesser extent, Tilapia (Ibale 1998). Approximately 40% of the total fish catch landed from the waters of Uganda are processed mainly using traditional methods of smoking, salting and sun drying.

Figure 1: Map of Uganda showing the major water bodies. (NET Networks, Inc. 2007)

The traditionally processed species include the mainly lean and often less fatty Tilapia, Alestes, Hydrocynus, and Bagrus. Immature Nile perch and Rastrineobolar argentea have been lately added. The main processing method is salt curing accounting for about 65% (targeting markets mainly in the Democratic Republic of Congo and southern Sudan. The smoked and sun-dried fish products (35%) are marketed within the country and other neighbouring countries like Rwanda, Sudan and Kenya (Nyeko 2004).

Table 1: Fish catch by water body.

Water body	Catch in thousands of Tonnes								
	1995	1996	1997	1998	1999	2000	2001	2002	2003
Victoria	103	106.4	106.6	105.2	111.4	175.4*	105.8	136.11	175.22
Albert	16.4	21.9	19.1	19.1	19.1	29.6	29	19.38	19.46
Albert Nile	4.7	4.6	3.4	3.5	3.7	3.7	nd	nd	nd
Kyoga	80.2	80.6	80.1	80.2	79.3	81.2	80.1	55.58	32.89
Edward, George & Kazinga channel	5.2	4.8	6.4	5.6	5.8	7.2	7.4	nd	nd
Others	3.7	3.7	3.7	3.5	3.7	4.5	4.5	10.82	14.13
Aquaculture	0.19	0.21	0.36	0.36	0.36	nd	nd	nd	nd
Total	213.4	222.2	219.7	217.5	223.8	301.6	227	221.89	241.70

Source: Nyeko (2004).Fish production and utilisation in IcelandThe major sources of seafood in Iceland are capture fisheries comprising of cod, haddock, halibut, herring and capelin caught mainly from the North Atlantic Sea, and aquaculture that accounts for a relatively small quantity.

The largest portion of seafood landed in Iceland is processed by both freezing and chilling (Figure 2) and exported mainly to the European Union market. This is followed by meal and oil processing. Traditional processing of seafood by salting and drying is also done and products are

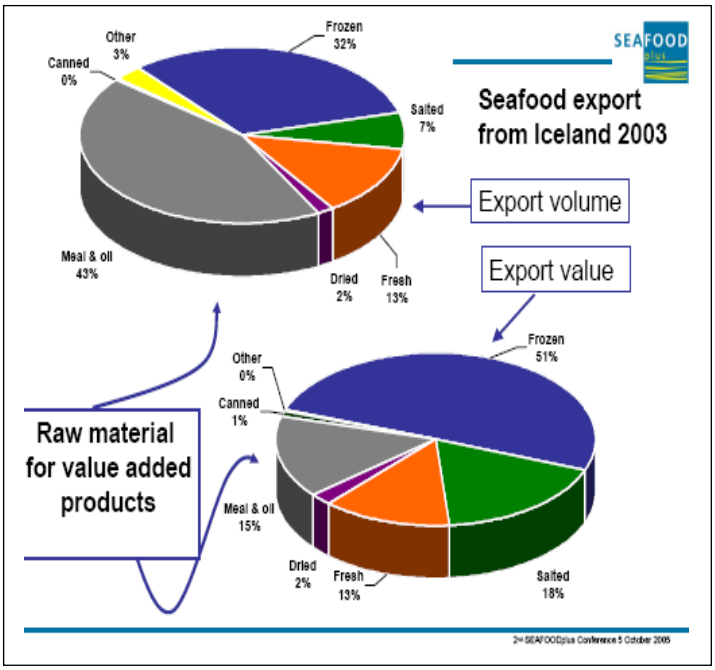

Figure 2: Seafood export from Iceland, 2003 (Seafood Plus 2005).

mainly exported with a little surplus consumed locally. In 2003, out of the (2.0 million tonnes) total catch landed, worth 68 billion ISK, 43% was processed into fish meal and oil, 32%, was exported frozen and 13% was sold fresh in a chilled form (Figure 2). The surplus was processed traditionally by salting (7%), drying (2%), and 3% was exported in other forms. However, although the biggest portion was processed into fish meal and oil, its export value (15%) was low compared to the revenues from fish processed by freezing, salting and that sold in a fresh state (chilled). The export value of the fish sold in a frozen state was 51% followed by that sold in a salted form (18%). The value from chilled, dried and canned fish products

was 13%, 2% and 1% respectively (Sigurgisladottir 2005). In 2004, a similar trend was observed (Magnusson 2006) (Figure 3). According to Magnusson (2006) and the Fisheries Association of Iceland (2006), the fishery sub-sector (1.7 million tonnes landed, worth 121.7 billion ISK) accounted for about 60% of the total exports from Iceland, of which 18% was from salted fish. The data show that the fisheries industry significantly contributes to the countries' GDP (Figure 4) and salting as a processing method is one of the major activities which heavily contribute to this income. Salted fish has been processed and exported from Iceland since the turn of the 20th century and it formed the bulk of the exports until the Second World War when modern fish processing by freezing and chilling took over (Magnusson 2006).
Figure 3: Seafood exports from Iceland in 2004 (Fisheries Association of Iceland 2006).

Salted cod (bacalao) from Iceland is mainly exported to Spain and Portugal.

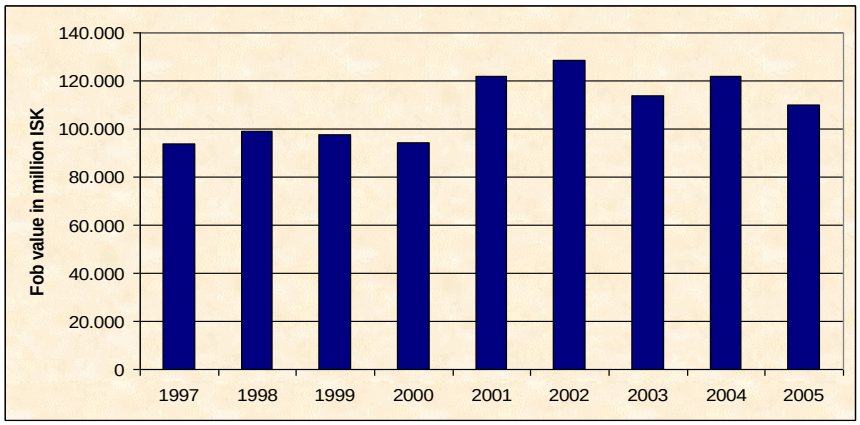

Figure 4: Value of exports accruing from marine products in Iceland (Fisheries Association of Iceland 2006). (F.O.B "indicates Freight on board")

2.2 The fish used as raw material in salt processing

In Iceland and most western countries, cod (Gadus morhua) (Figure 5) is the most widely used species. Others include haddock (Melanogrammus aeglefinus), ling (Molva vulgaris) and Pollack (Pollackin virens).

Figure 5: Atlantic cod (The New York Public libraries 2002).

In Uganda, the fish species most commonly used as raw materials in salting vary according to various factors such as availability, market demand, taste and state of the fish as far as fat content is concerned (section 2.1.1). In this study, North Atlantic cod was used as the raw material.

2.2.1 Chemical composition of the raw materials

The chemical composition of fish varies (Table 2). It can vary widely among species, individuals within species and among different body parts of fish (Murray and Burt 2001). Several factors are responsible for the variation among which, species, age, sex, environment and season have been ear-marked to contribute to the said changes in fish composition (Huss 1995). According to Huss, (1995), cod, for instance, has an average muscle chemical content in the range 78-83% water, 15-19% protein, 0.1-0.9% lipids and 1.2% ash. On the other hand, information about the chemical composition of most fishes in Uganda's lakes is very scanty. However, the fish used for salting could be similar to cod with less than 1% fat because most are lean and other fatty ones are used at the immature level when the fat tissues have not yet well developed. Whole fish muscle is very complex in its composition, which makes it difficult to evaluate the influence of different individual factors on for example lipid oxidation (Svensson and Almgren 2005). The growth history, structure and biochemical composition of the fish muscle influence the flesh quality and the raw material characteristics of fillets particularly for secondary processing to higher value products (Johnston 2003).

Table 2: The principle constituents of fish.

Constituent	Fish fillet (%)		
	Minimum	Normal variation	Maximum
Protein	6	16-21	28
Lipid	0.1	0.2-25	67
Carbohydrates	-	<0.5	-
Ash	0.4	1.2-1.5	105
Water	28	66-81	96

Source: Huss (1995).

2.2.2 Salt as a medium for curing fish

There is no doubt that salt has been used for a long time in the preservation of meat and fishery products because of its ability to prolong shelf life, and to enhance flavours and odours that many people seek. In addition, it inhibits the action of microorganisms by reducing the water activity in the food products. However, the numerous advantages associated with salt curing may be counteracted by some disadvantages that accrue from its use especially by the artisanal fisher folk whose level of knowledge about the effects of salting in many developing countries, such as Uganda, is low. Salting, for example, may lead to protein coagulation particularly if applied heavily and using much finer crystals. If done inappropriately, finer salt crystals dissolve very rapidly into the muscle resulting into an inferior product due to case or surface hardening which leaves the centre of the product still wet and susceptible to microbial deterioration. This defect is, however, in most cases avoided especially with heavy salting.

The quality of salt is yet another important factor to consider while curing fish by salting. Salt contaminants such as copper, calcium, magnesium and iron may affect the quality of the final product particularly if the quantities of the contaminants surpass the internationally accepted limits. Metals like copper and iron also accelerate the rate of oxidation common in both fatty and lean fish and often lead to yellow stains (discolorations) in the salted fish. Calcium also has a catalysing effect on the proteolitic enzymes. Although pure salt solution offers antiseptic properties because of its ability to extract free water and thus limit the growth of microorganisms (Hermes 2006), it does not completely deter the growth of all these organisms. Halophillic bacteria will instead grow and multiply whenever their optimum salt concentration of (5-30%) prevails (Magnusson 2006). These organisms may not be pathogens but their presence in large numbers makes them able to break down the muscle tissues and cause bad odours and even discolour the muscle surface of the salted product. A combination of this and enzyme activity often render the final salted product inferior in quality.

Different salting methods also have varying magnitudes of influence on the structural and mechanical features of the fish muscle. Usually brining offers a better salt penetration than kenching and pickling and the product is of a superior quality (sensory) and yield. Dry salting and pickling may be responsible for high levels of thiobarbituric acid (TBA) and volatile basic nitrogen in the fish product.

2.2.3 Nutritional characteristics of fish

Fish is one of the most important valuable sources of high grade protein as well as other nutrients available to man. Knowledge of fish composition is therefore essential if the fullest use of fish is to be achieved. There are varying reasons for considering the chemical composition of fish. Like for other foods, the processor may be interested in knowing the nature of the raw material so that they are able to choose the appropriate method of preservation and processing. The nutritionist may wish to know what contribution the particular food (fish) can make to the diet and the health of individuals and the cook must know whether the fish in question is normally lean or fatty. Similarly, the consumer would be interested in knowing the taste and, to some extent, the nutritional value of the food. The fishmeal manufacturer's concern too would be the composition of the whole fish, and the oil processor may be interested in knowing what is in the liver. Measurement therefore, of the constituents of the products is necessary to meet specifications or compliance with the regulations (Murray and Burt 2001). The regulations referred to include, for instance, the Hazard Analysis Critical Control Point (HACCP) and the Good Manufacturing Practices (GMP) which are internationally accepted.

2.3 The structure of fish muscle

The muscle function is said to be similar throughout the animal kingdom, although some differences occur. In fresh fish muscle, blocks are fairly attached to the connective tissue, and the surface of a cut fillet is smooth and continuous. There are also tiny blood vessels that run through the muscle. Because of the less connective tissue in the muscle of fish compared to that of beef muscle, fish is generally less tough to eat than meat (Murray and Burt 2001). The blocks of muscle forming the

individual flakes, especially in cooked fish, are separated by thin sheets of connective tissue. They run from the backbone to the skin. Fish muscle is of two kinds, light and dark muscle. In white fish, such as cod and haddock, there is a small strip of dark or red muscle just under the skin on both sides of the body, running beneath the lateral line. In fatty fish, like herring and mackerel, the strips of dark fish muscle are larger in proportion and contain higher concentrations of fat. The proportion of dark muscle to white varies according to species, and increases with swimming activity (Rome 1981). Most fish that rest most of the time at the bottom of the sea have less dark muscle compared to their pelagic counterparts that swim continuously throughout their lives. The tail and trunk consists of a series of muscle blocks called myotomes.

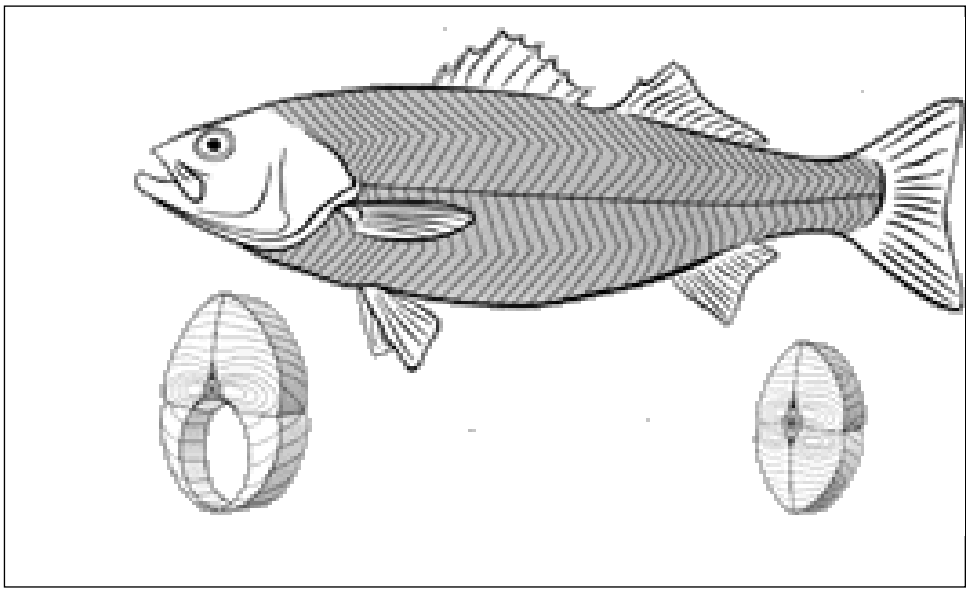

Figure 6: Muscle blocks called myotems are separated body tissues called myosepta (Rome 1981, SeaWorld 2002).

The myotomes resemble the letter "W" tipped at a 900◦C angle (Figure 6). The myotomes are separated by connective tissues called myosepta. The horizontal septum separates the myotomes into dorsal (or epaxial) myotomes and ventral (or hypaxial myotomes).

The myomeres and myosepta in fish have different functions. The myomeres facilitate steady swimming at slow and steady speeds while the myosepta facilitate burst swimming and escape responses using high amplitude axial undulations (Rome 1981). The myosepta effectively transmit the contractile force to the backbone and skin and this keeps the posterior fibres from lengthening. According to Rome, the geometry of the muscle fibre is related to the fibre types or colour. The white muscle is arranged helically with fibres pointing into or away from the midline at high angles. The red muscle is arranged in a longitudinal band near the junction of the horizontal septa and skin. This muscle uses the highly efficient oxidative phosphorylation pathway. The oxidative phosphorylation path produces a slow twitch while the glycolytic path produces a fast twitch, thus supporting Rome's suggestion that red muscle is used for slow and steady swimming while the white muscle is used for rapid bursts and escape swimming.

2.4 Classification of muscle proteins

Muscle proteins are divided into three groups based on their solubility in aqueous solutions. They include: structural proteins (myofibrillar proteins), sarcoplasmic and connective tissue proteins (stromal proteins). The structural proteins make up the contractile apparatus responsible for the muscle movement. Structural proteins (actin, myosin, actomyosin and tropormyosin) constitute 70-80% of the total protein content (compared to 40% in mammals). They are soluble in neutral salt solutions of fairly high ionic strength (> 0.5). Myosin is the most abundant protein in the contractile process and has been studied intensively (Watanabe 2002). It consists of two heavy chains of approximately 200kDa and four light chains of approximately 20kDa (Harrington and Rodgers 1984).

The sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic concentrations (< 0.15) constitute 25-30% of the total proteins. They participate in cell metabolisms such as anaerobic energy conversion from glycogen ATP. Sarcoplasmic proteins tend to change when the organelles (endoplasmic reticulum, mitochondria and lysosomes) are broken particularly during freezing and other preservation methods. These proteins can also be used to distinguish between different species of fish. The connective tissue proteins (collagen) on the other hand constitute approximately 3% of the total protein in teleostei and about 10%. Collagens form the most wide spread protein component of the extracelullar matrix of the muscle (Bruggemann and Lawson 2004). They, in fish muscle, constitute the main component of the connective tissue membranes joining individual myotomes and are responsible for the integrity of the fillets (Sikorski et al. 1984). Collagen content in fish muscles is about 0.2-1.4% and in squid mantel, about 2.6%. Fish and some invertebrate collagens contain slightly more essential amino acids than intramuscular bovine connective tissue collagen.

The chemical and physical properties of collagen proteins are different in tissues such as skin, swim bladder, and myocommata in muscle. Although, there are few published investigations into specific changes that occur in collagen (Bremner, 1997), the collagen in fish is said to be more thermo-labile and forms more labile cross-links than from warm blooded vertebrates (Huss 1995). According to Skorski et al. (1984), thermal changes are the necessary result of the cooking of fish, squid and minced fish products and contribute to a desirable texture of the meat. On the hand, they may lead to serious losses during hot smoking due to a reduction in the breaking strength of the tissues when heating is done at high relative humidity. Generally, the conformational structure of the fish proteins is easily changed by changing the physical environment. Huss (1995) noted that treatment with salt concentrations or heat may lead to denaturation of the proteins which ultimately may be irreversible.

2.5 Salting fish (salt curing)

The salt curing procedure is a simple one dating back many centuries, as early as 3500-4000 B.C, in its use to preserve foods and it reached a peak in the 18th to 19th centuries (Sikorski 1990). Salt has long been used both as a primary ingredient, and in combination with other methods such as drying and smoking (Turner 2005). In recent years, salting has become a less popular technique due to the development of quick freezing, canning and smoking preservation methods for fish (Sikorski 1990). Today,

the market preference is towards processing techniques that allow retention of the freshness characteristics of fish products as much as possible. Thus, frozen and chilled products have tended to surpass the demand for traditionally processed fish products. However, although the demand for fresh fish products is increasing, in many parts of the world especially in the developing countries of Africa and Latin America, salting in combination with natural drying processing methods remain widely in use today (Sikorski 1990). In Uganda, for instance, fish is mostly cured by the oldest methods of kenching and pickling although to some extent a combination of brining and drying can be applied depending on the market demand and the nature of fish (fat content). The reason for this can be traced to deficiencies in freezing and cold facilities due to limited supplies of electric power requirements.

Sodium chloride is one of the important components that determine the taste of salted fish, direction, and intensity of chemical processes during ripening. It is essential not only because of the rate of the above stated processes but also for product stability during storage. A study conducted on a group of consumers in the Soviet Union (now Russia) about the preference rating for salt concentration in salted fish using herring, revealed that a majority of consumers preferred low salt concentrations in the range 4-5%, while a relatively smaller proportion preferred concentrations above 6% (Sikorski 1990). It should be emphasised that in this study salted cod is first rehydrated before consumption. The dilemma in the Russian study, however, was that the preferred lightly salted fish had shorter storage stability (shelf life) compared to the heavily salted fish products. But with the technological advancement, where freezing and cold storage facilities were available to cater for the preservation role, the production of light salted products could continue.

2.5.1 Pre-handling of fish for salting

Before salt curing, the raw material is appropriately handled beheaded and gutted, washed and then chilled before splitting or filleting. After splitting, the fish is again washed thoroughly and then immersed in brine tanks (brine salting depends on the method of brining to apply)

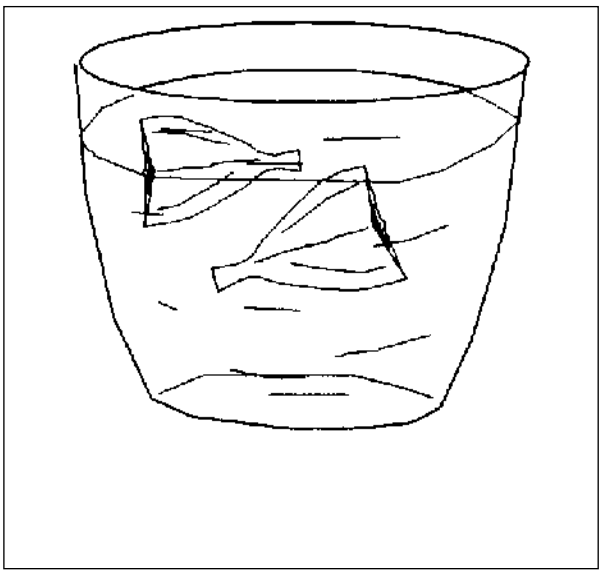

Figure 7: Traditional brining method (Essuman 1992).

for 2-3 days before being laid in alternating layers of salt and fish (dry salting) as shown in Figure 7and Figure 8 respectively, below, for 1-2 weeks. When the product is fully salted, it is packed and stored at chill temperature before dispatch.

2.5.2 Salting methods

Salting is done usually by the various methods among which, brining and dry salting take centre stage both in developed and developing countries . This is not particularly for preservation, especially in the western world, but for development of special products with desirable odours, flavour tastes and textures. It also provides special and traditional dishes in many countries, for example bacalao in southern Europe and Latin American countries. Brining involves

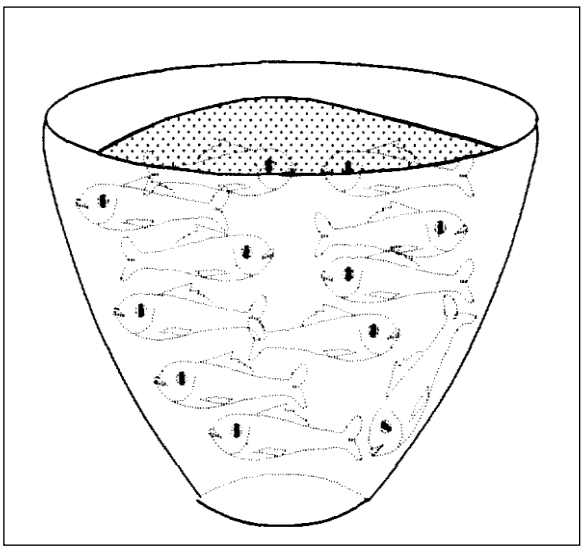

Figure 8: Traditional dry salting of fish (Essuman 1992).

placement of dressed fish in prepared salt solutions of known salt concentrations for a couple of days before they are removed and left to dry at ambient temperatures or further dry salted. Dry salting involves the application of salt crystals on to the dressed fish in layers usually in a salting vat or drum and left over a period of time (Figure 8).

These two main methods of salting fish may be modified depending on a number of factors such as weather, market demand, and desire for a longer shelf life, among others. In dry salting for example, one may desire to retain the brine liquor that oozes out of the salted fish into the salting vat (pickling) or can opt to drain it away. Therefore, during dry salting, the granular salt is applied directly to the fish either in the gills, on the surface or, in the case of split fish, on the entire split-open surface. The exudate from the fish may be allowed to drain away or be retained. In the latter case, the fish becomes immersed in the exudate and this is often referred to as "pickling" (Essuman 1992). In dry salting, usually plenty of salt (heavy salting) is applied resulting into a product with approximately 20% salt content. An improved way of dry salting is shown in Figure 9. These methods are more traditional than those of wet salting or brining. In brining, the developed world currently uses the injected brining method (Figure 10), a method that ensures quick penetration of the salt into the fish muscle and effects drying much faster than any other method.

Figure 9: Modern method of heavy dry salting in plastic containers after brining for 2-3 days. An improved method used for salt curing of "bacalao" in the western world.

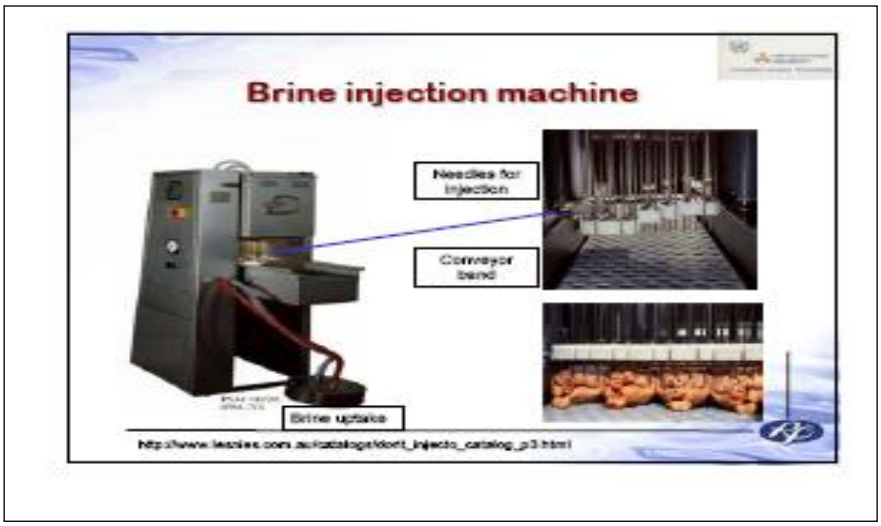

Figure 10: Modern brining equipment (http://www.lesnies.com.au/catalogs/dorit_injecto_catalog_p3.html **2000).**

2.5.3 Factors that affect salt up-take

At the stage of fish salting, sodium chloride ions are transported from brine into fish and water dipoles from fish to brine through the processes of diffusion and osmosis, as most scientists believe (Sikorski 1990). However, another view is that sodium chloride and water dipole transportation proceeds with differing intensities during salting and ripening, and depends on the value of the chemical potential of the organic system in fish and brine (Sikorski 1990). Therefore, knowledge of the factors that influence the rate of intake of sodium chloride and removal of water is important. The factors include: concentration of sodium chloride in the fish muscle tissue and brine is the most important factor determining the rate of transportation of these constituents. The sodium chloride concentration gradient can be increased by changing the quantities of salt relative to fish. By increasing this concentration, conditions are established when the higher salt concentration in brine promotes a more intensive sodium chloride and water transport, hence salt uptake. According to Sikorski (1990), with an increase in salt amount from 9-21% relative to fish mass in salting mackerel, salt up-take increased 1.5 times. The size of fish and thickness are equally important factors influencing the rates of sodium chloride and water transport. Beheading, gutting and splitting open the fish or filleting can promote the salt uptake.

The chemical composition of fish muscle also plays important roles in determining the rate of salt up-take. The skin and scales on the fish body reduce the salt-in rate by 1.6 (Sikorski 1990). Hypodermic fat also impedes the sodium chloride and water transport. It functions as a peculiar hydrophobic barrier for sodium chloride transport during salting. Other factors are the state of the fish, such as during rigor mortis. Salt uptake during this state is lower than at the final stage of rigor mortis.

Freezing and defrostation of fish also favours salt uptake Stoknes et al. (2005). The size of salt crystals equally plays a critical role in the up-take in this process. The smaller the size, the easier the penetration but small size may also be associated with

case hardening. Therefore, a combination of bigger and smaller size crystals would be recommended (Sikorski 1990, Hermes 2006).

2.5.4 Influence of salt on the muscular components of fish

Virtually all reactions in the body and muscles are mediated by enzymes, which are protein catalysts that increase the rate of reactions without being changed in the overall reactions. Enzymes channel reactants, called substrates into useful pathways and thus direct all metabolic events (Harvey and Champe 2005). Fish muscle, just like other animal muscle contains a number of enzymes which continue being active even if the fish has been killed as long as optimum conditions for their activity still prevail.

Such conditions include temperature, pH, and salt concentrations. The fish muscle contains proteases which degrade the fish muscle proteins after death and results in the weakening of the myofibrillar structure. The collagens are equally degraded in the fish muscle the same way the myofibrillar are. The enzymes called the collagenases are responsible for the degradation of this important and major connective tissue in the muscle structure that determines the textural attributes of the raw flesh (Bremner 1997). Similarly, serine proteases and heterogeneous group of proteases can also degrade some collagen types. These collageneases are metallo-endoproteases having an optimum pH in the range of 7-8 and are activated and stabilised by calcium ions, a possible reason being that their optimum activity is when the pH levels are high which in this case, presence of the Ca2+ seems to fulfil. However, they are inhibited by metal chelators such as EDTA (Bremner 1997). Stoknes and Rustard (1995) found out from their study on purification and characterisation of a proteinase from Atlantic salmon muscle that the purified proteinase was able to hydrolyse myosin heavy chains. The same study suggests that proteinases can degrade a variety of protein and peptide substrates. In a related study, Choi et al. (2004) noted similar actions of hydrolysis by the chymotrypsin and cathepsin L- like enzyme in the myofibrillar proteins of anchovy. High proteolytic activity in anchovy intestines and muscle tissues accelerates autolytic degradation of tissue proteins and may shorten the fermentation time during anchovy sauce production. Trypsin and chymotrypsin are quantitatively important in the digestion due to their highly proteolytic activities.

Various other studies have also noted that the activity of enzymes is favoured by low ionic strength, but the more you increase the salt concentration for instance, the more the increase in the ionic strength. This, however impacts the rates during their activities. An observation by Stoknes et al. (2005), in their study on the proteolytic activity in cod muscle during salting noted that chymotrypsin-, trypsin-, collagenase- and elastase-like activities increased during the first five days in salt/brine but thereafter activity decreased again as the salt concentration increased. These findings also correspond to those of Choi et al. (2004) during their work on properties of proteases responsible for degradation of muscle proteins during anchovy sauce fermentation. This is probably because the high salt concentrations may denature the proteins which mostly occur when the concentration exceeds 10% by mass as noted in this study and the corresponding increase in ionic strength may affect the proteases activity, hence the decrease.

Another phase of salted fish which forms their structural, plastic, flavour, and odour properties that add to their quality and make the fish a ready-to-use-product without

any need for additional processing is called “ripening”. It is a complex physical and chemical process of transforming proteins, lipids, and carbohydrates, by enzymatic systems into peculiar taste, flavour and consistency of salted fish (Sikorski 1990). The changes that occur during ripening of salted fish are based on hydrolysis of muscle proteins under the influence of peptide hydrolase complex. During ripening salted products from dressed fish, the hydrolysis of proteic substances is influenced by the action of peptide hydrolases of cathepsins A, B, and C types. This is due to the occurrence of their optimum pH conditions and those of fish muscles tissues.

In ripening salted whole fish, the role of ripening agents is played by the peptide hydrolase complex in the viscera, particularly the pyloric appendages containing a highly active complex of peptide hydrolases of trypsin and peptidase types. When this occurs, the complex of these peptide hydrolases penetrates further into the fish muscle, combines with the muscle enzymic system and subsequently promotes the hydrolysis of muscle proteins resulting into release of free amino acids. The released free amino acids and peptides then undergo complex reactions with lipids and carbohydrates transformation products. The higher the activity of the visceral and muscle peptide hydrolase complex, the more intensive the hydrolysis of proteins, and the faster the ripening process in salted fish is. In a study by Olsen and Skara (1997), they noted higher protein breakdown products in the muscle during ripening of North Sea Herring. A related study by Nielsen and Borrescen (1997) also suggests that one of the causative factors of ripening in salted fish is the action of endogenous intestinal proteases that hydrolyse the muscle proteins and create a softer texture and taste-active peptides and free amino acids. They also noted an increase in total protein content into the brine after particularly two weeks of salting, an indication that the high molecular weight protein materials diffuse from the muscle tissue into the brine as well as the low molecular weight nitrogen compounds. This was relatively high in the ungutted herring. In their conclusion, they noted that intestinal trypsin-type endopeptidases may not be always the activated enzymes during the salting and storage and that these proteases may not always be the main enzymes responsible for the proteolitic breakdown during ripening of traditionally heavily salted herring. It is important to note here that although these observations may relate in some way to the current study they were based on other fish species and different salting methods but the researcher is optimistic that the principle applies for cod too.

2.6 Water activity

The total water or moisture content in a particular type of food is the total water in a product, including the molecularly bound water, and is often expressed as a percentage of the total weight. The relative humidity means the relative product equilibrium humidity, the freely available water for microorganisms to grow; its measuring value is called water activity value (aw) (Novasina 2005). Water activity (aw), therefore, measures how efficiently the water present in a food material can take part in a chemical or physical reaction. It is therefore defined as a ratio of the partial pressures of the water above the food (p) to the pure water (p0) under identical conditions of mainly temperature (aw = p/p0) (Fontana 1998). It is sometimes also defined as “free water”, “available water” or “unbound water” in a system. Multiplication of the aw by 100 gives the percent equilibrium relative humidity (ERH) of the atmosphere in equilibrium with the food sample. If half the aw is so tightly bound to a protein molecule that it could not take part in a hydrolysis reaction,

the overall water activity would be reduced. The tightly bound water has no tendency to escape from a food as vapour and therefore exerts no partial pressure and has an effective water activity of zero. Water activity is clearly a function of composition but is also a function of temperature. Water activity instruments usually measure the amount of free (unbound) water present in the sample. A portion of the water content present in a product is strongly bound to specific sites on the chemicals that comprise the product. Such sites include the hydroxyl groups of polysaccharides, the carbonyl and amino groups of proteins, and other polar sites. The hydrogen bonds, ion-dipole bonds, and other strong chemical bonds tightly bound water. Many preservation processes such as salting attempt to eliminate spoilage by lowering the availability of water to spoiling agents such as microorganisms, and other chemical changes that result from enzyme activity on foods. Water activity is a property that can be used to predict the stability and safety of a food with respect to microbial growth, rates of deteriorative reactions and chemical/physical properties (Fontana 1998). The ability for water to act as a solvent, medium and reactant has a tendency to increase with increase of water activity in food materials (Labuza 1975). By measuring the water activity of food stuffs, one is able to tell the type of microorganism that will potentially spoil the food and the most probable infection that may arise from that, (Staphylococcus aureus) for example, requires a minimum water activity of 0.86 for growth and toxin production (Novasina 2005). Water activity can also play a role in determining the activity of enzymes and vitamins in foods. It thus helps in predicting some of the physical properties like texture and shelf life of most foods. By so doing, the processor can ably decide on the most appropriate methods to apply in processing and preservation of the different types of food depending also on the various interests of the market, nature of food and the respective positions of the food in as far as food security is concerned. Water activity also serves the important role of ably telling the safety of the food especially when used as a control measure in quality control aspects of the Hazard Analysis Control Point (HACCP – ISO 9001). It is also used as an indicator of whether certain government regulations (European-GMP, United States-FDA, and the Japanese) are fulfilled. It also serves as a guarantee of the product's quality, good taste, colour and crispness (Novasina 2005). Two food ingredients may have the same moisture content, but totally different water activity values. Usually water migrates from regions of high aw to those of low aw and not between areas of unequal moisture content. Water migration between different layers of a multi-component food causes textural changes. Because the humidity of air is typically between 50-80% (aw = 0.5-0.8), foods with lower aw tend to gain water whilst those higher aw tend to lose water (Chaplin 2006). Salted fish products often have water activity between 0.6 – 0.7 and have a 60 -70% relative humidity which only exposes them to Xerophile moulds, Osmophile yeasts and Halophilic bacteria (Figure 11). The effects of these microorganisms usually include changes in the final products that affect the flavour, taste, texture and appearance, all of which are consequences of high or lower aw (Marsili 1993). Controlling the water activity does not only inhibit the growth of microorganisms but also help to maintain the chemical stability of foods. Foods that contain proteins and carbohydrates, for example, are said to be prone to non-enzymatic browning reactions, called Maillard reactions (Marsili 1993) whose likelihood of occurrence increases as the water activity of foods such as salted fish increases reaching a maximum at aw in the range of 0.6 to 0.7. In a study by Eichner and Karel (1972), about the influence of water content and water activity on the sugar-amino browning reactions in model systems under various conditions, they noted that the browning rate decreased with increasing water content, except in systems in which

mobility of the reactants became substantially impeded at low water contents. On the other hand, a further increase in aw hinders the Maillard reactions, thus it is quite important to measure the aw to keep good quality of food products.

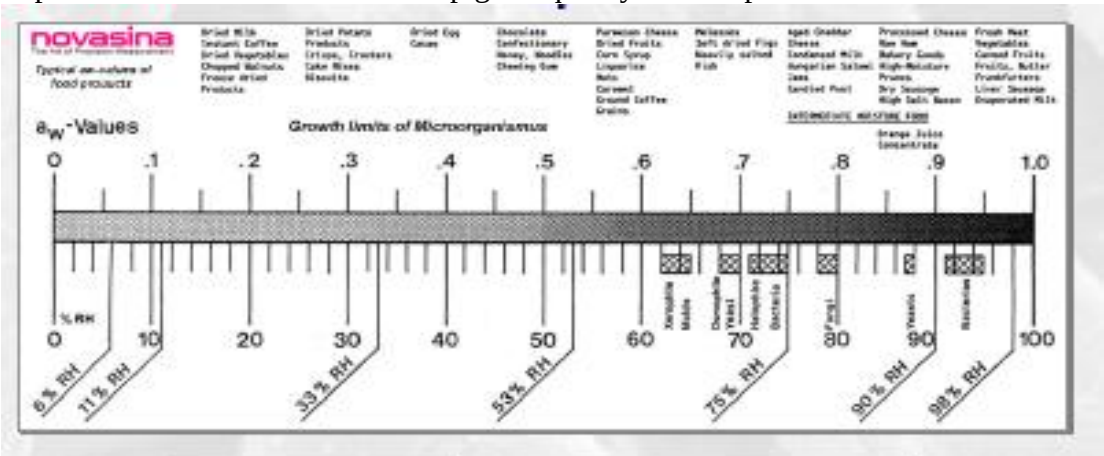

Figure 11: aw values (Novasina 2005)

2.7 Microbial load

In Africa and Uganda in particular, salting and drying of fish for preservation is accompanied by fermentation although the period is short and the product is not transformed into a paste or source. The products, however, are characterised by a strong odour and, for that reason, they have been described as "sink" fish (Essuman 1992). The characteristic smell of salted/fermented fish is the result of enzymatic and microbiological activity in the fish muscle. The fish in their natural habitats often have a natural microflora all over the bodies, gills and viscera. This microflora is not dangerous until the fish dies and they start to invade the flesh of the fish and with the influence of enzyme action, result in degradative changes in the fish muscle. The microorganisms in the salt medium also contribute to the said changes in the fish. Like other spoiling agents in fish, microorganisms require water in a free form for their growth and metabolism. Any control done to inhibit their access to water will obviously retard their activity and lead to death. This is exactly what salt does to these organisms although the salt loving ones (halophiles) will keep thriving at high salt concentrations as high as 25% (Magnusson 2006). Halopiles grow optimally at the said salt concentrations but are unable to grow in salt free-media. The halotolerant organisms grow best without significant amounts of salt but can also grow at concentrations higher than that of sea water. Xelophiles are those that grow rapidly under relatively dry conditions or below aw 0.85 while Osmophiles grow under high osmotic pressure. Salt curing methods can ably retard the growth of most pathogenic microorganisms (Table 3) especially in salted fish (Essuman 1992). Sufficient salt levels can inhibit Clostridium botulinum growth and toxin formation particularly in a 3.5% water phase salt in thickness portion of loin (Anonymous 2001).

Table 3: Growth of microorganisms in salted fish

Water activity (aw)	Sodium chloride Concentration (%)	Microorganisms grooving	
		Pathogens	Spoilage organisms
0.98	< 3.5	All known food - borne	Microorganisms pathogens of concern in foods particularly the gram negative rods.
0.98-0.93	3.5-10	Bacillus cereus, Clostridium botulinum, Salmonella spp., Clostridium perfringens, Vibrio parahaemolyticus	Lactobacilalliaceae, Enterobacteriaceae, Bacilliaceae, Micrococcaceae, Moulds
0.93-0.85	10-17	Staphylococcus	Cocci, yeasts, moulds
0.85-0.60	> 17	Mycotoxic, xerophilic moulds (no mycotoxin is produced at aw less than 0.80)	Halophilic bacteria, yeasts, moulds (dun =Wallemia sebi)

Source: Essuman (1992),

3 MATERIALS AND METHODS

3.1 Materials

The raw material used in the study was salted cod (Gadus morhua) which was provided by the Icelandic Fisheries Laboratories as part of an on-going study. The fresh cod had been caught at the end of October and November 2006, gutted and iced in tubs until processed.

Substrates (Z-Gly-Pro-Arg p-nitroanilide acetate salt, Succinyl-Ala-Ala-Pro-Phe p-nitroanilide) and Bio-Rad Protein assay dye were purchased from Sigma Company and BioRad respectively.

Pre-prepared 20mM Tris, 1mM CaCl2 buffer were obtained from the Icelandic Fisheries Laboratories, having been stored at about 0◦C for close to six months. All chemicals used in the analyses were assumed to be of analytical grade.

3.2 Salting

After 4-6 days from catch the fish were beheaded and splitted (Figure 12). The fish was salted by traditional and modern methods of salting by four different processes:
1. Injection of brine (17% salt and 5% phosphate), brine salting and kench salting
2. Injection of brine (17% salt and 18% minced cod muscle), brine salting and kench salting
3. Brine salting and kench salting
4. Kench salting.
The fish was brine salted for 2 days and then dry salted or kench salted (at 3-5°C), for 5 weeks, one group was only kench salted. After the 5 weeks, the samples were taken out of the salt and divided into two batches for rehydration and drying.

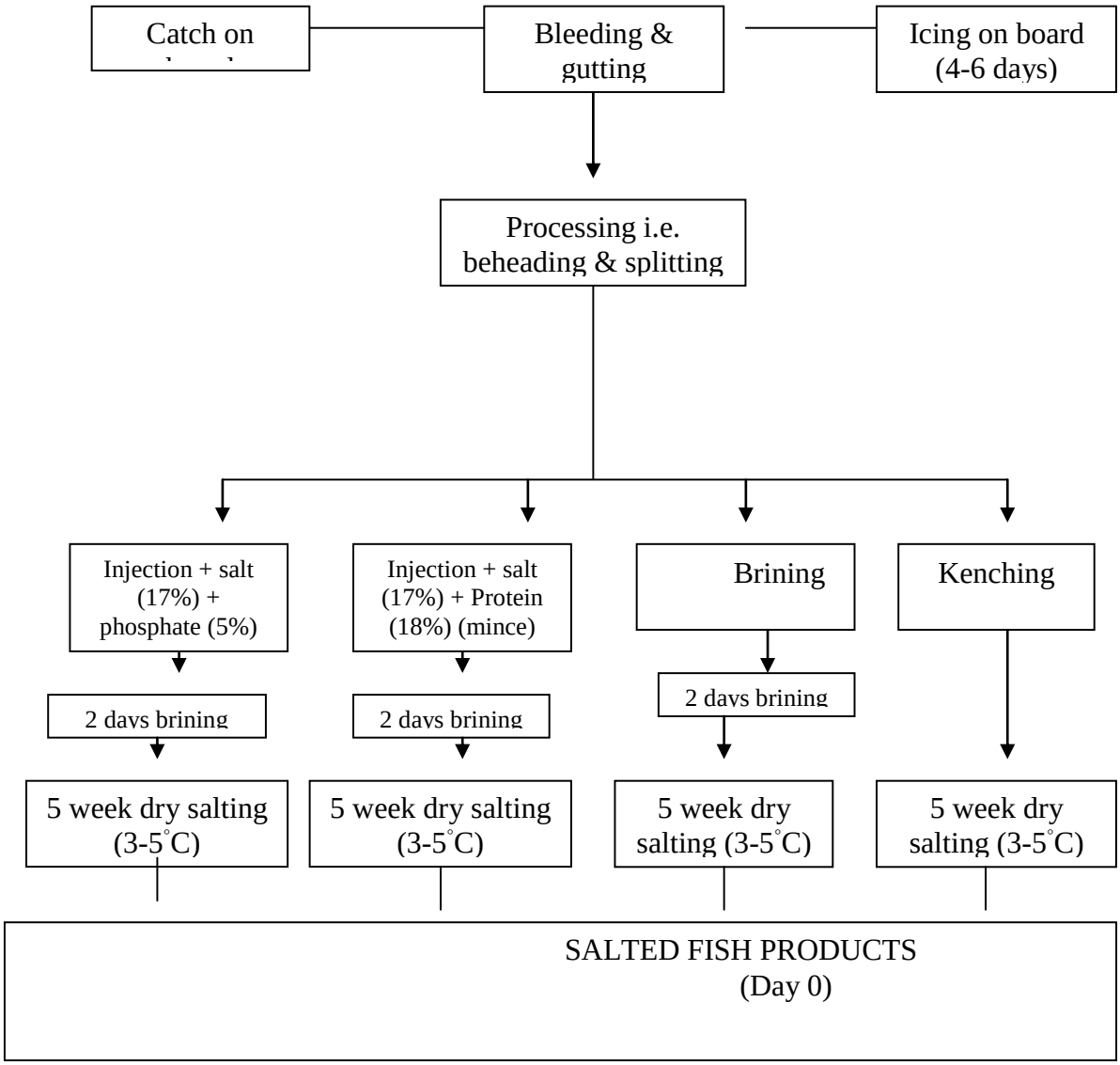

Figure 12: Activity flow diagram for handling and processing of the fish before laboratory analysis.

3.2.1 Rehydration

One of the batches was rehydrated (Figure 13) using a fish to water ratio of 1:5 for one day, then the water was replaced by fresh water and the fish allowed to rehydrate for 3 days using fish to water ratio of 1:5,5 at 4 ± 1°C

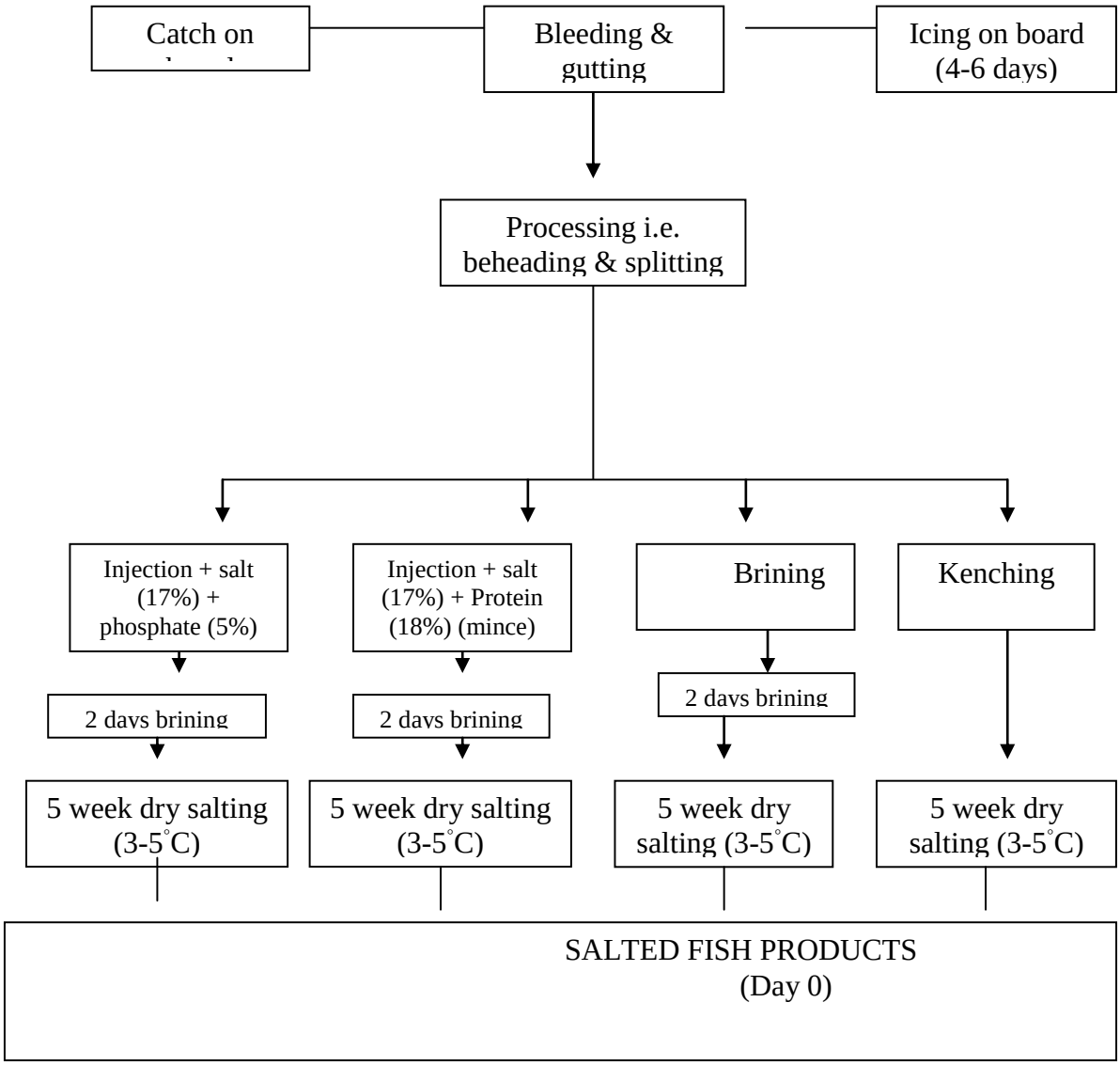

Figure 13: Sampling and laboratory analysis

3.2.2 Drying

The fish was placed on racks (Figure 15) and dried for 10 days. The air velocity was approximately 1 m/s and the temperature 17°C. The drying process was delayed due to power failure on days 5 to 6 (Saturday-Sunday) as shown in Figure 14.

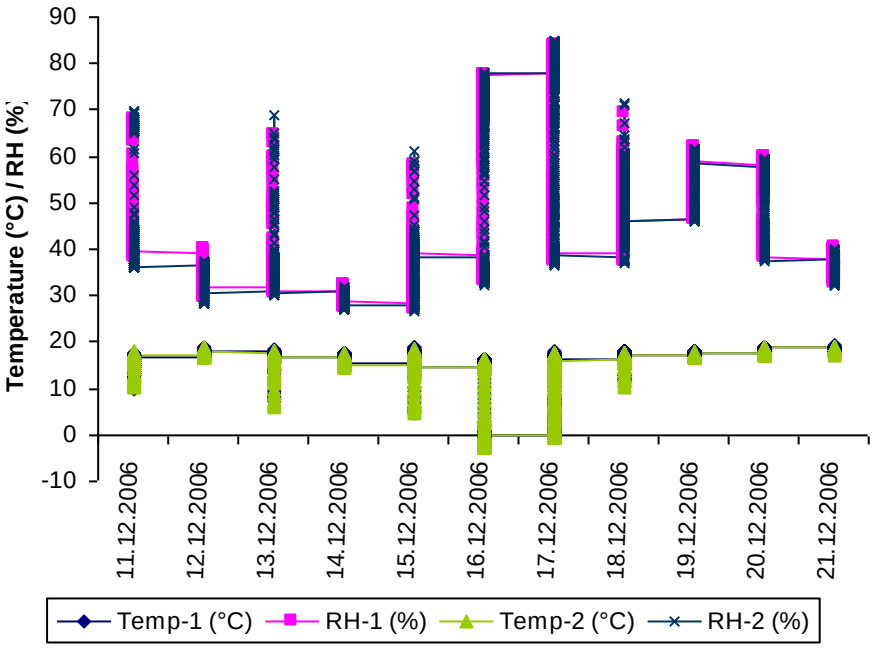

Figure 14: Temperature and humidity (RH) profiles during the drying process (logger 1 and 2 in front of and behind the drying racks).

Figure 15: Dried salted product on trolleys in a drying chamber.

3.2.3 Sampling procedure

Cod loins (Figure 16) from 3 fishes per group were collected after each processing step, salting (day 0), rehydration and drying (days 2, 4, 7, and 10) also shown in (Figure 13). They were kept at 1.5°C just before and during the time analyses were conducted. The analyses conducted on the samples were for water activity, salt, moisture and protein contents, microbial load and enzyme activity.

Figure 16: Cod loins sectioned from one of the dry salted bacalao.

3.3 Sample preparation for enzyme activity and protein content determination

60-100 g of the salted and rehydrated sample portions were weighed in triplicates and minced for about 30 seconds using a food processor at room temperature. Distilled water, about double the weight of the samples (120-200 ml) was added depending on the weight of the sample. The mixture was further homogenised for 10-20 seconds before the extract was transferred to a beaker and kept on ice for 20-30 minutes. 10 g of the extract were weighed into centrifuge tubes and centrifuged at 12,000 g for 5 minutes at 4◦C. The supernatants were decanted off into labelled eppendorf tubes, frozen and stored at -79 ± 1°C. The extracts were later used as sample material for both enzyme activity and protein contents.

3.4 Determination of enzyme activity

Enzyme assays in foods are conducted to determine the soundness of the food, to follow up the changes that occur while processing such as in salting or heating. In this study, a spectrophotometric method was adopted to quantitatively determine the activity of trypsin-like and chymotrypsin-like enzymes in salted fish muscle. 10µl of 12.5 mM Z-Gly-Pro-Arg pnitroanilide acetate salt and the same volume of 12.5 mM N-Succinyl-Ala-Ala-pro-phe p-nitroanilide substrates were used. 10µl of the substrates, 495 µl of Tris, 1mM CaCl2 buffer (ph 8.0) and 495 µl of samples were pipetted and dissolved into a set of 3 cuvettes (triplicates) per sample. Micro-pipettes of appropriate volumes were used and the mixture left to incubate for about 20 minutes. A blank was prepared by adding 10 µl of the substrates to 990 µl of the buffer into a cuvette. Enzyme activities were then measured spectrophotometrically at 410 nm. The measurements were made at time 0, followed every 20 minutes, for a period of 100 to 120 minutes for each sample extract. Absorbance values were recorded by a computer and a graph of absorbance against time was plotted to determine the slopes of the curves which were later used to compute the Kfactor. This Kfactor is important in computing for the total activity of the enzyme as shown in the two equations below:
Kfactor = Amount of substrate used in cuvette x 106/ ε (m-1cm-1) x b x c

Where ε is the extinction coefficient of the product (8800 at absorbance of 410 nm), b is the width of the cuvette (1.0 cm) and c is the volume of the sample in the cuvette.

Total enzyme activity (U/ml) = Slope x Kfactor.

3.5 Determination of protein content

The protein contents of the extracts were measured according to the Bradford dye-binding method (Bradford 1976, Chang 2003) using Bovine Serum Albumin (BSA) as standard and Bio-Rad Protein assay dye. Bio-Rad Protein assay dye solution was prepared by diluting 1 part dye reagent concentrate with 4 parts distilled water. The solution was filtered through a Whatman#1 filter paper to remove the particulates. In order to prepare a protein standard curve, seven dilutions of a representative of the protein solution to be tested were prepared. 100 µl of the BSA standard solution were pipetted into a set of three clean, dry test tubes (triplicates) for each dilution and 5 ml of the dye reagent solution added to each of the test tubes and vortexed to mix thoroughly. A blank was also prepared by adding 100 µl of the distilled water and 5 ml of the dye in a test tube. All test tubes were incubated for 10-15 minutes at room temperature. Spectrophotometric measurements using cuvettes were done at 595 nm. Absorbance was plotted against strength of the BSA standard to obtain a standard curve which was later used for obtaining appropriate dilutions, slope and the Y-intercept for protein content computation (a linear range of the assay for BSA solution was from 0.2 to 0.8 mg/ml). The protein contents for each of the sample extracts were then determined at a dilution of 50 (i.e. 50 µl of the sample: 950 µl of distilled water). The mean absorbance of the different samples were also calculated before finally computing for the protein contents using the equation below:

Protein content (mg/ml) = ((absorbance x dilutions)/ Slope) – Intercept

3.6 Determination of water activity

Water activity was determined using the Novasina IC-500AW-LAB instrument as described in the laboratory procedure by (Wilson 2006). This instrument relies on measuring the amount of moisture in the equilibrated headspace above a sample of food product which correlates directly with the sample water activity aw (Bradley 2003). About 10 g of the minced fish samples were placed in a small closed chamber at almost constant temperature (about 25◦C). Readings were made when the instrument stabilised after approximately 30 minutes for every sample. The means of the triplicate samples were then calculated.

3.7 Moisture and salt content

Water content was determined by drying in an oven at 103 ± 2°C for 4 hours (ISO 1999). Salt content (NaCl) was determined using the potentiometric method (AOAC 2000)

3.8 Microbial load

Total viable counts and counts of H2S-producing bacteria were done on iron agar (0.5% NaCl) as described by Gram et al. (1987) with surface plating. Plates were incubated at 22°C for 3 days. Iron agar with 10% NaCl was also used. These plates were incubated at 22°C for 5 days.

3.9 Statistical analysis

Data in some cases were analysed by Analysis of Variance (ANOVA) particularly adopting Duncan's test to detect the mean differences at $p<0.05$, using NCSS 2000 software.

4 RESULTS AND DISCUSSIONS

4.1 Drying yield

There was a generally observed decreasing trend in the yield within all the products in the different curing methods during the drying period. There were significant differences in yields particularly between the traditional (brine and kench) and modern methods (injected products) in the first 7 days (Figure 17). Significant differences were also observed between the two modern methods. The yields of both kench and brine cured methods were significantly less than the yields of the injected methods (ANOVA, Duncan's test; $p<0.05$).

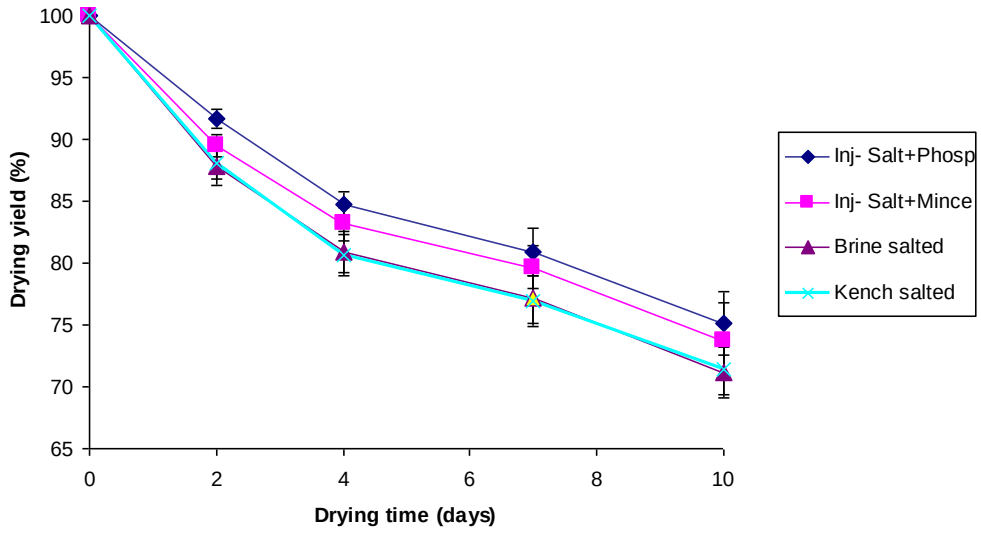

Figure 17: Drying yield in the different curing methods

The rehydration yield was in the range of 121-131%, where the kench salted fish had significantly higher yield than other groups (Figure 18).

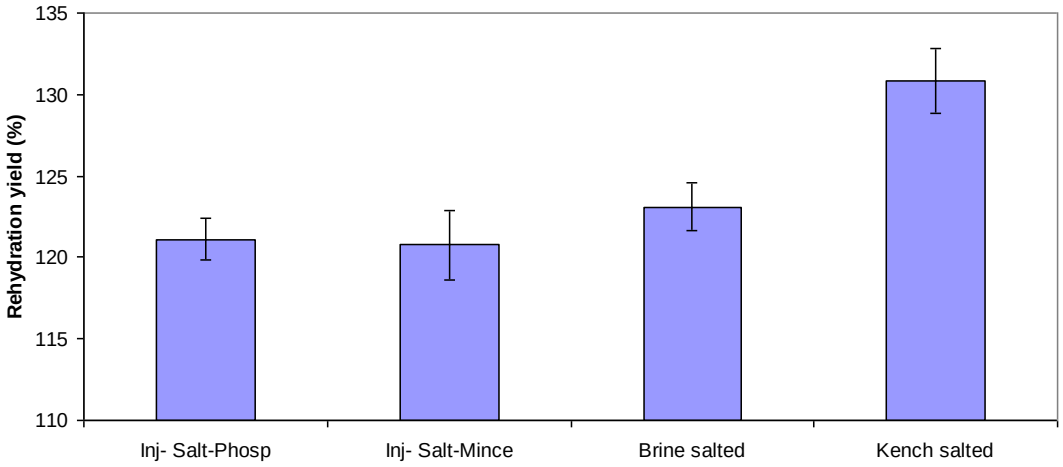

Figure 18: Rehydration yield in the different curing methods.

4.2 Salt, moisture and salt-free dry matter

The salt content in the salted and splitted fish (Figure 20) on (day 0) ranged from 19.5 (in kench salted) to 21.4% (inject-salted + mince). The range of moisture content (day 0) was between 56.2 (kench salted) – 59% (injected-salted + phosphate) products. The muscle of the products further dehydrated to a range of 44.7 – 54.3% respectively by the 10th day of drying (Figure 19). There was a gradual increase in the salt-free dry material as moisture content reduced in the products during the 10 days of drying and storage (Figure 19). There was a general increase in the salt concentration in the muscle during the drying period as water was removed.

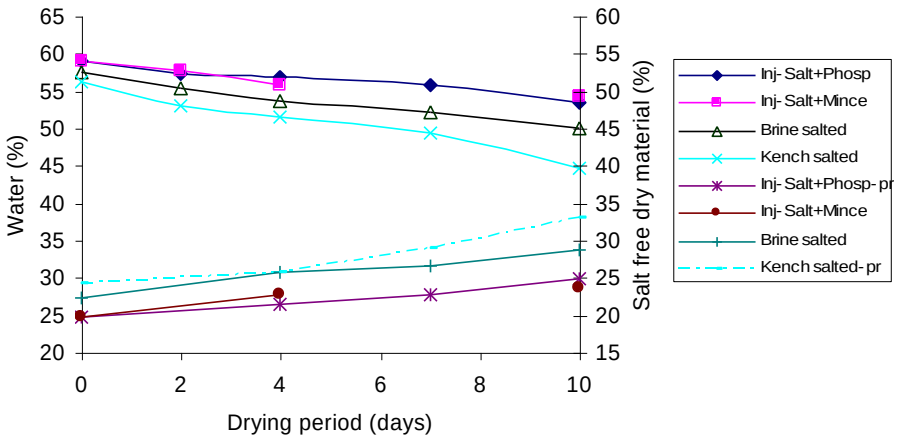

Figure 19: Changes in moisture content of the different salted products during the drying period.

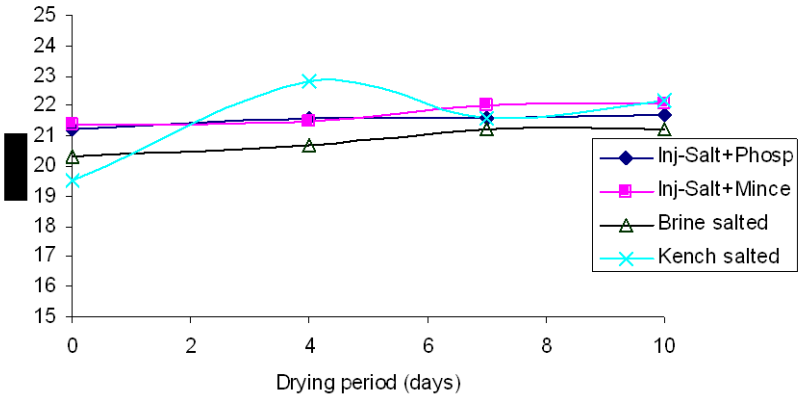

Figure 20: Changes in salt content of the different salted products during the drying period.

Figure 21 shows how the trends of different salting methods were in response to the salt saturation within the muscle. All the methods indicate an increasing trend superseded by kench salting with higher difference in saturation (about 32%) than the injected methods and brining (about 27-29%) respectively for the entire drying period.

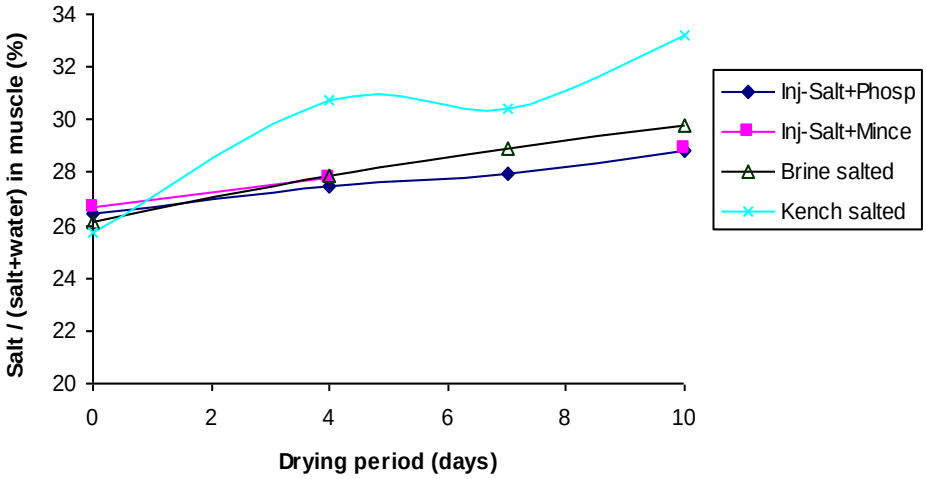

Figure 21: Influence of saturation effects of salt in fish muscle on enzyme activity and protein content.

During rehydration, the salt content of the various products reduced drastically, with the inject-salted + phosphate (6.2%) registering the highest compared to brine salted (4.6%) (Figure22). Although the moisture content after rehydration was high in all salting methods, the inject-salt + phosphate and kench salting registered both the lowest (76.9%) and highest (79.8%) respectively (Figure 23). The water and salt content in the raw material before salting was 0.2% and 82.5% respectively.

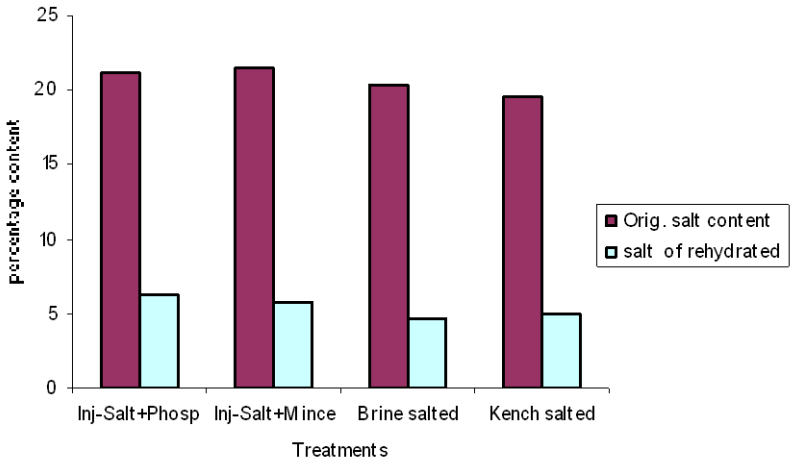

Figure 22: Changes in salt content in both salted and rehydrated products at 5 weeks of dry salting.

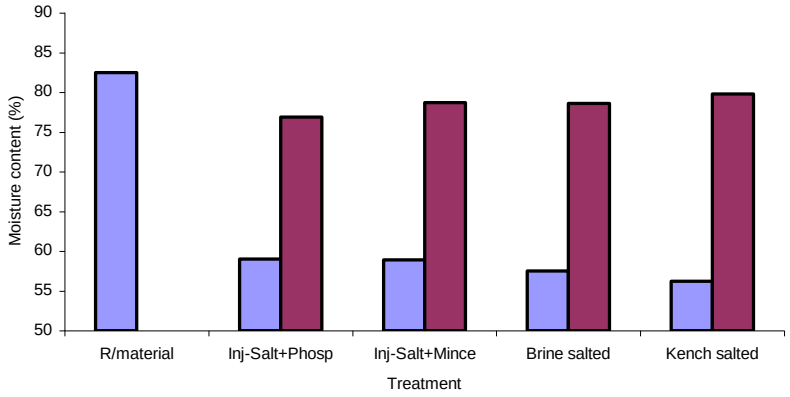

Figure 23: Moisture content in the different salted and rehydrated products after 5 weeks.

4.3 Water activity

Figure 24 shows that the water activity on the first day of drying period was about 0.74 in all the methods. There was a general reduction in the water activity in virtually all curing methods with the traditional kench curing registering the highest reduction to (about 0.72) by the 10th day. The reduction in the water activity for the modern methods of salt curing (inject- salting) was lower compared to brining and kenching by the last day. The water activity for the kench cured products was significantly lower than the two modern methods in the first 4 days and significantly lower for all other methods by the 10th day. On the 7th day, brine salting was equally significantly lower than the inject-salt minced products (ANOVA, Duncan's test; $p<0.05$).

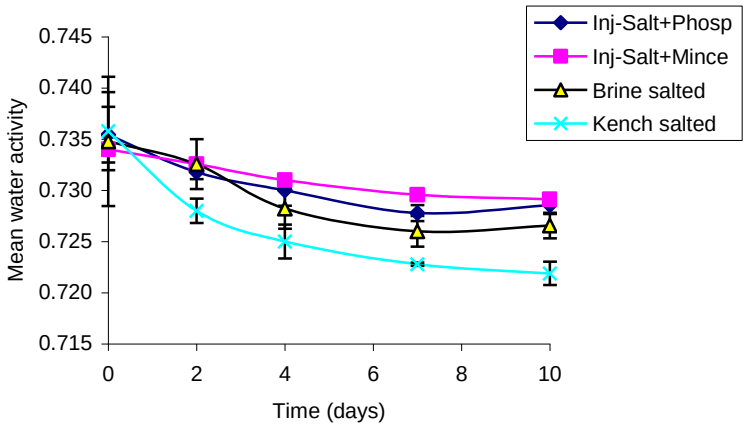

Figure 24: Changes in water activity of the different salted products during the drying period.

The water activity in the different rehydrated salted products increased from about 0.73 in the salted products to just above 0.97 in the brine salted products. The range was from 0.95 to 0.97 which was similar in all products (Figure 25). The highest moisture attainment was in the brine salted products whose water activity value rose from 0.73 to 0.97 an increase of about 32.9% compared to 29.7% in inject- salt + phosphate, inject-salted + mince (30.1%) and kench salted (31.1%).

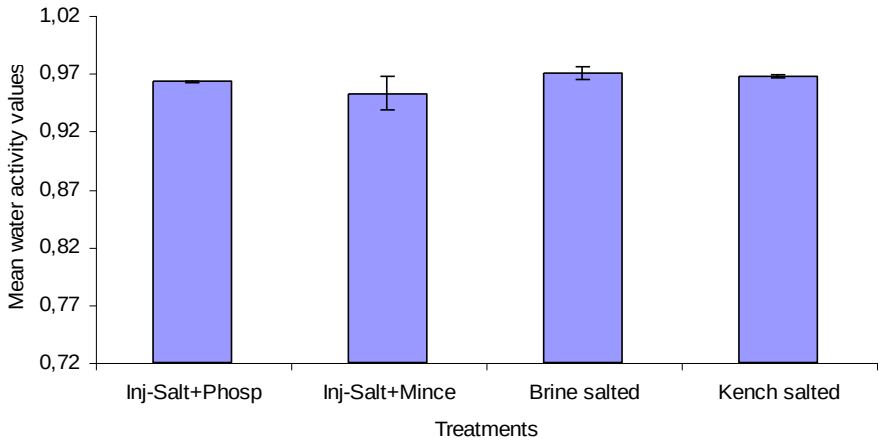

Figure 25: Water activity in the rehydrated products

4.4 Microbial load

Total viable counts and the hydrogen sulphide producing bacteria (black colonies) on both 0.5 and 10% NaCl using iron agar were evaluated. The range for the microbial load of the salted products at 5 weeks was from 4,600 to 9,200 cfu/g for TVC and 100 to 120 black colonies respectively (Figure 26) at 0.5 % NaCl. Almost the same was true in the 10% NaCl (Figure 27) in the same products although slightly fewer loads were observed (750 – 2600 cfu/g for the TVC and only 10 black colonies). The variations within the different curing methods were observed with magnitudes of thousands. The difference between the raw material fish used for salting was low

microbial counts, (50 cfu/g TVC and 10 black colonies) compared to the salted products. It must be mentioned that samples were taken from the gutted fish before splitting.

However, the rehydrated products had high levels of microbial load ranging from as low as 43,000 and 20 to as high as 1,000,000 and 60 TPC and black colonies respectively (Figure 28). The proliferation of these organisms was even higher in the 10% NaCl concentration (Figure 29) except for the black colonies which instead reduced and kept at a constantly low level of about 10 colonies suggesting that the higher the concentration the lower the hydrogen sulphide producing colonies.

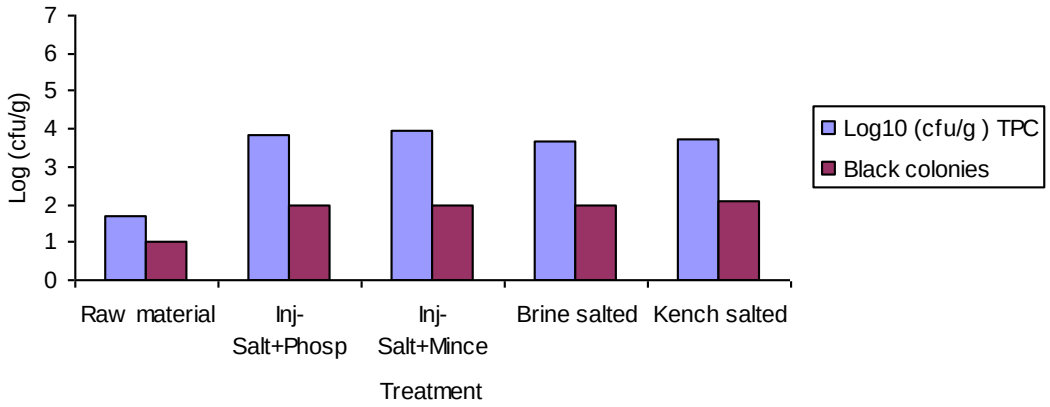

Figure 26: Microbial load in the various salt cured products in 0.5% NaCl iron agar.

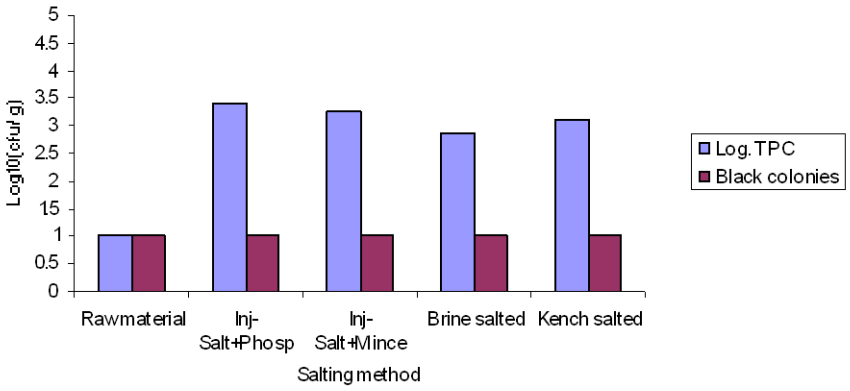

Figure 27: Microbial load in the various salt cured products in 10% NaCl iron agar

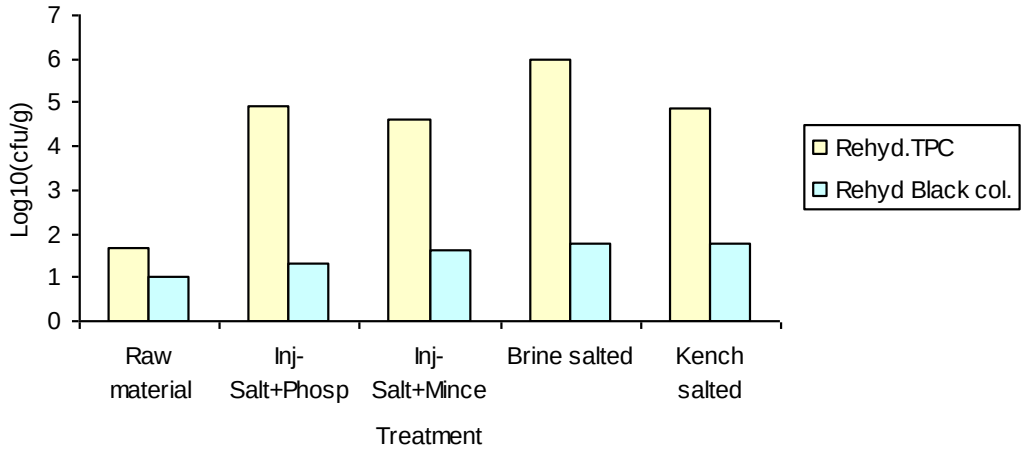

Figure 28: Microbial load in the various rehydrated products in 0.5% NaCl iron agar

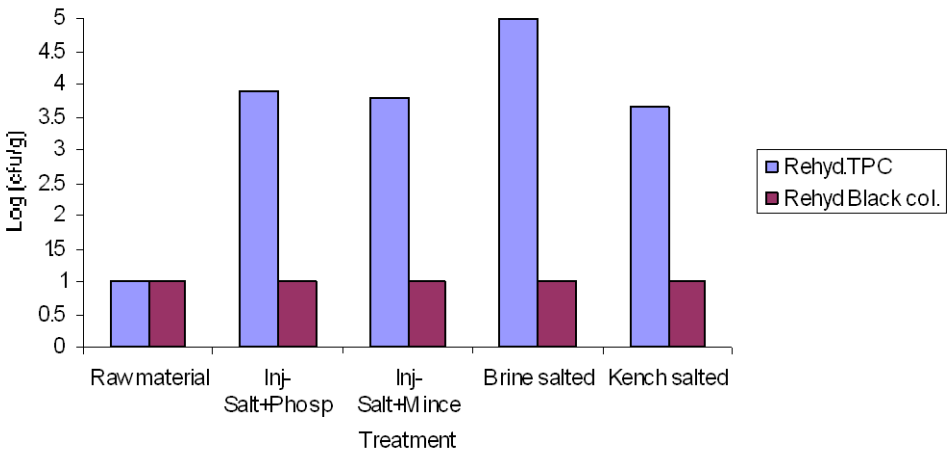

Figure 29: Microbial load in the various rehydrated products in 10% NaCl iron agar

4.5 Protein content

During the first day of the drying period, the kench and brine salted products registered the highest protein contents (about 25mg/ml) compared to the modern methods (inject-salt, + phosphate; + mince) with about 18mg/ml (Figure 30). During the drying period, all the methods registered an increasing trend in the muscle protein content as the moisture content reduced. This trend was also shown by the increase in the salt-free dry material in all the salt curing methods (Figure 19).

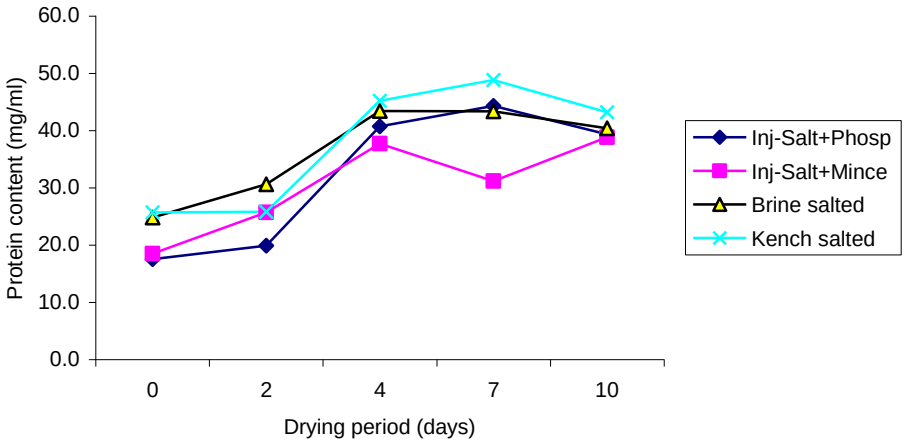

Figure 30: Changes in protein content with increasing drying period.

Figure 31 shows the protein content of the rehydrated products was greatest in the brine salt cured products followed by the kench salted products. The modern methods (inject-salt +phosphate and mince) had the least protein content by the end of the 5th week of dry salting when rehydration was conducted.

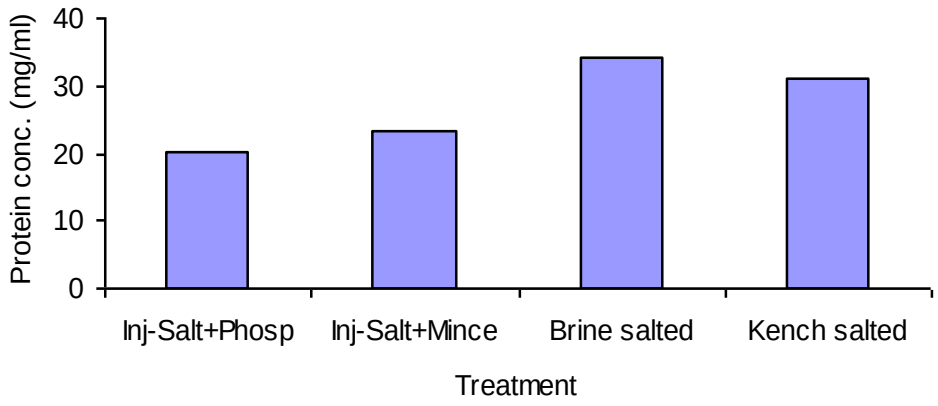

Figure 31: Protein content in the rehydrated salted products

4.6 Activity of enzymes on muscle proteins

The activity of the two proteolytic enzymes (trypsin and chymotrypsin) after salting was low and generally decreased further during the drying period (Figure 32andFigure 33). Because of the relatively low activity in these enzymes, the activities within the different salt curing methods were also very low.

Figure 34 shows the trypsin-like activity in the rehydrated products. The activity was highest in inject- salt + phosphate but almost minimal in the other three methods studied. The trend in the Chymotrypsin-like activity in the rehydrated products

(Figure 35) was almost similar to that in trypsin-like activity although small variations could be observed particularly within the individual curing methods.

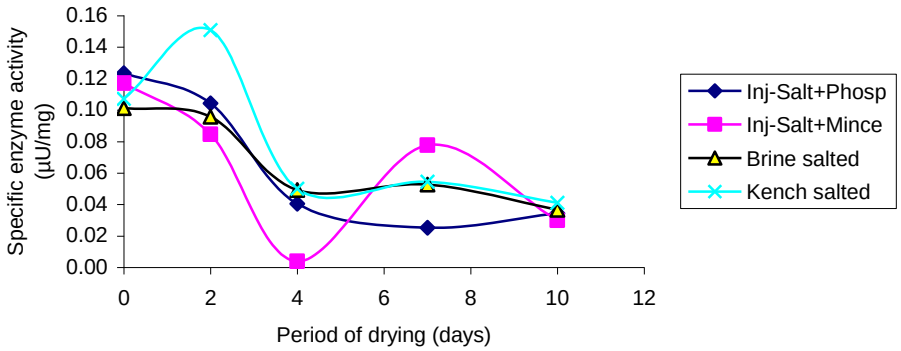

Figure 32: Variations in the specific trypsin activity with increase in the drying period.

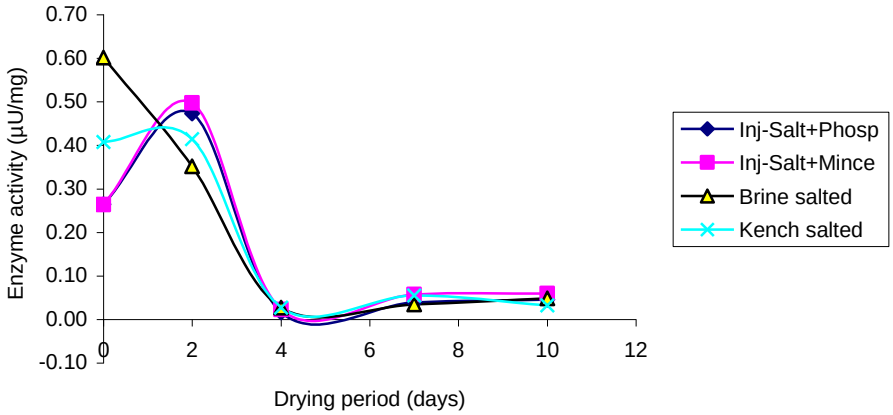

Figure 33: Variations in the specific chymotrypsin activity during the drying period.

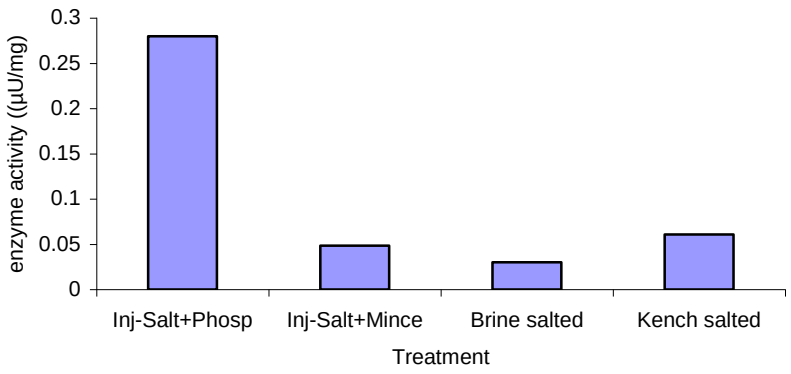

Figure 34: Specific trypsin activity in the different rehydrated products

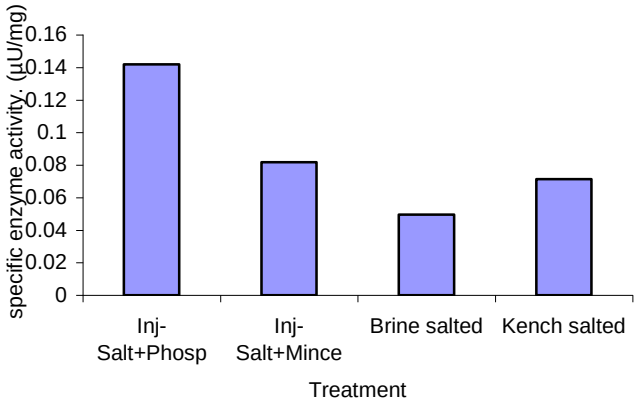

Figure 35: Specific chymotrypsin activity in the different rehydrated products

5 DISCUSSION

The significant differences noted in the yields of the different methods particularly the kench and the modern methods of injected salting could be associated with loss in the water content especially in the drying period. This suggests that as you continue the drying process after heavy salt curing, the fish muscle loses its water holding capacity, thus drying the muscle. This does not only affect the yield in terms of weight, but also affects the nutritive value of the dry salt cured products. The muscles would lose the soluble proteins and other micronutrients in the process. The defects were more pronounced in the traditional methods of kenching and brining as shown in Figure 17and Figure 24 because of the generally high dehydration during the curing and drying period. Earlier studies by the Icelandic Fisheries Laboratories (not dated) have shown that the weight yields in heavily brine salted cod increased in the first couple of days and later started declining gradually as the salt concentration increased, water reduced with increased salting time. The same was true for the lightly salted products except that the products had a better water holding capacity which kept intact relatively longer than could be observed in heavily brined products. Similar observations were made by Wheaton and Lawson (1985) when they noted an increase in the yield because of in take of some water in the brining process. Brines with small concentrations of salt also promote the yield and water holding capacity more than saturated brines of more than 25%, according to (Barat et al. 2002, Hamm 1960). Deng (1977) also noted that some fishes including cod do not only take up salt, but also lose water when salt concentrations in the brine exceed 13-15%, particularly in the early stages (Hamm 1960).

Although the yield in the modern methods was better than in kench and brine cured products, the fact that all methods had had heavy salting (concentration up to 20%), much higher than the average 5-6% for an optimum yield, may have affected the final yield in this study. In general, the water holding capacity in injected products was better compared to that of the traditional methods.

The significantly high yields in almost all the rehydrated products could be attributed to the addition of water before the drying period due probably to water re-absorption during rehydration. This suggests that most probably the salted fish reached an

optimum level of curing in as far as yield was concerned before it could further be dried. This meant that the salt curing methods at that salt composition would have been sufficient after 5 weeks of dry salting. The reason for the significantly high increase in the yield of the kenched products could be attributed to the lower water activity and high salt concentration which influenced the water uptake by the products because of the probably a larger concentration gradient between the water and the muscle. However, this observation contradicts the findings by Telis et al. (2003) when, during their study of the salting kinetics and salt diffusivities in farmed Pantanal caiman muscle, observed that as salt uptake increased and the moisture content reduced, the density of the muscle increased.

The relatively high increase in salt content (Figure 20) could be linked to the equivalent loss in moisture during this period. The differences in salt concentrations, osmotic pressures and the salting medium results in salt-water exchange (salt-uptake by the fish muscle). The relationship between changes in weight, salt and water contents is almost linear. The slight increase in the salt free dry material could be attributed to the loss of moisture in the various salted and stored products. Usually, as the moisture content reduces, the concentration of other nutrient components increases (Pigott and Tucker 1990). This observation was consistent with earlier work related to a study in changes in myofibrillar proteins during processing of salted cod as was determined by electrophoresis and differential scanning calorimetry by Thorarinsdottir et al. (2001).

The lower degree of dehydration experienced in all these products during the drying period may be attributed to the speculation of the saturation effect since the salt content of the products ranged from 19.5 to 21.4% in all the salting methods. The slight variation in water intake during rehydration of the products could be explained by the size and thickness of the fish muscle.

The variations within the microbial loads (Figures 26-29) in the different products could be attributed to the different types of microorganisms and how they thrive in the salted conditions of such intensities. The rise observed between the raw material (before splitting) and the salted products could be due to the favourable conditions for particularly the halophiles, the processing method and equipment on deck and the time between catch and processing. However, the differences between salting methods especially the modern methods of inject-salting and the traditional brining and kenching in Figure 26 and Figure 27 could be linked to the respective differences in water activities and salt concentrations although the differences could be small. At high salt concentrations in the salted fish muscle, the water activity goes down and yet it is this free water which facilitates the growth and activity of the microorganisms (Eichner and Karel 1972). The traditional methods had slightly lower water activity than the modern methods. Thus, the variations in the levels of free water (high water activity) in the modern methods favoured the growth of these microorganisms compared to the traditional methods whose free water was much lower (low aw) which could not sustain and support their growth and activity. In brine and kench salted products, the load was much lower in both NaCl concentrations (5 and 10%), possibly because of the lack of sufficient oxygen that may have been desired by the bacteria (aerobic) yet the conditions in the brine are typically anaerobic. It should be noted here that for the microorganisms whose growth and mortality rates are high, a small difference in the conditions might reflect a significant change. This may explain

why the loads in other salt curing methods were higher than that in the brine cured. Another observation was related to the black colonies (H2S producing bacteria) which reduced dramatically from the magnitudes of hundreds in 0.5% NaCl to just tens in the corresponding 10% NaCl. The cause of the decline and possible inhibition could be related to the differences in the agar. The increase could also be due to various reasons including a reduction in the concentration of salt due to rehydration, presence of anaerobic microorganisms which had initially been inhibited by the lack of suitable conditions. Rodriguez et al. (2003) conducted an investigation characterising and identifying bacteria presented in soaked cod (desalted) that had been produced from green salted and dried salted cod and the findings gave clear indications that rehydrated salted products could equally harbour a high level of microorganisms including pathogenic microflora. However, in their study on the keeping quality of desalted cod fillets, Magnusson et al. (2006) noted a rapid increase in the microbial numbers mainly in fillets stored aerobically. This means that probably the lowest microbial load would be most prevalent in the brine salted products compared to the other methods.
The findings in the protein contents (Figure 30) were also in agreement with related studies by (Thorarinsdottir et al. 2001, 2004) where quite related trends were observed with indications that the amount of protein was a function of salt concentration and water content. The increase in protein content of most of the salted products during the first 4 days could be attributed to a reduction in water content. This observation could also be related to similar findings by Alvarez and Guillen (2005) and Olsen and Skara (1997), although the latter's investigations were based on North Atlantic Sea herring (Luccia et al. 2005). The decline in the protein contents in most of the salt cured products after the 4th day could be attributed to the activity of salt concentrations and low water content within the muscle protein cells which may have led to denaturation particularly when salt concentrations were in the range of 20-21%. Alvarez and Guillen (2005) stated that water loss and high salt concentrations might be closely related to the denaturation of cod muscle proteins. Similarly, this high salt concentration may have initially caused the protein loss by the osmotic effect of the salt (Luccia et al. 2005).

Although phosphates are said to have a stabilising effect on the proteins (Thorarinsdottir et al. 2001), the protein content in this curing method was lower than that in kenched products. The dramatic changes that were also observed in the inject-salt + mince could not easily be explained.

The decline in trypsin-like activity may be associated with the already high salt concentrations in the cod muscle and probably the decreased water activity (free water). Consequently, as the free water was reduced and the salt concentration had increased during the drying period, the enzyme activity reduced drastically to quite low levels. Alternatively, according to Michaelis-Menten Kinetic model, the decline may also be assumed to be a result of high levels of protein (substrate) in the initial drying period. If the substrate increases beyond the enzyme level, the enzyme becomes saturated with the substrate (protein) and the activity of the enzyme then reaches a maximum and the reaction remains constant. This seems to be the case in this study because, when the protein content begins to decline at the later stages of drying, the enzyme activity gradually increases (Figure 32 and Figure 33). It is also worthy noting that since the enzymes are catalysts, they remain unaffected during the reaction but in this case only fix themselves to the substrates forming enzyme-

substrate complexes and when conditions change, they get transformed back to enzyme and products at the end of the reaction (Birch 2006).

The possible reason for the observed activity of the enzymes in this treatment could be linked to the suitable ionic strength of the Na+ and Cl- in the solution and the saturation effect of the salt in the muscle as water activity reduced. In kenching for instance, the salt concentration in the products was slightly higher than in the rest of the products in the other methods (Figure 20), yet the water content was equally lower. This suggests that trypsin in particular whose magnitude of activity was the lowest of the three methods works best in slightly higher ionic strengths compared to chymotrypsin, a confirmation of an observation noted by Stoknes et al. (2005). Alternatively, the other possible cause of the observed pattern of activity could be linked to the substrate saturation in the solution that may have inhibited the reaction probably due to the low amounts of the enzyme. The high concentrations of Na+ and K+ ions in the solutions, which may inhibit the activity of enzymes especially at high pH (Stoknes et al. 2005), could not be ruled out.

6 CONCLUSION AND RECOMMENDATIONS

The yield in all the salt curing methods decreased with increasing dehydration and salt concentration during the drying period. The highest reduction in the yield was in the traditional kench salting followed by brining and least in the two modern methods. The inject-salt+ phosphate would be the better method in as far as salt curing is concerned because of the high yield outcomes but for some reasons, it may not gain the deserved popularity compared to the inject-salt+ mince because of the attitude of many consumers to denounce non organic additives like phosphates which in this case enhances the colour of salted products.

The water content, water activity reduced very slowly during the 10 day drying period with the modern methods registering the highest compared to the traditional method. The reason for the slow reduction was that the products were virtually dry by the end of the fifth week since they already had a 20% salt content in the muscle particularly the modern methods. In terms of product quality, the modern methods were the better alternative despite the differences in water activity of the final product.

The protein contents also increased as the water content decreased. However, this was only true for a short while when they started declining as the moisture content reduced further, possibly due to denaturation in kenched, injected-salted methods and hydrolysis mainly in the brine salted methods.

The microbial loads in all the methods increased during the salting period most probably due to increasing salt. The rehydrated products however increased in microbial content because of the replenishment of the much desired water.

The activity of enzymes in general was too low to base the conclusions on the findings in this study although indications may be that as the water content reduces in the salt cured products, the salt concentration increases leading to saturation in the muscle which may retard the activity of the said proteolytic enzymes. This could be advantageous though, in as far as preservation of the nutrient composition of the product is concerned. No major differences in the rehydrated products were observed because of the similarity in magnitudes of the activity of both enzymes.

6.1 Recommendations

Ensure application of salt curing methods that would control as much as possible the agents of spoilage so that the salted product retains its potential to supply the nutrients in their wholesomeness to the benefit of the consumer. This will be achieved if the following points are adhered to:

Application of the salting methods that ensure faster penetration of the salting medium and facilitate an optimum salt-water exchange within the most ideal time.
The drying period should be that which should ensure a high water holding capacity but regulate the activity of halophilic microorganisms.
Although the main idea in salt curing in Uganda is to preserve the fish, the drying conditions and period should not compromise the nutrient composition.

The kench curing method mostly used during salt curing in Uganda should be regulated so that the drying conditions and period retain a relatively substantial amount of water content to ensure retention of the nutrients (proteins) for a longer time and higher yield.
Above all, yield and final quality should be taken care of while considering the best salt curing option.

ACKNOWLEDGEMENTS

In the process of compiling this document, I am deeply indebted to the following people and organisations: my supervisors, Ms. Kristin A. Thorarinsdottir and Mr. S. Arason, who despite their always busy schedules, found time to help me in the technical work. The Director, the Deputy Director and staff of the UNU-FTP for the wonderful and successful organisation of the programme, Mrs. S. Ingvarsdottir for her kindness and tireless provision of all necessities during the programme and Ms. Ragnhildur for her assistance in the laboratory and with literature material. The UNU for the Fellowship, the entire staff of the Marine Research Institute, Icelandic Fisheries Laboratories (MATIS), University of Akureyri and all the private enterprises and fish processing plants in Iceland whose cooperation in one way or another, enabled me to come up with this document. Of course the UNU-FTP fellows who were always by my side to comfort me during this endeavour and my family back in Uganda who kept encouraging me while at this noble work.

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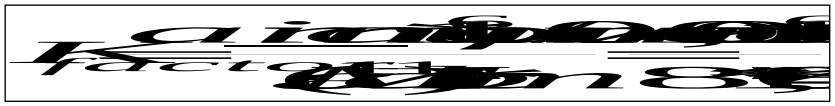
APPENDIX I:PROCEDURE FOR EXTRACTION OF SAMPLE AND ENZYME ACTIVITY ANALYSIS USING A UV-VISIBLE SPECTROPHOTOMETER.

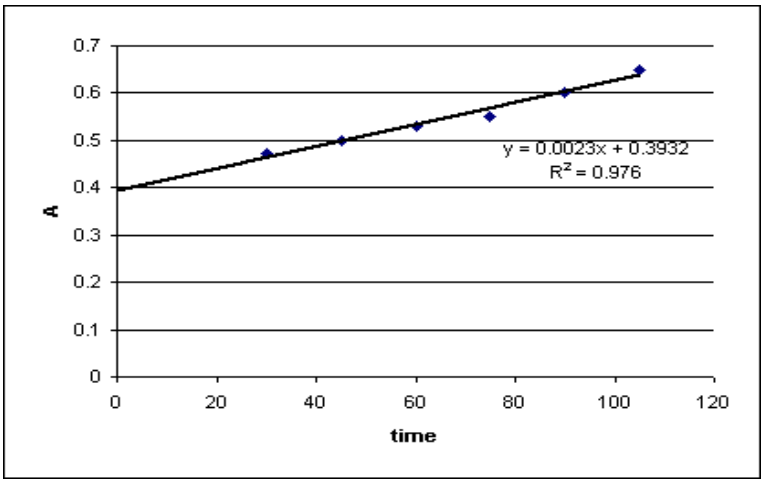
Take salted fish steak (stored at chill temperature) in a chill room.
Cut approximately 100g of the flesh from the steak using a knife.
Weigh 100g of the cut portions carefully using an analytical balance.
Blend the 100g portion in a blender to have the portion reduced to small pieces
Add 200ml of distilled water (i.e. double the weight of the sample) and mix well before transferring to a beaker.
Keep the blend into ice for about 30 minutes. Stirring in the solution every 5-10 minutes.
Weigh about 10g of the extract into each of the prepared centrifuge tubes in triplicates or duplicates and place the tubes on ice. (duplicates are enough)
As you are waiting for the 30 minutes of sample extract on the ice, switch (turn) on the centrifuge to warm and set it to run at 12,000 revolutions per minute for 5 minutes at 40C. ((It's important to do this because the centrifuge takes a long time to cool down).
Place the tubes of sample extract in the centrifuge in opposite arrangement to balance the rotation and let it run as above.
While the centrifuge is running, prepare the eppendorf tubes (1.5ml) in which the extract will be transferred and kept on ice or frozen until spectrophotometer reading for enzyme or protein analyses.
After 5 minutes, the extract should be removed from the centrifuge and carefully transferred into the measuring cylinder (100ml) avoiding solid particles. Do this for the same sample extract tubes.
Transfer the sample extract into eppendorf tubes. Cover and keep iced until reading.
Or the extract should be frozen; this is may be better because you will be able to control your time better.

Spectrophotometer reading (enzyme activity analysis)

Turn on the spectrophotometer and set it to read Absorbance at 410nm. The computer too should be turned on.
Transfer about 100ml of the buffer into a beaker.
Arrange 3 micro-pipettes, together with their respective pipette tips for use in measuring the samples and other reagents.
Also arrange the required volumes of the cuvettes (1.5ml and or 4.5ml capacity). Disposables are better.
Using a pipette, measure 495 µl of the assay buffer into 4 of the cuvettes. (One of these without the sample extract and substrate will work as a BLANK).
The blank: 990 µl buffer, 10 µl substrate.
Measure 10 µl of the substrate stock solution into each of the 3 cuvettes above. (This is done using a different pipette from that used to transfer the assay buffer).
To the 3 cuvettes above, add 495 µl of the sample extract.
Place the blank into one of the slots of the spectrophotometer.
Then start reading the other cuvettes with the sample one at a time in succession.

This should be done for about 2 hours at an interval of 20-30 minutes respectively. (20-30 min interval should be good enough to get a good slope).
The data read is then saved and imported to Microsoft excel where a curve of absorbance against time is drawn and a slope determined. The K factor is obtained using the computation below.
How to make the slope: Right click with the mouse on one of the data points in the graph, choose "add trend line", choose a linear model, then go to the flip "options" in the window and fill in the boxes "add equation on chart" and "display R value on chart". Then you have the slope and with the R-value you see how good the line fits with your data





Standard curve to demonstrate the instruction. Data is indicated in the table below

Illustration table

Read	Abs	nm			
1	0.1006	410			
2	0.1008	410			
3	0.0874	410			
4	0.1229	410			
5	0.1268	410			
6	0.1118	410			
7	0.1402	410			
8	0.1418	410			
9	0.1272	410			
11	0.1418	410			
time		A			
0	10		410		
15	12		410		
30	13	0.4708	410		
45	14	0.4994	410		
60	15	0.5297	410		
75	16	0.5507	410		
90	17	0.6007	410		
105	18	0.6501	410		
slope	0.0023				
	0.22956		0.00052		
Kfactor	8		8	U/mL	(Kfactor*slope)
			0.52800		
			7	mU/mL	

APPENDIX II:PREPARATION OF THE BUFFER (20 MM TRIS, 1 MM CACL2, PH 8,0)

Weigh 2.42 ± 0.05 g Tris base and 0.147 ± 0.002 $CaCl_2 \cdot 2 H_2O$ and place in a 1000 mL graduated cylinder. Add approximately 900 mL distilled water and dissolve.
Stir the solution, (i.e. place on a magnetic stirrer) and dip the pH electrode into the solution. Adjust the pH to 8.00 ± 0.05 with 4 M NaOH at room temperature (about 21°C).
Fill to 1000 mL ± 10 mL with distilled water. Mix the solution well for approximately 15-20 minutes to thermally equilibrate, recheck the pH and readjust if necessary.
Pour the freshly made solution into containers labeled with “20 mM Tris, 1 mM $CaCl_2$, pH 8.0”, the date of preparation, the expiry date, and the initials of the maker.
Store in the refrigerator at 2 to 8°C for up to 6 months.

APPENDIX III: PROCEDURE FOR MEASURING WATER ACTIVITY USING (NOVASINA IC-500 AW-LAB.)

The sample (4g) to be measured is placed in a shallow plastic dish (in triplicates).
(Make sure the plastic dish is wiped dry so that no water contamination occurs).
The plastic dish together with the sample is placed into another metallic trough
Carefully, the metallic trough and contents is fitted into the water activity machine such that it fits well or grips well on to the handle.
To start analyzing, hold down the start button until an orange blinking light comes on, on the analyzing button. This means that it has started analyzing.
The analysis will be over when the green light appears on the OK button.
Read off the water activity value (usually which takes place about 30 minutes).
Having done that, then remove the finished sample, empty the dish, place another sample and start again. It is important to first calibrate the equipment and set the temperature to read 25degrees Celsius.

APPPENDIX IV: PROCEDURE FOR MEASURING PROTEINS IN THE SAMPLES

Mark eppendorf glasses with the name of the sample and dilution.
Make dilutions as per instructions below: (Make dilutions of x20, and x50)
Dilute the samples (see table), then follow the instructions below the table.
The dilution should have an absorbance that is on the standard curve. If the dilution is higher or lower, the step needs to be done again, with a different dilution.

dilution	ratio	sample µL	water µL
x10	1:9	100	900
x20	1:19	50	950
x50	1:49	20	980
x100	1:99	10	990

Transfer 100micro-litre of the diluted sample into test tubes (triplicates)
Using a 5ml pipette, transfer 5 ml of a prepared Dye (Bio-Rad Protein Assay) to each of the test tubes and vortex to mix and wait for 10-15 minutes.
Then transfer to the 4.5ml cuvettes for the spectrophotometric reading. Reading should be at an absorbance of 595 nm.
Make an extra test tube into which 100 micro-litres of water are added together with 5ml of the dye only. Do not add the sample and the standard to this test tube. This will act as the blank for all the readings.
The above should be done having put on the spectrophotometer and the computer.
To save the work appropriately to end with the extension of file (rtf).
You can then import the file (through) excel to the working computer for Computations.