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EFFECT OF CHILLED TEMPERATURE AND SALT CONCENTRATION ON SHELF LIFE OF HERRING *(CLUPEA HARENGUS)*

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ABSTRACT

Keeping the quality of fish and fish products at its best is the most important issue in fish processing. Based on the trend demanding that salted fish reduce salt content for dietary reasons as well as for further processing, this project focused on the extension of shelf life by investigating quality changes during brining in the cold storage of herring (*Clupea harengus*), the one of the popular foods in DPR Korea. Instead of the traditional salting method, an innovative method has been suggested called cold brining. Cold brining is a processing method expected either to provide a good salty flavour or to extend shelf life for further products by delaying microbiological growth and the chemical changes of the co-operation of ice and salt. In both experiments using fresh whole herring and fillets, five different brine concentrations of 8, 12, 14, 16 and 18%, and five different temperatures of 2, -1, -2, -4, -8 and -24 °C were used for 25 and 18 days, respectively, to select the optimum conditions for cold brining. The results were revealed that the 14% brine at -8 °C can be regarded as optimum condition for pre-cooling of herring. The cooling time will take about 20 minutes in the brine, in which the ratio of fish to brine is 1:2. The cold brining of herring should be done with a mixture of salt and brine. The amount of salt is 10% to the weight of raw fish and the ratio of fish to brine is 1: 0.2.
Keywords: herring (Clupea harengus), salting, shelf life

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1 INTRODUCTION

In the Democratic People`s Republic of Korea (DPRK) seafood is regarded as a very important food resource not only for the satisfaction of protein demands but also for the food security and the improvement of people`s living standards, as arable land areas are extremely limited comprising only 15% of DPRK territory.

DPRK is a maritime country which has sea both on the east and west side. The mixing of the cold and warm water currents of the ocean and the enrichment of nutrients from rivers and streams create favourable conditions for developing marine fisheries in these waters. The Korean East Sea, which has a surface area of about 970,000km^2, has both cold and warm water species totalling approximately 650 species. The cold-water species of commercial importance caught in the DPRK water area include Alaskan pollack, herring, flounder and silverfish. The warm water species include sardines, anchovies, mackerel and half beak (FAO 1998).

At present, the importance of product quality in seafood processing has been emphasized reflected in the high domestic demands for good quality and the trends of modernization in the processing industry. Both along the east and west coasts, modern fish processing plants have been built or are being built with the support of the government.

Keeping the quality of fish and fish products at its best is the most important issue in fish processing. The deterioration of fish is caused by the actions of microorganism and enzymes. Maintaining the freshness of fish is thus to some extent done by controlling or minimizing their actions, by the principle of cold storage. Proper handling, quick cooling and storage of fish on board ensure the quality of the fish. In addition, proper processing types and methods based on capacity and demand are also the key factors for real benefits.

The fatty fish species such as herring (*Clupea harengus pallasi)* and sardine **(***Sardinops melanostictus)* are very popular and important for domestic consumption because of the good nutritional properties of polyunsaturated fatty acids. Products are fresh, frozen, salted, dried and canned fish. Herring containing 12% of fat in muscle is used for making canned products after chilled in refrigerated sea water (RSW) and the spiced canned herring is also popular. The salted herring has become one of the most popular traditional foods and is in high demand. But the fatty fish tends to be more susceptible to spoilage compared to other species because most of them are small and are caught in large quantities, rarely eviscerated immediately on capture and often chilled inadequately. The polyunsaturated fat is easily spoiled (becomes rancid). Delayed cooling, with environmental temperatures between 15 °C and 20 °C, will shorten the shelf life by several days (Burt and Hardy 1992). Under the unfavourable conditions autolysis enzymes in the muscles bring about hydrolysis of protein and lipid as well as other chemical changes that influence the flavour in the post mortem state and the quality of fish is reduced very quickly.

Fatty fish catches sustain great post harvest quality losses with insufficient cooling and non-optimal storage on board. So the fishermen are trying to bring enough ice on board for the fishing trip but the amount of ice for fishing trips is commonly insufficient due to the low capacity of the old cooling system on landing sites. To overcome this disadvantage the fishing boats which do not have sufficient ice for fishing trips select group fishing. During the fishing season, fishing groups organized by five or seven boats approach the fishing grounds together and one or two boats of the group return to the landing site by collecting all the group products to reduce the storage time on board. The catches, in most cases, are put into sea water to prevent decomposition before they are landed.

The principle of salting and storage is also to control or minimize the fish deterioration by killing or reducing the growth of microorganisms in the fish. The high concentration of salt has been shown to prevent microbial spoilage in similar products (Andersen *et al*. 2007). As

mentioned above, salting herring has been popular and practiced for centuries. The primary purpose of salting is preservation but during salting the fish are transformed into a salted well appreciated delicacy. A well ripened salted herring has a soft, tender consistency and a pleasant taste and odour (Gudmundsdottir and Stefansson 1997). The methods are traditional and largely based on experience. The industrial salting methods regarded as suitable for fatty fish like herring are dry salting and brine salting with saturated salt water.
Brining of herring has two functions: first, to give the product the desired taste and second, to delay spoilage (Bohdan *et al*. 1986). There are several methods in salting, among them the brine salting with saturated salt water which is also one of the most commonly used ways. Definition of salted products has been clarified by salt content in fish muscle. The definition of salted products has been clarified by salt content in muscle in Table 1 (Jong 2007).

Table 1: Classification of salted fish products by salt content in flesh

	Heavy	**Medium**	**Light**
Salt content in muscle (%)	>20	15-20	10-15

Adequate storage techniques that efficiently cool the fish material before salting could reduce post-capture losses. Today, the trend in domestic demands in salted fish is moving away from the traditional processing towards the use of less salt for dietary reasons as well as for further processing like marinating or drying. Based on high demands of lightly salted products and the common capacities of present cold storage in the DPRK, the cold brining is recommended as a better way. The following is a brief description of the methods of which are going to be recommended for cold brining:

- Preparing the cold brine.
- Immersing the herring in the cold brine to rapidly lower the body temperature to 1 °C ~ 3 °C.
- After removing some brine adding salt at 10% of fish weight.
- Storage this in the cold storage at around -10 °C.

Compared with previous salting methods, this method could be expected to have many advantages such as the prevention of protein decomposition and deterioration, increasing yield, saving salt, preserving good taste and the hygiene of the products as well as the optimum usage of current cooling capacity. Therefore, the selection of conditions for effective pre-cooling and storage in cold brining is the key factor needed to increase the shelf life and to improve the quality of salted products in brining.
The aim of this project is to study the quality changes in Atlantic herring during brining and cold storage.

2 LITERATURE REVIEW

2.1 Background

The demand for high quality fish is generally increasing in the world. This has put pressure on fishermen to fish more and maximise the quality of the product (Burt and Hardy 1992).
Herring is an important commercial fish (Stroud 2001). Total world catch of herring in 2006 was 2.6 million tons (FAO 2006). A large proportion of the herring catch has traditionally been reduced to meal and oil. However, it is used mostly as a food supplement for animals

like pigs, chickens and salmon. There has been a trend to increase the proportion of herring for human consumption: from 57-64% in 1991-1993, to 74-75% in 1994-1996, and 82-86% in recent years of the total catch in the Northeast Atlantic (Herring network 2003).
Herring is also a common fish species in the DPRK (Hong 2007). This fat fish species is used for producing many delicacy products such as being salted, kippers, marinated and canned in oil etc. (Stroud 2001).

2.2 Characteristics and chemical composition of herring

Herring is a small, oily fish of the genus *Clupea* found in the shallow, temperate waters of the North Atlantic, the Baltic Sea, the North Pacific, and the Mediterranean. There are 15 species of herring, the most abundant of which is the Atlantic herring. They move in vast schools, appearing in spring at the shores of Europe and America, where they are caught, salted and smoked in great quantities.
In the Pacific, herring migrates along the cold current of about 2 °C - 10 °C within the depth of 0 m – 150 m of the shallow waters of the Northwest Pacific Ocean including the Korean East and West Seas and south to Japan. The average body length of an adult is close to 30 centimetres. The fish interior is quite bony and fatty. Frozen and salted herring have been very popular in the domestic market of DRSK while being exported for industrial use (Hong 2007).

Figure 1 shows the weight of a herring in relation to its length (Stroud 2001).

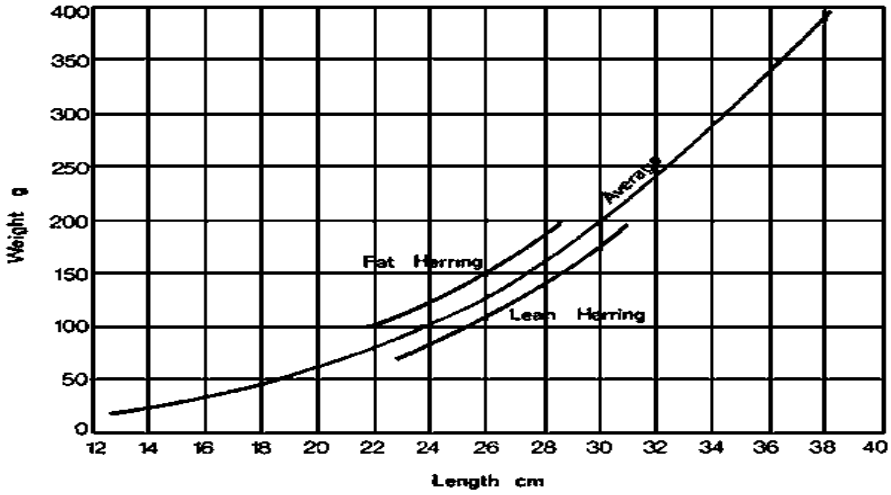

Figure 1: Length and weight of un-gutted herring

The chemical composition of herring varies considerably with the season and the breeding cycle. The fat content of herring may be less than 1% (right after spawning), or more than 20% (before spawning season). Table 2 shows the water, fat and protein contents of herring (Burt and Hardy 1992, Stroud 2001).

Table 2: Chemical composition of herring

	Water %	**Fat %**	**Protein %**
Whole herring	60-81	1-24	17-21
Herring	57-79	0.8-24.9	14-17

The chemical composition of skinned herring fillets caught southeast of Iceland in September 2001 was 68% water, 18.1% protein, 12.4% lipids, 0.2% salt, and 1.4% ashes (Geirsdottir *et al.* 2007)

There is a correlation between the water content and fat or protein content of herring (Figure 2 and Figure 3). Therefore it is possible to estimate the fat and protein content of the fish based on its water content (Stroud 2001).

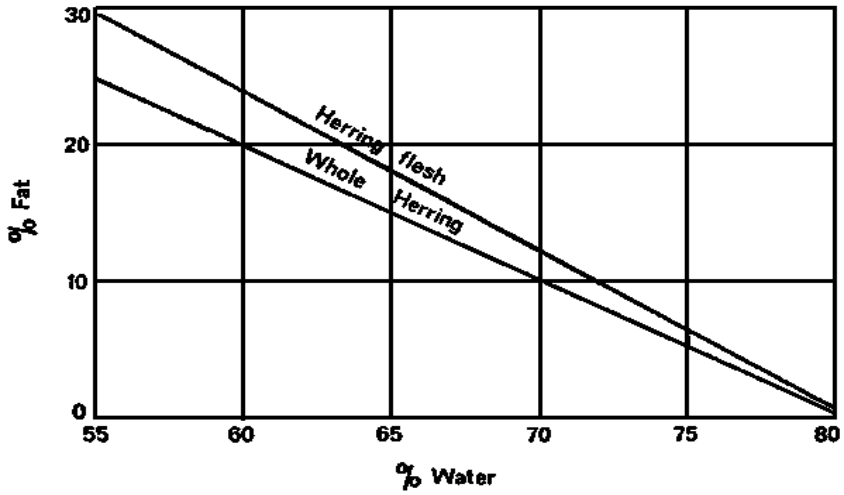

Figure 2: Fat content of herring in relation to its water content

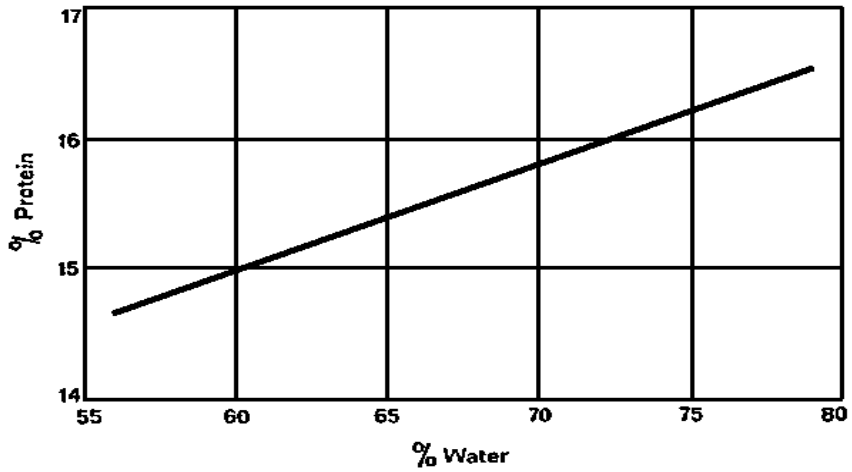

Figure 3: Protein content of herring in relation to its water content

In herring, the fat is mainly in the flesh. Raw flesh of a moderately fat herring, containing 11% fat, has an energy value of about 7.4 kJ/g (Stroud 2001). Herring lipid is rich in n-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) which have positive effects against cardiovascular disease, cancer, etc. (Burt and Hardy 1992, Underland 1998). Herring is also rich in minerals (e.g. iron, calcium and iodine) and vitamins (Stroud 2001), as indicated in Table 3.

Table 3: Vitamins in herring mg/kg

A	D	B vitamins						
		Thiamine	Riboflavin	Niacin	B6	B12	Pantothenic acid	Biotin
6-120	7-25	0.1-1.3	0.9-3.3	20-63	3.5-4.2	0.08-0.14	9.3-9.7	0.09-0.16

2.3 Handling and storage methods from catch to processing

2.3.1 Handling on board

Care in handling is important because unnecessary damage of the fish can provide access through cuts and wounds for spoilage bacteria (Graham *et al.* 1992).
Storage time depends on the fat content of the fish and the amount of food in the gut. Herring with high fat content will keep for a shorter time than herring with low fat content (Stroud 2001). Shelf life in ice for fat (summer herring) and low fat (winter herring) fish is 2-6 and 7-12 days, respectively (Huss 1995).
The traditional method of chilling herring on board is in ice. The fish is stowed in boxes with layers of ice above, below, and between the fish. The ratio of ice to fish should be about 1:3 in summer. If the catch is of large quantity, herring is sometimes stowed in bulk in the fish-room with or without ice. Sometimes the fish is not iced adequately, and some of the fish might be damaged in deep bulk stowage (Stroud 2001).
There are other ways of chilling besides using ice. Fish can be immersed in chilled water or can be exposed to cold blowing air. Seawater, cooled mechanically (RSW-refrigerated seawater) or by the addition of ice (CSW-chilled seawater) is a suitable alternative to rapidly chill large quantities of small pelagic fish (Graham *et al*. 1992).
Most common chilling methods used at sea are:

I) Icing the fish (flake ice or tube ice)
II) Fish stored in liquid ice
III) Refrigerated seawater (RSW)
IV) Chilled seawater (CSW)

An alternative method of chilling is stowage in fixed tanks with refrigerated seawater (RSW) or portable tanks containing ice and seawater (CSW). Herring stored in tanks keeps as well or even better than in ice for the first 3-4 days, but then it starts to spoil more quickly (Stroud 2001, Kelman 2001).
Ice made from fresh water has played a major role in the chilling of fish on board. The amount of ice required depends on the amount of fish to be chilled. Fish that is completely surrounded by melting freshwater ice will be chilled to about 0 °C. Fish temperature slightly below 0°C can be achieved by adding salt to the water, lowering the melting point of the ice. Using ice alone, however, the fish temperature cannot be reduced to the point at which freezing begins, between –1 to –2 °C. Each fish should be in constant contact with melting ice so that fish temperature is reduced as quickly as possible and maintained as low as possible. The melting rate of ice is higher when the fish are warmer or in warmer climates (Graham *et al.* 1992).
When refrigerated seawater (RSW) is used, a mechanical refrigeration unit cools the water from seawater temperature down to below 0 °C. The main advantages of using RSW has been the greater speed of cooling, reduced pressure on the fish, low holding temperatures and it is suitable for large quantities of fish. The RSW system has been used for sardine, salmon, halibut, menhaden, shrimp, mackerel, herring, blue whiting and capelin (Graham *et al*. 1992).

The advantages of chilled sea water (CSW) are that the catch can be cooled rapidly, the process of loading and unloading is easy and less affected by handling (being crushed and bruised). Other advantages are effective washing and a tendency to firm the fish, which can aid further processing (Kelman 2001).

2.3.2 Handling at landing site

For long lorry journeys, the fish should be well iced. The ratio of ice to fish should be about 1:3 for long trips in warm weather, especially if the lorry is not insulated. A mixture of ice and salt is sometimes used to lower the temperature of the fish and thus reduce spoilage during long journeys. This treatment is called "klondyking", and is also used for transhipments consigned by sea on carrier vessels to land (Stroud 2001).
At a low uniform temperature and reduction in available oxygen, the development of oxidation rancidity is retarded. Textural deterioration is also retarded.
Temperature fluctuations from -28 °C to -10 °C produced a significant increase in peroxide value and free fatty acids following a prolonged storage period. No difference was noted in the peroxide value of whole herring stored before freezing in chilled sea water agitated either by air or nitrogen. The use of carbon dioxide for extending the storage time of whole herring in chilled sea water has the potential danger of accelerating the oxidative changes during frozen storage (Bilinski 1981).
Time is also an important factor in reducing the deterioration of raw material. The growth rate of micro flora and the activity of enzymes are affected by temperature. As temperature rises more microbial activity is observed (Burt and Hardy 1992).

2.4 Quality changes due to spoilage-relevant factors and variables

2.4.1 Microbiological spoilage

The leading role of microorganisms in marine fish spoilage is well known. Bacteria degrades fish constituents, particularly non-protein nitrogen compounds, thus inducing the development of off-odours and flavours typically associated with fish spoilage (Ababouch *et al*. 1991).
Under favourable conditions bacteria grow rapidly, utilising non-protein nitrogenous compounds such as free amino acids, volatile nitrogen bases, ammonia, trimethylamine, creatine, betaines, and uric acid (Jay 1986). Utilisation of these substances normally leads to the production of a slightly alkaline condition especially in stored fish products (Liston 1980). Histamine-producing bacteria decarboxylate histidine develop during temperature abuse of stored raw fish. Scombroid poisoning in humans occurs from ingesting fish containing toxic histamine levels. The Food and Drug Administration (FDA) has established toxic level of histamine in tuna at 500 ppm (FDA 1995). Additionally, an action level has been set as a measure of decomposition and is a decrease from the 200 ppm limit enforced previously. The FDA's Fish and Fisheries Products Hazards and Controls Guide have included herring in the group of fish capable of containing scombrotoxin (FDA 1998). Fermented Atlantic herring products have caused human illness in Europe after consumption of fish stored at abusive temperatures (Taylor 1988). The amount of histamine in herring can vary depending on the product form and storage conditions. Pacific herring (*Clupea harengus pallasi*) dried for 3-4 days at 15-20 °C resulted in low biogenic amine production with histamine below 5 ppm (Suzuki *et al*. 1994). Baltic herring (C. h. harengus) stored on ice for 12 days contained less than 20 ppm histamine (Kolakowska *et al*. 1992). When stored at room temperature for 24

and 48 hours, Baltic herring contained 80 and 239 ppm histamine (Gajewska and Ganowiak 1992).
Some bacteria are active spoilers like some strains of the genera Alteromonas, Shewenella and Pseudomonas. Accumulation of metabolic products of bacteria are the primary causes of the organoleptic spoilage in raw fish, producing the characteristic fishery ammonia and sulphide odours, and changing texture to the slimy and pulpy characteristics of spoiled fish. Trimethylamine oxide (TMA-O) usually present in marine fish is typically reduced to trimethylamine (TMA) by spoilage bacteria even at low temperatures. It produces the characteristic fishy smell of spoiled fish. Increased storage temperature results in faster spoilage. As the temperature rises above 0 °C, different groups of bacteria are able to grow. Above 5 °C the Gram-positive flora will become increasingly important. However, different strains of the same species will have different metabolic rates at the same temperature (Gormley 1990).
Halophilic bacteria are adapted to saline conditions and require a level of 2% - 8% sodium chloride to grow. Halophilic bacteria occur naturally in the outer layer of the skin, on the gills and intestines of marine fish. They usually use protein and amino acids for their growth (Prescott *et al*. 1996). Spoilage bacteria grow even at low temperatures in refrigerated seawater (RSW) systems in the presence of NaCl because they are adapted to the environment where the fish is caught (Silva *et al*. 1998).

2.4.2 Chemical spoilage

Herring fillets are rich in polyunsaturated fatty acids, which are very susceptible to peroxidation. In addition, herring contains a high level of catalytic haeme-proteins and has a relatively low post mortem muscle pH. Low pH activates haeme as a catalyst of lipid oxidation and thus the combination of highly unsaturated fat and active catalysts makes herring very susceptible for lipid oxidation (Asbjorn *et al.* 2007).
A major reason behind quality problems arising during post harvest handling of herring is the high content of compounds that efficiently catalyse the development of rancidity (Underland and Lingnert 1999), pigmentation, texture changes and a loss of nutritional value (Bosund and Ganrot 1970).
In fatty fish, such as herring, the most important changes are taking place in the lipid fraction and they are oxidative processes of a purely chemical nature. These changes may give rise to serious quality problems such as rancid flavours and odours as well as discoloration. Two types of rancidity are found, auto-oxidation and lipid autolysis. Auto oxidation is a reaction involving oxygen and unsaturated lipid which is accelerated by heat and light (especially UV). Lipid autolysis is an enzymatic hydrolysis with free fatty acid and glycerol as major products (Huss 1988). The release of the fatty acids and breakdown of sulphur-containing ammonium acids to methyl mercantan, dimethlsulphide and hydrogen sulphite contributes to the characteristic smell of spoiled fish (Gram and Huss 1996). Reduction of the peptide to ammonia gives off the ammonia and sulphate odours. In most cases, small and medium sized fatty pelagic fish such as herring, sardine and mackerel are caught in large numbers. They are not eviscerated immediately after catch which gives rise to a problem due to acceleration of rancidity (Huss 1988).
The concentrations of the various lipid classes are initially 2 - 5 times as high in dark muscle as in white muscle. Storage for up to 12 weeks at -15 °C resulted in an increase of the free fatty acid content from 50 to 1000 mg/ 100 g in the dark muscle, and from 17 to 280 mg/ 100 g in white muscle (Ingmar 1969).

2.4.3 Autolytic spoilage

The naturally occurring enzymes remain active after the fish dies, and cause self-digestion especially in small fatty fish. The rate of self-digestion by enzymes depends on temperature, the time of the year and species. The enzyme activities and other related reactions do not immediately cease in the fish muscle upon death (Howgate 1982). Due to self-digestion the belly wall becomes weak (Johnston *et al*. 1994).
Autolysis in combination with rough handling can result in belly bursting which is dependent on storage time and temperature. Unless properly cooled, herring caught in summer can become unsuitable for smoking in 1 day (Hansen *et al*. 1980, Hansen 1983).
Fish spoilage can be caused by nucleotide catabolites from autolytic changes. The first autolytic process in the fish muscles involves carbohydrates and nucleotides. Following this process rigor mortis sets in, which is a basis for further autolytic spoilage. In ungutted fish particular digestive proteolytic enzymes are involved in the autolytic spoilage. The concentration and activity of digestive enzymes are high in the gut and upon death the enzymes soon begin to digest the gut walls and surrounding tissues (Howgate 1982).
If caught during a period of heavy feeding the belly of certain fish (e.g. herring, capelin, sprats and mackerel) is very susceptible to tissue degradation and may burst within a few hours of catching (Huss 1988).

2.4.4 The relationship between spoilage and temperature

The rate of self-digestion by enzymes depends on temperature, the time of the year and species (Johnston *et al*. 1994).
There are three important ways of preserving the freshness of fish: cooling, good hygienic practices and good handling (Graham *et al*. 1992).
By cooling the fish to around 0 °C, some of the bacteria groups responsible for the spoilage will stop growing. Therefore, cooling the fish to around 0 °C will reduce the rate of spoilage (Burt and Hardy 1992). Table 4 shows the increase of microbial flora on whole Pacific herring (*Clupea harengus pallasii*) during storage at 10 °C (Charles and Brian 1998).

Table 4: Aerobic plate count (APC) of whole Pacific herring stored at 10 °C

Genus	**Day 0**	**Day 4**	**Day 10**	**Day 14**
Enterobacter	5	0	0	0
Enterococcus	10	15	35	45
Lactobacillus	45	50	50	45
Providencia	0	0	10	10
Psedomonas	10	35	5	0
Serratia	5	0	0	0
Yeast	25	0	0	0
APC	2.3×10^3	1.4×10^5	1.1×10^7	2.7×10^7

2.4.5 Methods for preventing spoilage

A number of operations can be used to reduce spoilage rates. Proper handling and chilling of catches on board under anoxic conditions (vacuum packed or modified atmosphere packed) can prevent chemical spoilage or the development of rancidity. The effect of hygiene in the

control of spoilage varies depending on the type of contamination, which may take place. Great effort to reduce the general contamination during handling of the catch on board does not lead to any significant delay in spoilage as only a very small part of this general contamination is made of specific spoilage bacteria.
The fish at higher ambient temperatures has to be processed sooner than the fish kept in a cool environment. The easiest and the best way of doing this are to use plenty of cooling media (Burt and Hardy 1992).

2.5 Effects of salt and key factors

Enzyme action can be reduced and controlled to some extent by other methods than cooling, such as salting, drying and marinating (Johnston *et al*. 1994).
The amount of salt penetration (NaCl) into the fish muscle depends mainly on size and species of fish (Graham *et al*. 1992).
The effect of the addition of different kinds and amounts of salts on the thermal behaviour of proteins has been widely studied. The results obtained to date show that meat proteins such as myosin and actin were destabilised by increasing salt concentrations. The addition of salt in the range between 1 and 4% lowered the enthalpies and denaturation temperatures in actin and myosin of chicken and fish. The thermal stability of collagen was reduced at low salt concentrations (lower than 0.3 M) but at higher levels (0.3–2.0 M) Tmax (peak maximum temperature) was increased, indicating stabilisation (Schubring 1999).
The preservative effect of salt has been recognised according to a decrease in water activity, less availability to microbial attack, and enhancement of functional properties, leading to an increase of the shelf-life time (Santiago and Maurizio 2002).
The salt in the solvent can kill or control the microorganisms by forming high osmotic pressure and causing plasmolysis. The osmotic pressure is proportional to the difference in salt content between the brine and the cell of microorganisms. Table 5 shows the relationship between the salt content of brine and water activity and table 6 shows the relationship between the purity degree of salt and fish salt contents during salting (Jong 2007).

Table 5: Salt concentration and water activity

Salt content (%)	**0.9**	**1.7**	**3.3**	**7.0**	**10.0**	**13.0**	**16.0**	**19.0**	**22.0**
Water activity (A_w)	0.995	0.990	0.980	0.960	0.940	0.920	0.900	0.880	0.860

Table 6: Influences of purity degree of salt on salt content of fish

Purity degree of salt	**Fish salt content (%) during salting (day)**			
	Day 1	Day 4	Day 7	Day 10
NaCl 100% (purified salt)	9.8	18.0	19.7	22.4
NaCl 99% + $CaCl_2$ 1%	2.5	7.9	14.1	14.4
NaCl 99% + $MgCl_2$ 1%	6.5	15.7	18.7	19.0
NaCl 95.4% + $MgCl_2$ 4.6%	5.9	12.7	17.1	18.0
NaCl 90% + $NaSO_4$ 10%	7.1	10.5	15.3	17.1

Rate of salt uptake at 10 °C was statistically different (higher) from brining at 0 °C, but the difference in salt uptake by the herring was exceedingly small. Delayed salting appeared not to have an effect on salt uptake (Bohdan *et al*. 1986)
Many factors are believed to affect the quality of the final product, including the condition of the raw material, the type, quality and concentration of salt as well as the salting method. When the fish is surrounded with brine the rate of salt penetration into the fish muscle is higher than that obtained by dry salting. It is also believed to render both better quality and higher weight yield. Table 7 shows the chemical composition of salt from Torrevieja and Almeria, Spain (Thorarinsdottir *et al*. 2004).

Table 7: Chemical composition of salt from Torrevieja and Almeria, Spain (SIF)

Factor analysed	**Torrevieja**	**Almeria**
Water (%)	2.5-1.8	3.0
NaCl (%)	97.3-97.7	95.7
$CaSO_4$ (%)	0.15-0.32	0.44
$MgSO_4$ (%)	0.00-0.10	0.31
$MgCl_2$ (%)	0.00-0.12	0.31
$NaSO_4$ (%)	0	0
Matter insoluble in water (%)	0.10	0.01
Iron (mg/kg)	12-15	4.8
Copper (mg/kg)	<0.01	0.02

2.6 Commonly used salting methods of herring

The salting of herring has been practiced for centuries. Barrel salted herring is an important product in the Nordic fisheries industry and the manufacturing process is bound by tradition, based on human knowledge and experience (Voskrensensky 1965).
The traditional production of salted herring in Northern Europe is a process that takes several months. Briefly, whole herrings are placed in barrels with salt and 24 h after a natural brine (or blood brine) is formed. Subsequently, the barrels are filled up with saturated brine and stored for up to 12 months at chilling temperature and the product is allowed to ripen. The blood brine and brine are believed to be important for the development of the characteristic organoleptic properties of salted fish during ripening (Andersen *et al*. 2007).
The term “marinades“ or “marinated fish“ is used to define fish products which consist of fresh, frozen or salted fish or portions of fish processed by treatment with an edible organic acid, usually acetic acid, and salt and put into brines, sauces, or oil (Meyer 1965).
Whereas salting in former days was carried out on fresh material to avoid spoilage, today frozen herring is increasingly used as raw material for further processed and salted products. The main salted herring products these days are marinated products. The use of brining is for an intermediate step in marinating, smoking, and canning of herring. Commonly the saturated brine (26.5%) was used for salting of skin-on or skin-off herring fillets and stored at ambient air temperatures of 3.3 ± 2.0 °C or 17.7 ± 0.7 °C for 7 days (Sveinung *et al*. 2005)
The whole herring was used to prepare different styles of salted herring with different salt: fish ratios, i.e. 1:4 (heavily salted), 1:7 (moderately salted) and 1:10 (lightly salted). Salting was performed by a two-stage process. In the first step, the herring was dry salted, i.e. mixed with salt in a tub, then packed in the traditional way, by hand, into barrels and allowed to stand at ambient temperature until brine was formed (about 24 hours). Then the barrels were

filled completely with herring, and brine of different salt concentrations depending on the salt: fish ratio used until the barrels were well filled, and then the barrels were closed. After frequent rolling for better homogeneity of the salt distribution, the barrels were stored at a constant temperature of 4 °C (Reinhard and Jorg 1997).
The herring was salted in a traditional method by a herring manufacturing company. One hundred kilograms of whole-headed herring was mixed with 10 kg of salt. After 1 day the barrel was filled with saturated brine and stored at 0 - 5 °C (Vibeke *et al*. 2004).
In Iceland the industrial practice is to salt partially gutted fish. The industry has shown interest in salting fillets instead of partially gutted herring as that would be more economical (Gudmundsdottir and Stefansson 1997).
Maatjes herring is a lightly salted and fermented ready-to-eat fish product that is very popular in the Netherlands, being characterized by a distinct level of sub-cutaneous fat of 16-20%. After being caught, the herring is partly gutted and cured. The remaining intestines produce enzymes, which stimulate a fermentation process resulting in the typical maatjes product characteristics. After brining, the fish undergoes a ripening period of up to one day and it is then vacuum-packaged and stored frozen until further use. The product is thawed, filleted and sold unpacked or packaged under modified-atmosphere (MAP) and stored at chilled temperatures (Ulrike and Rian 2005).

3 MATERIALS AND METHODS

3.1 Design of Experiments

3.1.1 First experiment

Experiment 1 was conducted to get some quality parameters to compare the differences between fillet and whole herring in various types of brine and temperature (figure 4).

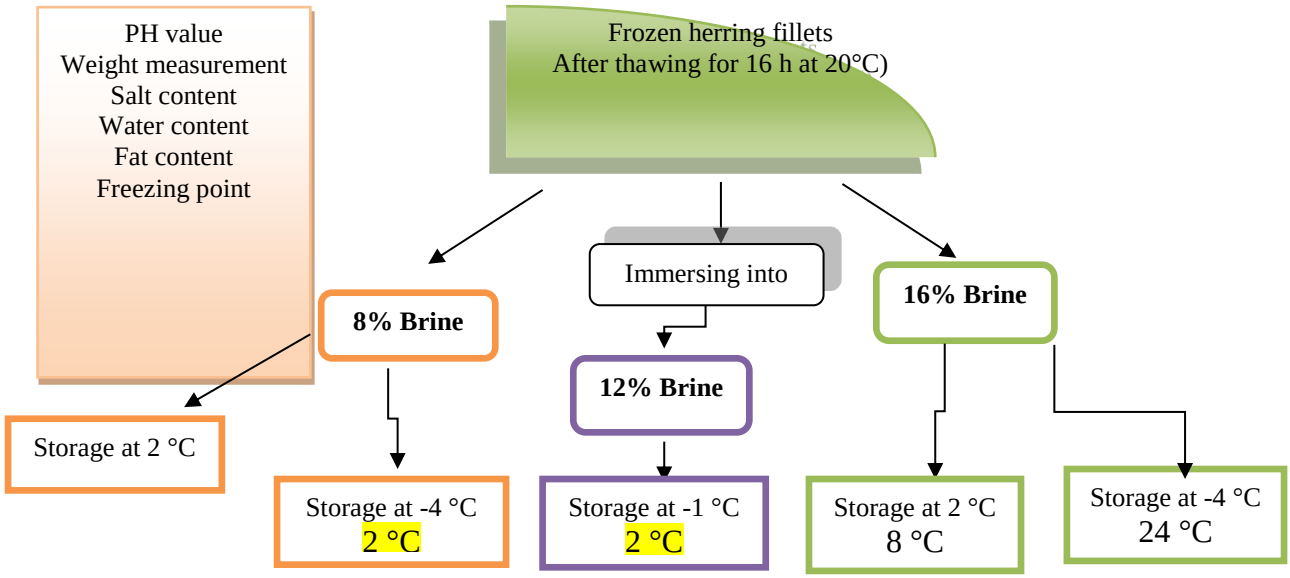

Figure 4: Flow chart of first experiment

- Fish material

The herring used for the first experiment was caught in Breidafjordur, South-west of Iceland on 19 November 2008 by purse-seine. The catch had been stored in slurry ice on board until it was landed on 22 November 2008. After landing, the raw materials were beheaded, gutted, skinned and filleted and kept in block ice. A total of 26 kg of herring fillet was used and immersed in 52 kg of different brines in each of the three cooling systems. All the samples were divided into five groups; the first group was immersed in 12% brine at -1 °C cooling chamber, and the other four remaining groups were immersed in 8% and 16% at the cooling chambers of 2 °C and -4 °C, respectively (Table 8). The ratio of fish to brine was 1: 2.

Table 8: Preparation of brines in Experiment 1

Bucket No.	Block	Salt content (%)	Temperature (°C)	Ratio (fish: brine)
5	Block 1	16.00	-4.00	1 : 2
1	Block 1	16.00	-4.00	1 : 2
12	Block 1	16.00	-4.00	1 : 2
9	Block 1	16.00	2.00	1 : 2
2	Block 1	16.00	2.00	1 : 2
6	Block 1	16.00	2.00	1 : 2
13	Block 1	8.00	-4.00	1 : 2
10	Block 1	8.00	-4.00	1 : 2
3	Block 1	8.00	-4.00	1 : 2
15	Block 1	8.00	2.00	1 : 2
7	Block 1	8.00	2.00	1 : 2
14	Block 1	8.00	2.00	1 : 2
4	Block 1	12.00	-1.00	1 : 2
11	Block 1	12.00	-1.00	1 : 2
8	Block 1	12.00	-1.00	1 : 2

-Sampling

Analyses were carried out on the samples from every group and triplicate measurements were conducted to achieve the statistical study. The quality parameters monitored in each cooling system during salting and storage time:

- Acidity (pH) in herring
- Salt content of herring stored in each cooling system.
- Fat content in herring
- Water content in herring
- Weight changes of fish during salting
- Freezing point measurement in relation to different salt content in fillet

The measurement of weight change was conducted by choosing five samples randomly from each bucket and marking them. Changes in weight during salting were determined by weighing the marked samples based on the sampling schedule. For salted herring fillets sampling was carried out on days 0, 0.5, 1, 1.5, 2, 3, 5, 7, 12, 18 and 24 after immersion into

the brine. At the beginning of the experiment, day 0, three herring fillets were chosen and tested for pH and all the chemical and physical parameters listed in Table 9.
At every sampling occasion two herring fillets were also randomly taken out from each bucket for salt, water and pH determination. Simultaneously, the same ratio of the brine was removed from buckets to maintain the same balance of fish to brine from the beginning.

Table 9: Sampling schedule in Experiment 1

Days	Weight	Water content	Salt content	pH	Fat content
0	x	x	x	x	x
0.5	x	x	x	x	x
1	x	x	x	x	x
1.5	x	x	x	x	x
2	x				
3	x	x	x	x	x
5	x				
7	x	x	x	x	x
12	x				
18	x	x	x	x	x
24	x			x	

3.1.2 Second experiment

Based on the first trial, the second experiment was carried out to determine the optimum pre-cooling conditions in brining of whole herring by choosing different brines and cold storages from the experience of Experiment 1 (figure 5). To make the results potentially useful in the DPRK, the experimental conditions were made comparable to the local conditions, including current freezing capacity.

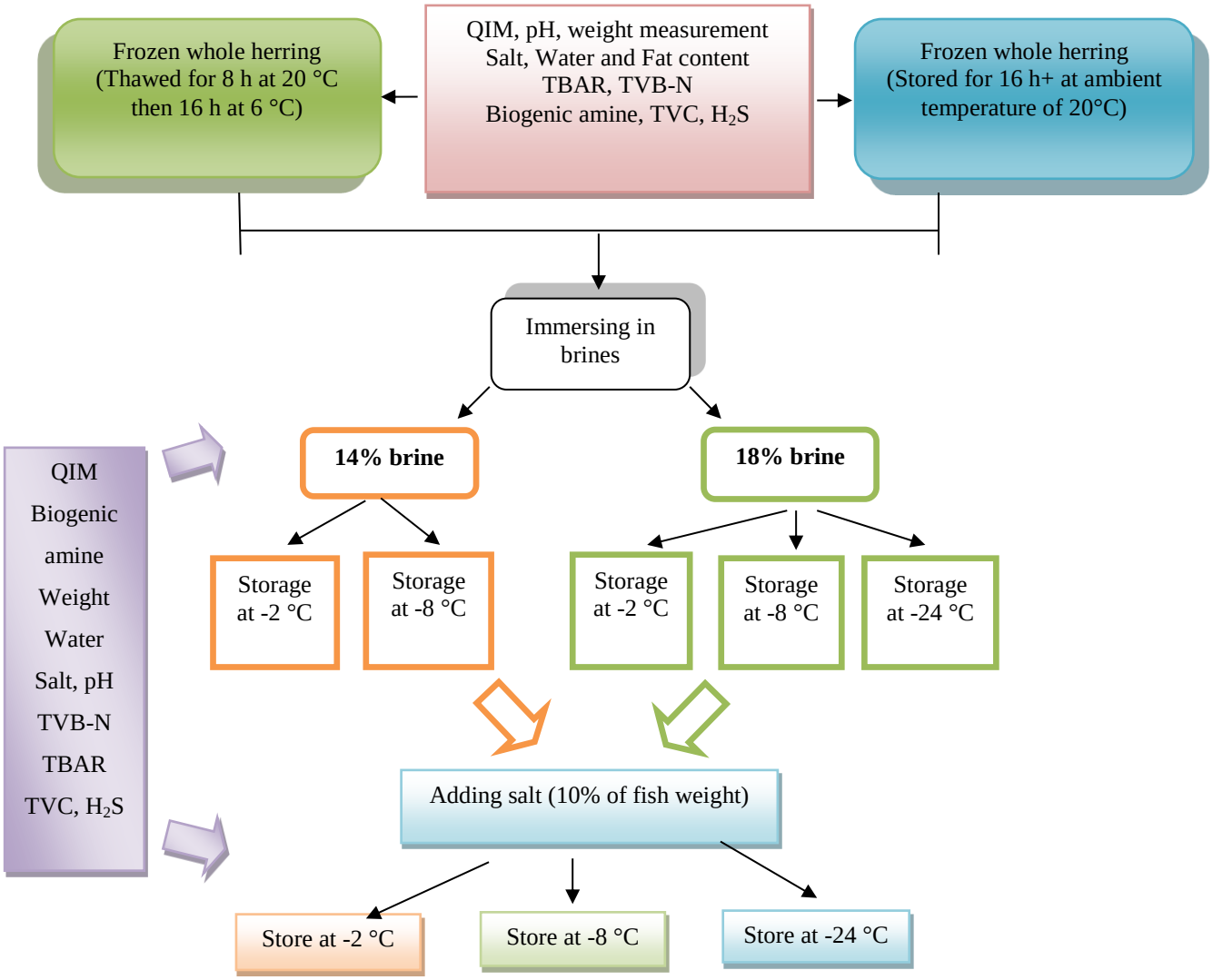

Figure 5: Flow chart of second experiment

- Fish material

The whole herring used for the Experiment 2 were caught in Breidarfjordur, South-west of Iceland on 12 December 2008 by purse seine. The catch had been stored in slurry ice on board until it was landed. At landing site on 14 December 2008, the temperature of the fish was -1.5 °C. In Experiment 2, a total of 140 kg of whole herring was used for the salting trial with 50 kg of industrial salt.

- Cold storage and brines

After receiving the raw materials, one group was thawed at 19.5 ± 0.5 °C for 8 hours then at 6 ± 0.5 °C for 16 hours. The thawing of another group was prolonged for 16 more hours at 19.5 ± 0.5 °C of room temperature. The cold chambers were prepared to keep constant temperatures of -24 °C, -8 °C and -2 °C and the brine salt contents were 18% and 14%. Table 10 shows the target brines in different groups.

The initial ratio between fish and brine was 1: 2. After removing some brine after 24 hours of immersing, the ratio between fish and brine was 1: 0.2, which was followed by adding salt. The amount of salt added was 10% of fish weight.

Table 10: Preparation of brines in Experiment 2

Bucket No.	Block	Salt content (%)	Temperature (°C)	Ratio (fish: brine)
5	Block 1	14.00	-2.00	1 : 0.2
1	Block 1	14.00	-2.00	1 : 0.2
12	Block 1	14.00	-8.00	1 : 0.2
9	Block 1	14.00	-8.00	1 : 0.2
2	Block 1	18.00	-24.00	1 : 0.2
6	Block 1	18.00	-24.00	1 : 0.2
13	Block 1	18.00	-8.00	1 : 0.2
10	Block 1	18.00	-8.00	1 : 0.2
3	Block 1	18.00	-2.00	1 : 0.2
8	Block 1	18.00	-2.00	1 : 0.2
7	Block 2	18.00(Ambient)	-2.00	1 : 0.2
14	Block 2	18.00(Ambient)	-2.00	1 : 0.2
4	Block 2	18.00(Ambient)	-8.00	1 : 0.2
11	Block 2	18.00(Ambient)	-8.00	1 : 0.2

-Sampling

Seven trial groups were formed, with different brine salt content and storage temperature as follows; Group 1 (14% salt, -2 °C), Group 2 (14% salt, -8 °C), Group 3 (18% salt, -2 °C), Group 4 (18% salt, -8 °C), Group 5 (18% salt, -24 °C), Group 6 (18% salt, -2 °C) and Group 7 (18% salt, -8 °C). Two groups from the second block were thawed over a longer time at room temperature marked with (Ambient) in Table 10.

The following quality parameters monitored in each cooling system during salting and storage time:

- Sensory analysis by QIM
- pH value of herring
- Salt content
- Fat content
- Water content
- Weight changes
- Biogenic amine analysis (histamine, tyramine, putrescine and cadaverine)
- Total Volatile Base-Nitrogen (TVBN) determination
- Lipid oxidation of herring by analyzing thiobarbituric acid reaction (TBAR)
- Total viable counts (TVC)

For weight determination, three samples of herring were randomly selected and marked from each sample bucket of the seven groups, measuring weight changes by weighing the marked samples and calculated by increased percentages. For salted whole herring sampling was

carried out on days 0, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 11, 12, 14, 18, 19, 21, 25 and 26 after immersing in the brine. After sampling all groups for 2 days, the groups stored at -8 °C and -24 °C were sampled on day 3, 5, 12, 19 and 26, and the groups stored at -2 °C were sampled on day 4, 7, 11, 14, 18, 21 and 25. At the beginning of the experiment, day 0, three fish were chosen and tested for pH and all the chemical, microbiological, sensory and physical parameters (Table 11).
At each sampling time two fish were randomly selected from each bucket for salt, water and pH determination. Simultaneously, the same ratio of the brine as fish was taken out from the buckets to maintain the same balance from the beginning.

Table 11: Sampling schedule in Experiment 2

Day	Weight	Water content	Salt content	pH	TVB-N	TBA	Biogenic amine	Fat	TVC H_2S	QIM
0		x	X	x	x	x	x	x	x	X
0.5	x									
1	x	x	X	x						
1.5	x									
2	x	x	X	x						
3	x	x	X	x						
4	x	x	X	x	x	x	x		x	x
5	x	x	X	x	x	x			x	
7	x	x	X	x	x	x	x		x	x
11	x	x	X	x	x	x	x		x	x
12	x	x	X	x	x	x			x	
14	x	x	X	x	x	x	x		x	x
18	x	x	X	x	x	x	x		x	x
19	x	x	X	x	x	x			x	
21	x	x	X	x	x	x	x		x	x
25	x	x	X	x	x	x	x		x	x
26	x	x	X	x	x	x		x	x	

3.2 Methods

3.2.1 Chemical analysis

3.2.1.1 Salt content (Volhard method)

The salt content was determined according to the AOAC Official Methods of Analysis. 5 g of minced herring was put into 250 ml bottle with 200 ml H_2O. The solution was shaken for 60 minutes. A 20 ml solution was pipetted into a 100 ml beaker with 20 ml of HNO_3. Upon

addition of nitric acid, the solution is titrated with silver nitrate and the end point determined potentiometrically (AOAC 2000).

3.2.1.2 Fat content (Soxtec method)

After drying, 5 g of minced herring was weighed and extracted with petroleum ether, boiling range 40-60°C. The extraction apparatus was 2050 Soxtec Avanti Automatic System (AOCS 1997).

3.2.1.3 Water content

5 g of minced herring was weighed and put into a dish and placed into the oven at 103 °C ± 2 °C for four hours. The sample was then removed from the oven and allowed to cool to ambient temperature in a desiccator for 30 minutes and weighed. The water content was determined as the percentage loss of weight after the sample was heated in the oven (ISO 1999).

3.2.1.4 Total Volatile Base-Nitrogen (TVB-N) compounds

100 g of samples were homogenized followed by adding 200 ml of 7.5% aqueous trichloroacetic acid (TCA) solution. After homogenizing for 1 minute at high speed by waring blender, the supernatant liquid was filtered through Whatman No. 3 paper. 25 ml of filtrate was transferred into a distillation flask, followed by 6 mi of 10% NaOH solution. TVB-N was determined through direct distillation by 10 ml of 4% boric acid. The acid is titrated with H_2SO_4 (AOAC 2000).

3.2.1.5 pH value

The measurement of pH was carried out by pH-meter probe pushed directly into the fish muscle (PHM 210 Radiometer Analytical S.A., Villeurbanne, France).

3.2.1.6 3.2.1.6 Thiobarbituric acid reaction (TBAR) test

TBAR test was performed by a modified version of the extraction method described by Vyncke (1970, 1975) with few modifications. The sample size was reduced to 15 g and homogenized with 30 mL of 7.5% trichloroacetic acid solution containing 0.1% of both propyl gallate and ethylenediaminetetraacetic acid (EDTA). The absorbance of samples and standards were measured at 530 nm. TBAR, expressed as µmol malondialdehyde per kilogram of sample (µmol methyldiamphetamine (MA)/kg), was calculated using malondialdehyd-bis-(diethyl acetate) as standard (Sorensen and Jorgensen 1996).

3.2.1.7 Biogenic amine analysis (histamine, tyramine, putrescine and cadaverine)

The measurement of biogenic amines in whole herring was carried out using method applied by the Icelandic Food Research Laboratory (Appendix 2).

3.2.2 Physical analysis

3.2.2.1 Measurement of weight increase

At the beginning of the experiment, the weight of three samples which were randomly selected in every bucket of each group were measured, marked and put into the middle of the

bucket. The weight changes were measured every 12 hours for two days from the beginning, and then it followed the experimental design. The calculation was performed by percentage. Measuring of samples was done after the fish had been dripping for 10 minutes after taking it out of the brine at 0 °C of room temperature.

3.2.2.2 Measurement of brine temperature in cold storage

The brine temperature in each cooling system was recorded every 12 minutes in Experiment 1 and every 5 minutes in Experiment 2, throughout the experimental period, using the thermocouple type T sensor, Optiz Stow Away thermometer (Computer Corporation, Massachusetts) put on the bottom of each bucket.
The time needed for pre-cooling depends on the temperature and quantity of brine and on the initial body temperature of herring. In Experiment 2, the determination of the time needed to lower down the body temperature of those sample groups which were kept for 16 more hours at 20 °C was conducted by putting the temperature loggers into the middle of the fish muscle under dorsal fin and measuring every 5 minutes. To determine the pre-cooling condition, the 18% brine at -2 °C and -8 °C were selected respectively, as the second refrigerants for cooling. And the time check for temperature decreasing of fish was measured after immersing them to the brine. The ratio between fish to brine was 1 to 2 and body temperature of fish was around 15 °C.

3.2.2.3 Measurement of freezing points during brine-salting

Measurement of freezing points of herring of different salt content during salting was done as follows; the minced fish was put into a small plastic pot (220 ml: height 220 mm, bottom diameter 40 mm) and fix the thermocouple type T sensor, Optiz Stow Away thermometer (Computer Corporation, Massachusetts) in the middle of the minced fish. After that the pots were put into the refrigerator at -26 °C and the temperature measured every minute to detect the prolonged time interval of unchanged temperature when the latent heat of fusion is absorbed.

3.2.3 Quality Index Method (QIM)

In the experiment, six to eight panellists, who trained during a specialist course of fish processing and quality management of UNU FTP participated in the sensory analysis according to international standards (ISO 1993, Durita and Grethe 2004, Appendix 1). They learned and practiced to perform the QIM method at Icelandic Food Research (MATIS) using this technique to frequently evaluate the fish including herring. The observations were carried out in the same room with as little interruption or distraction as possible, at room temperature, under white fluorescent light.
A total of 23 herring samples, only stored at -2 °C, were analysed with QIM during the evaluation period. According to the brining conditions they were divided into 3 buckets; 14%, 18% and 18% (kept for 16 more hours at 20 °C before brining). For the evaluation (8 sessions over 8 sampling days) 3 fish from each batch were used each time. The samples were collected from the buckets and placed on a clean table 30 minutes before assessment. Each herring was coded with a random 3 digit number.

3.2.4 Total viable count (TVC)

For the detection of TVC and H_2S- producing bacteria, 20 g of each sample was placed in a stomacher bag and 180 ml of peptone water solution added to obtain a 10-fold dilution. Blending (homogenization) was done in the stomacher for 1 minute. After homogenization serial dilutions were prepared (1/10). Total viable psychrotropic counts (TVC) and counts of H_2S-producing bacteria were evaluated on iron agar (IA) as described by Gram *et al*. (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. Plates were incubated at 17 °C for 4-5 days. Bacteria forming black colonies on IA produce H_2S from sodium thiosulphate and/or cysteine. Colonies on plates that had 25 to 250 colonies were counted and counts were multiplied with the relevant dilution factor.

3.2.5 Yield and other calculations

The weight changes in every step from the start (for ex. A to B) were calculated by the weight gained or lost. Weight yields (%) were calculated from the weight analysis of the samples marked and recorded in each step (Andres *et al*., 2004; Barat *et al*., 2002; Gallart-Jornet *et al*., 2006):

$$\Delta M_B^0 = \left(\frac{M_B^0 - M_A^0}{M_A^0}\right) * 100$$

Whereas the ΔM_B^0 : weight change

ΔM_A^0 : previous weight

The equation was changed from previous studies in such a way that the quantitative changes in water and salt content were calculated as a ratio of the intial total mass of water in the raw material, not as a ratio of the total mass. The salt concentration in the muscle was calculated after each step from weigth fractions of salt (χ^{NaCl}) and water (χ^{w}) (Andres *et al*., 2004, Barat *et al*., 2002, Gallart-Jornet *et al*., 2006) as follows:

$$Z^{NaCl} = \left(\frac{\chi^{NaCl}}{\chi^{w} + \chi^{NaCl}}\right) * 100 \qquad (3)$$

3.2.6 Statistical analysis

The obtained data of each variable (level of microorganisms expressed as log colony-forming unit (CFU)/g) were analysed by analysis of variances (ANOVA) in the Statistical Analysis Software, NCSS version (2007) for windows (Statistical solutions Ltd, Ireland).
Data analysis was also performed by the principal component analysis (PCA) using The Unscrambler version 9.8 (Camo 2008, Norway). The various measurements on a sample constituted one row in the matrix X. Each column in X was mean-centred and scaled to a unit standard deviation.

4 RESULTS

4.1 Results of chemical analysis

4.1.1 Salt and water content changes

An increasing salt content was found both in the fillet and whole herring, according to the increase of brine solution concentration and temperatures employed in the pre-cooling. The salt was progressively transferred from the brine to the herring. The salt content of the herring

muscle was almost equilibrated after about 18 days and 25 days in the fillet and whole fish, respectively.

Before starting the salting in experiment 1, the salt content in each herring fillet was 0.47 ± 0.01%. Within 12 hours after immersing the herring in the brine in each temperature and salt content, the salt content of the fillet in every group increased rapidly. After three days of the salting experimental period, the salt content changes showed a smooth increasing trend in every group. The salt increase of groups in temperatures below 0 °C was slower than in the other groups (Figure 4). In the groups of fillet samples, the salt content increased rapidly within 3 days after immersing in the brine, and then increased more slowly at all temperatures and brine concentrations.

The initial water content was almost the same both for the fillets and whole herring but the changes during salting differed considerably. The water content of herring fillet at the beginning ranged between 65% and 68% in all samples. The overall change after 18 days of salting was small for all treatments except for the 8% salt content brine, where the water content increased from 66.8% to 74.2% (Figure 6). But overall the trend was increasing water content.

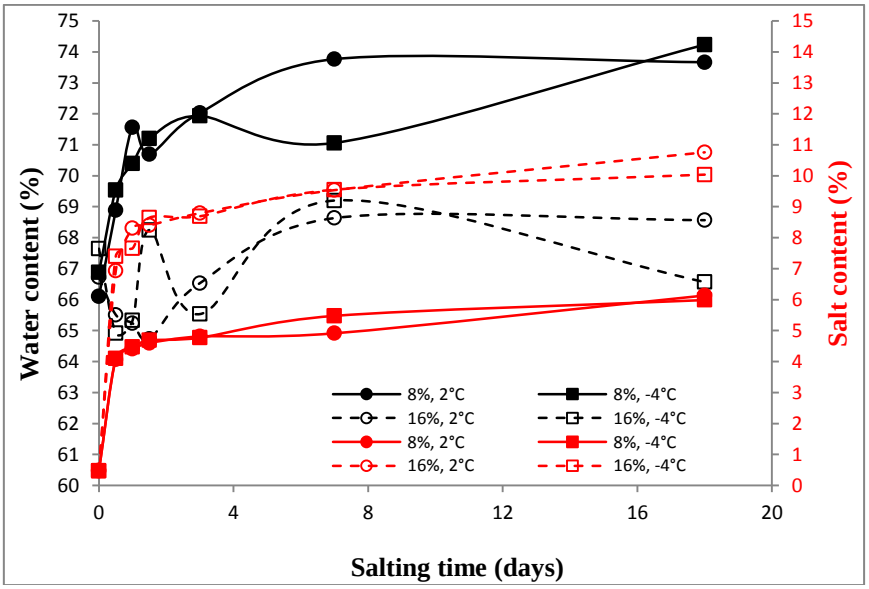

Figure 6: Water and salt content changes of herring fillet during brining and storage, the red colour lines indicate salt content changes

In Experiment 2 using whole herring, the initial average salt contents in every group was 0.60 ± 0.05% and they increased during salting (Figure 5 and 6). But the increasing ratios were not as rapid as in Experiment 1. The whole herring kept at -2 °C and -8 °C with salt added after 24 hours mixed with the salt of 10% to the fish weight. The ratio between fish to brine was 1: 0.2. After mixing, they were put into each chamber at -2 °C and -8 °C, and the salt was progressively transferred from the brine. The salt content increased more rapidly in the groups at -2 °C than the others during the first days of brining showing a constant increase. At the storage temperature of -8 °C, the salt content of all groups nearly reached the salting equilibrium after 25 days of brining while the one at -2 °C was reached after 14 days.

In Experiment 2, the initial water content in every group was 69.2 ± 0.7% and it decreased showed decreasing trends during salting (Figure 7 and 8).

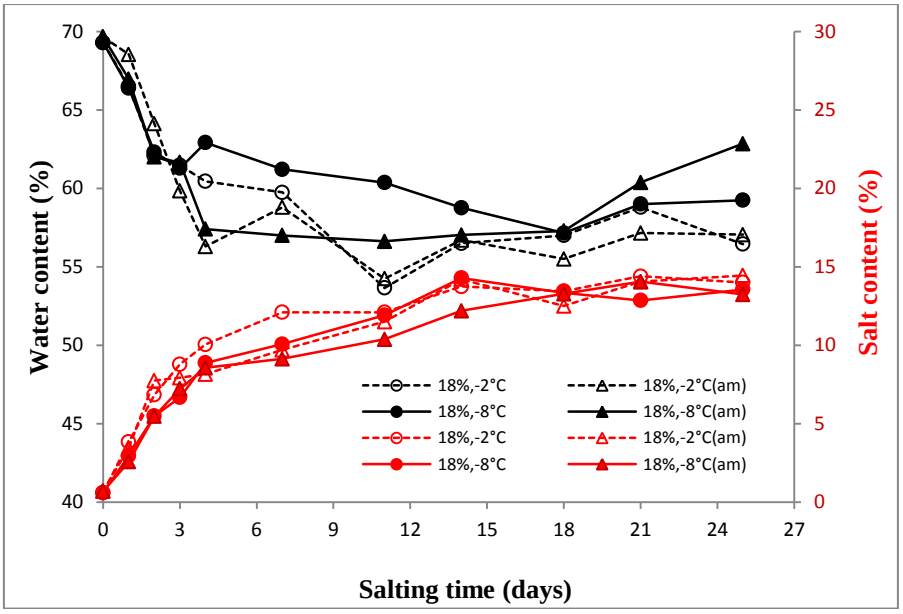

Figure 7: Water and salt content changes of whole herring in the brines of 18%, red colour lines indicate the salt content, treatment marked (am) - herring kept more 16 h at 20 °C

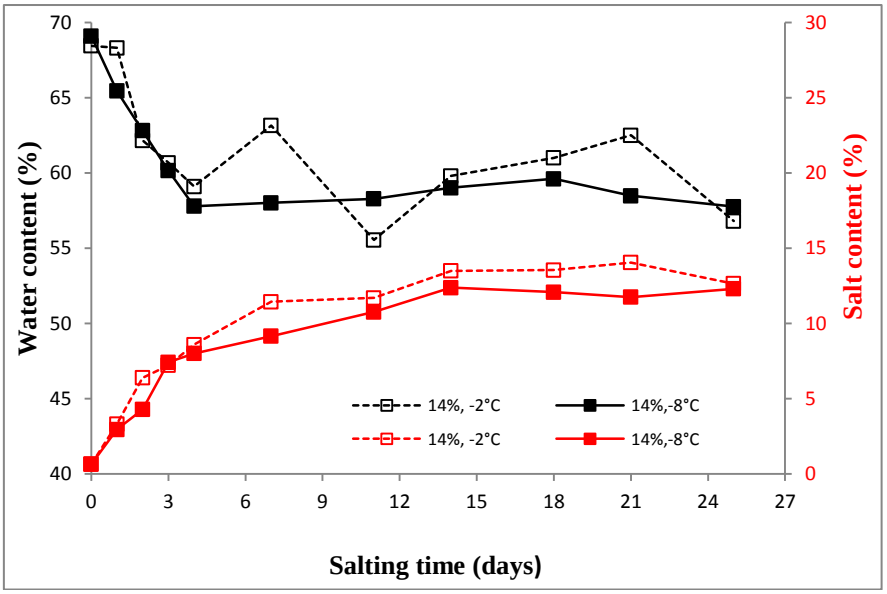

Figure 8: water and salt content changes of whole herring in the brines of 14%, red colour lines indicate the salt content changes

4.1.2 Fat content

Fat content was 13.40 ± 0.1 %, 13.80 ± 0.8 % respectively, in fillet and whole herring before brining. The fat contents as percentage in the fillets had decreased after 18 days of salting (Figure 9).

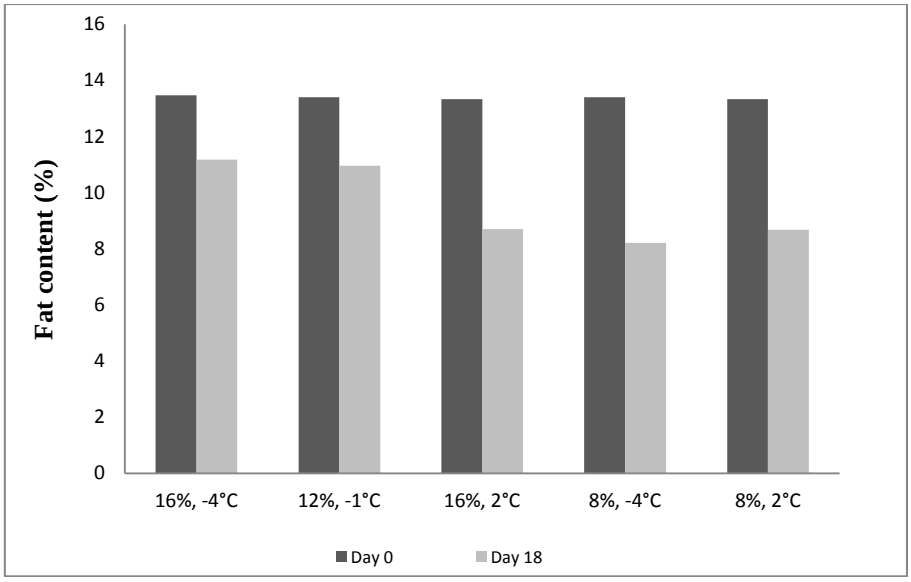

Figure 9: Fat content changes of herring fillet in different brine conditions

4.1.3 4.1.3 TVB-N

The initial TVB-N contents in raw whole herring was 16.43 mg/100g fish and the contents in the samples which were kept for 16 more hours at 20 °C before brining was 18.9 mg/100g (Table 14 and 15). Brining produced a little reduction in the TVB-N values during the experiment, only a minor decrease (0.4–2.25) was recognized in the groups stored at -2 °C even so they fluctuated widely. But in the group named 18% (amb) the TVB-N value was unstable.

Table 12: TVB-N changes in whole herring stored at -2°C (treatment amb- samples kept at 20°C for 16 h more)

day	0	4	7	11	14	18	21	25
14%	16.43	12.74	14.57	15.26	15.46	15.72	14.23	15.66
18%	16.43	11.61	15.21	17.05	15.81	16.07	16.71	16.11
18%(amb)	18.98	14.29	17.71	16.46	15.07	19.03	17.25	15.37

Table 13: TVB-N changes in whole herring stored at -8 and -24 °C (treatment amb- samples kept at 20°C for 16 h more)

day	0	5	12	26
14%	16.43	14.29	14.47	14.67
18%	16.43	13.94	14.67	16.43
18%(amb)	18.98	17.19	16.11	14.92
18%,-24°C	16.43	13.47	14.23	16.16

4.1.4 pH changes

The pH of the raw herring used in both trials was around 6.48 ± 0.03. Brining resulted in a small but significant reduction of the initial pH, while the pH in the fillets dropped during the first day to about 6.1-6.3 and stabilized after that (Figure 10).

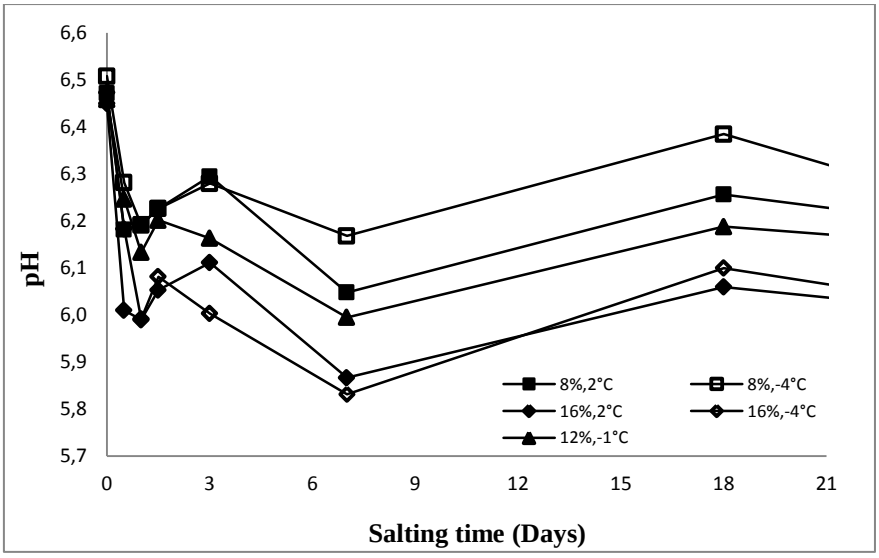

Figure 10: pH Changes of herring fillet during salting

The pH value in whole herring (Experiment 2) decreased sharply in every group during the first 3-6 days and stabilized after that. The levels of pH in all samples ranged from 5.7 to 6.5 (Figure 16).

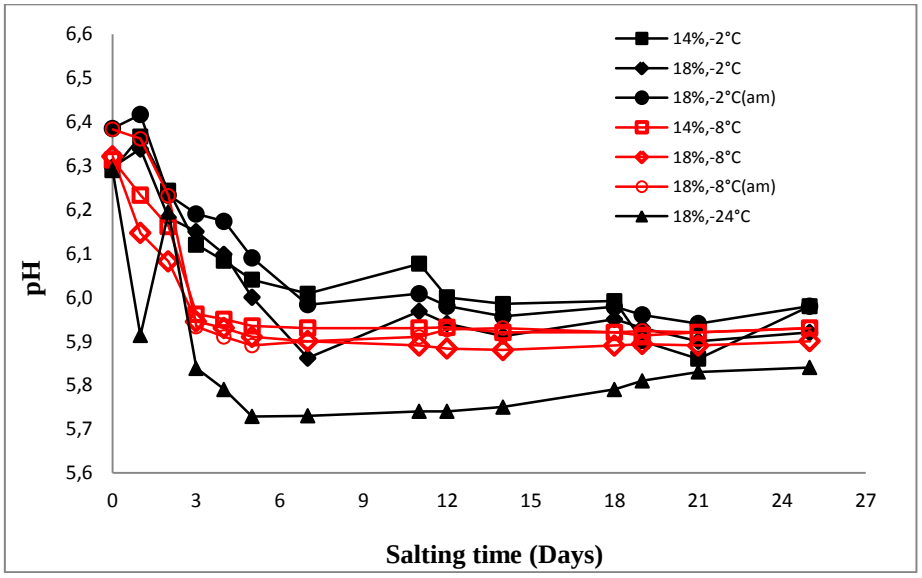

Figure 11: The pH changes in whole herring during brining

4.1.5 TBAR

In the present study, the TBA values of fresh raw herring was 0.075 and 0.114 mg MA/kg, and in those groups of samples which were kept for 16 more hours at 20 °C the TBA value was higher 0.139 MA/kg. During brining there was a tendency towards an increase in TBA values up to 0.103 and 0.094 mg/kg in the groups of 14% brine, while those of 18% showed stable or gradual decreasing trends (Table 16 and 17).

Table 14: TBA changes in whole herring stored at -2 °C (treatment amb- samples kept at 20°C for 16 h more)

day	14%	18%	18% (amb)
0	0.075	0.114	0.139
4	0.082	0.090	0.131
7	0.065	0.058	0.061
11	0.092	0.080	0.090
14	0.117	0.127	0.134
18	0.081	0.092	0.092
21	0.052	0.061	0.064
25	0.103	0.092	0.086

Table 15: TBA changes in whole herring stored at -8 and -24 °C (treatment amb- samples kept at 20°C for 16 h more)

day	14%	18%	18% (amb)	18%,-24 C
0	0.075	0.114	0.139	0.075
5	0.099	0.083	0.109	0.085
12	0.058	0.058	0.065	0.103
26	0.094	0.091	0.117	0.077

4.1.6 Biogenic amines

Table 16 summarizes the contents of biogenic amines measured in the fresh herring and those in the samples stored at -2 °C during brining. There was no detection of cadaverine and tyramine in any of the samples.

Table 16: Biogenic amines in whole herring during brining (unit-ppm, ND-not detected, i.e. amine level is less than 1 ppm)

days		**0**	**4**	**7**	**11**	**14**	**18**	**21**	**25**
14%	Cadaverine	ND	ND	ND	ND	ND	ND	ND	ND
	Histamine	9	9	9	9	11	12	13	15
	Putrescine	14	12	14	18	16	14	ND	ND
	Tyramine	ND	ND	ND	ND	ND	ND	ND	ND
18%	Cadaverine	ND	ND	ND	ND	ND	ND	ND	ND
	Histamine	11	11	12	10	13	12	15	17
	Putrescine	14	ND	ND	ND	14	13	ND	ND
	Tyramine	ND	ND	ND	ND	ND	ND	ND	ND
18% (amb)	Cadaverine	ND	ND	ND	ND	ND	ND	ND	ND
	Histamine	14	12	17	10	12	14	15	17
	Putrescine	15	16	16	14	14	16	ND	ND
	Tyramine	ND	ND	ND	ND	ND	ND	ND	ND

4.2 Results of physical analysis

4.2.1 Weight increment

The overall weight change in all fillet samples after 24 days of brining was very similar, a very rapid increase of about 30% during the first five days and slowly approaching a maximum of around 35% after that (Figure 17). Generally, the highest %-unit alterations in weight gain between days of brining were observed between day 0 and day 1 for all brining temperatures.

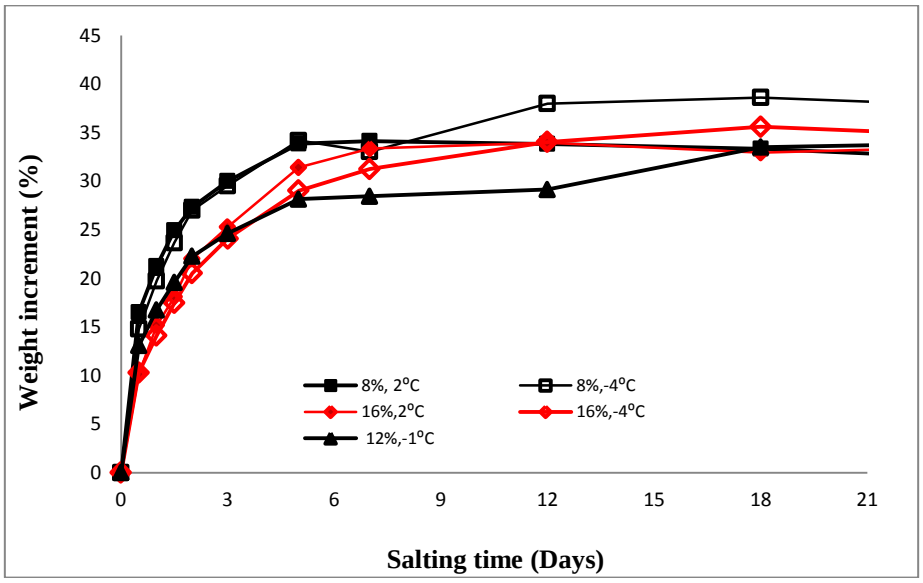

Figure 12: Changes in weight of herring fillet during salting

As shown below figures 13 and 14, the weight of all sample groups was increased as the salt content increased. But the increase in range was not as wide as for the fillet. The weight loss in the groups of samples which were kept for 16 more hours at 20 °C before brining was recovered after 25 days of brining.

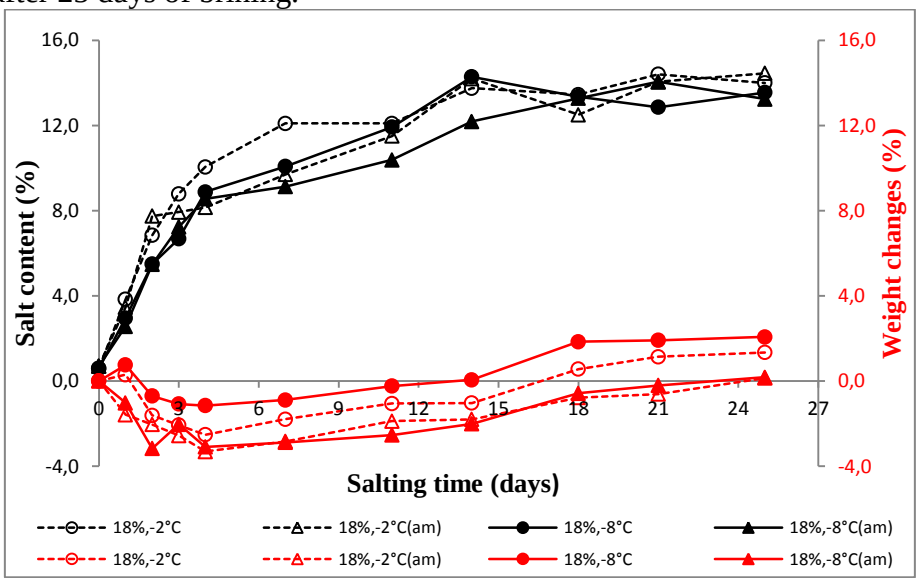

Figure 13: Weight changes in 18% brine comparing to the increase of salt content

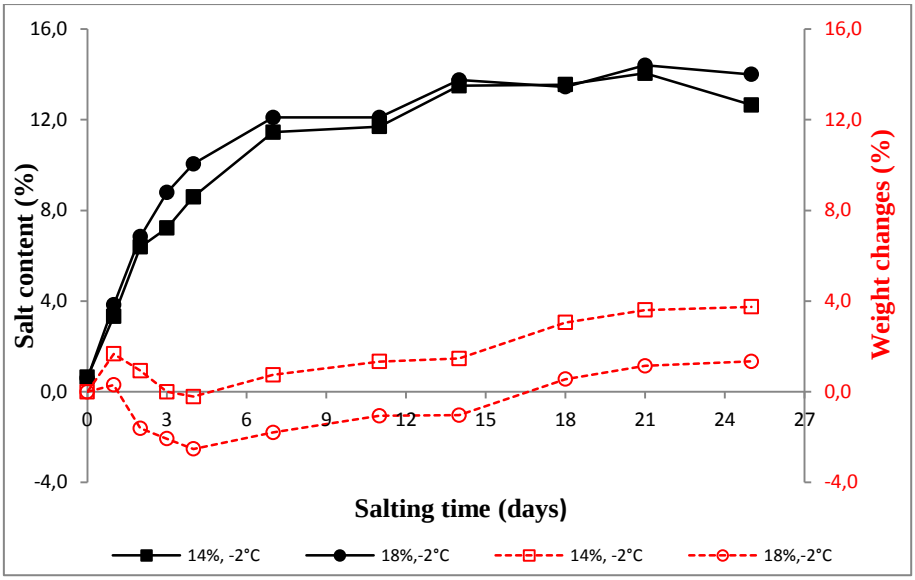

Figure 14: Weight changes in 14% brine comparing to the increase of salt content

4.2.2 Brine temperature fluctuation and pre-cooling time measurement

The temperature fluctuation in all storage chambers was very small ± 0.5 °C. Temperature differences in the upper and lower layers of the brine buckets in cold chambers were also small and probably did not affect the core temperature of the herring during salting and storage significantly.

Figure 15 shows the time needed to cool down the body temperature of samples which were kept for 16 hours at 20 °C before brining to the range of super-chilling temperature. The result shows that the brine at -8 °C and the ratio of 1:2 cooled down the fish core temperature from 15 °C to 0-1 °C within 20-25 minutes, while the brine at -2 °C could take almost 2 hours.

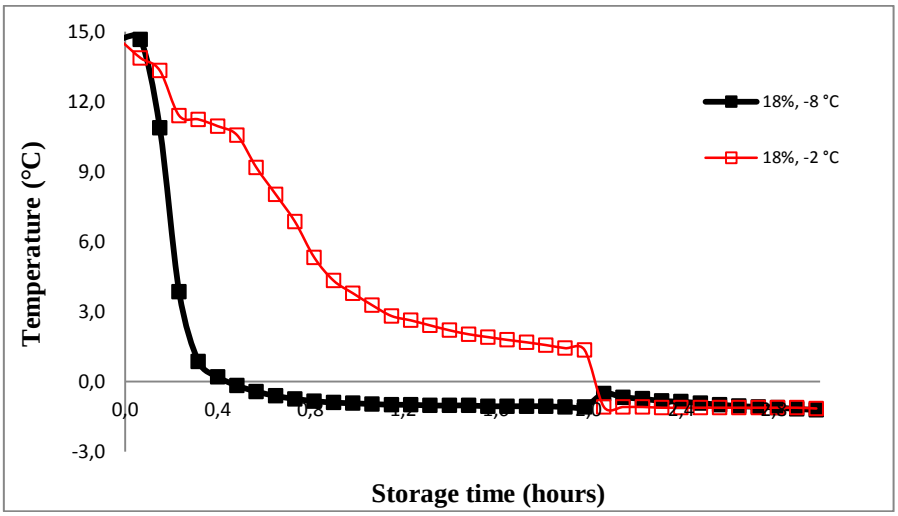

Figure 15: pre-cooling time determination of samples kept for 16 more hours at 20 °C

4.2.3 Freezing points during brining

To determine the salting condition by cold brine, the freezing points of herring muscle with different salt content was measured during different times during the experiments. The density and the freezing point of salt water changes with the salt content. With high salt content, lower temperatures are possible but care should be taken to choose optimum salt

content of brine to prevent freezing of the fish and increase a desired salt uptake. Table 17 and 18 show the results of measurements of freezing point by salting days in different conditions, as compared to the freezing point of flesh with the one of salt water. The freezing point of flesh is lower than of brine, at the same salt concentration. This is because of the soluble nitrogen compounds that are contained in the fish muscle (Figure 16; Ocean Water Freezing Point Calculator, 2008).

Table 17: Freezing points in fish muscle during brining (16%, -4 °C)

Brining time (days)	**0**	**0.5**	**1.5**	**3**	**7**	**18**
Salt content (%)	0.46	7.40	8.65	8.69	9.55	10.04
Freezing Point (°C)	-1.30	-7.51	-8.98	-9.22	-10.41	-10.63

Table 18: Freezing points in fish muscle during brining (12%, -1 °C)

Brining time (days)	**0**	**0.5**	**1.5**	**3**	**7**	**18**
Salt content (%)	0.48	5.45	6.28	6.59	7.19	8.54
Freezing Point (°C)	-1.20	-4.87	-5.88	-6.00	-7.22	-8.69

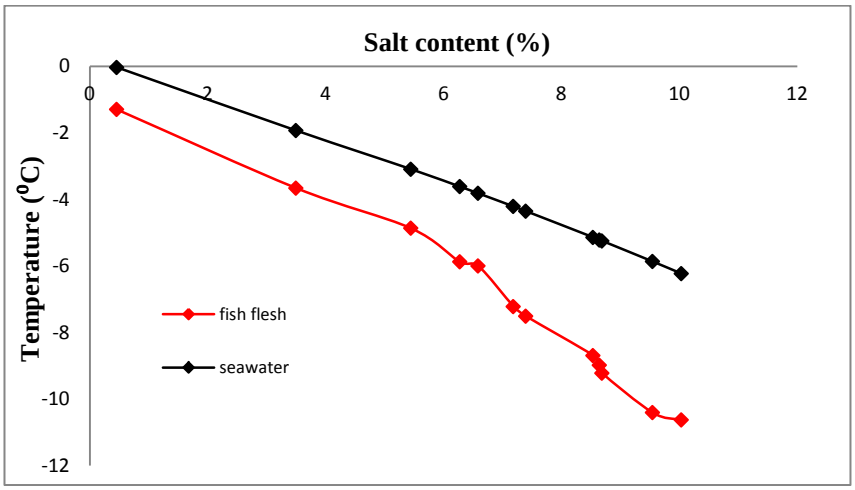

Figure 16: Comparison of the freezing point of seawater and the freezing point in herring by salt concentration (%)

4.3 Sensory analyses

Sensory attributes of brine salted herring at -2 °C are presented in Figure 17. The QIM scores at the beginning were about 6-7, but the original score of the samples which were kept for 16 more hours at 20 °C was about one unit higher than in the other groups. During the brine salting through 25 days at -2 °C the "normal" samples increased their score by two units, while the samples with prolonged time at 20 °C increased their score by one unit. All samples thus got the same final score. The average score ranged between 6 and 9.5. The group of samples which were kept for 16 more hours at 20 °C scored from 8.4 to 9.5.

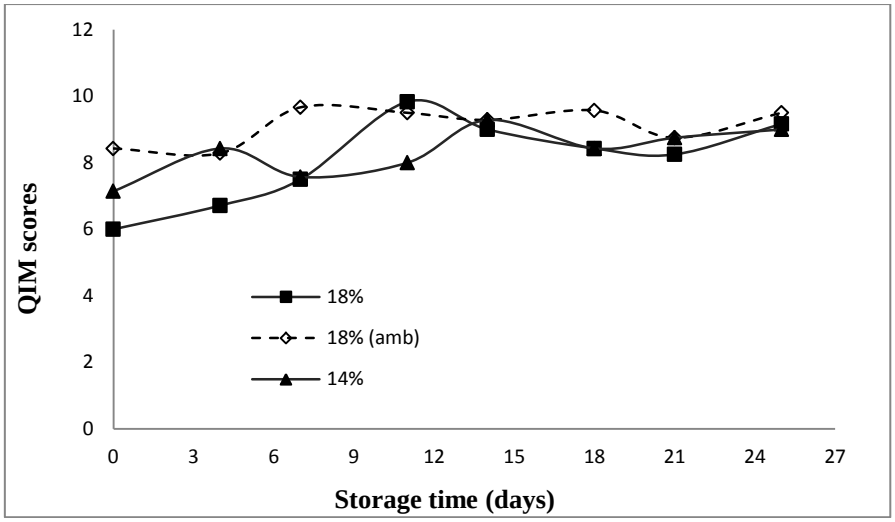

Figure 17: QIM scores increasing in herring stored at -2 °C

4.4 Total viable count (TVC)

Total viable bacterial count in raw fish material used in the second trial was 1.85 $\log_{10}$ CFU/g. And the TVC in the groups of samples which were kept for 16 more hours at 20 °C was 2.3 $\log_{10}$ CFU/g. By storage day 26, significantly ($P < 0.05$) lower counts were detected for the samples stored at -24 °C than at -2 °C. The sample group stored at -8 °C, which were kept for 16 more hours at 20 °C before brining, showed decreasing trends, while all the groups stored at -2 °C took a significant increase by the end of salting (Figure 18 and 19).

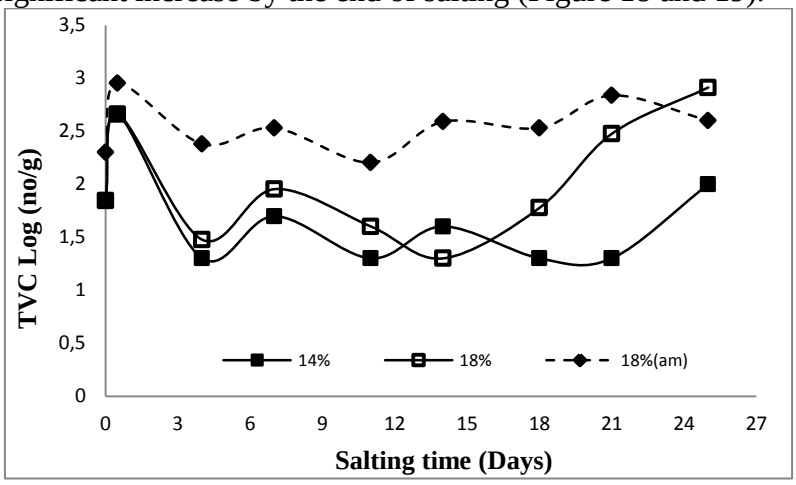

Figure 18: total viable microorganisms in salted herring stored at -2 °C

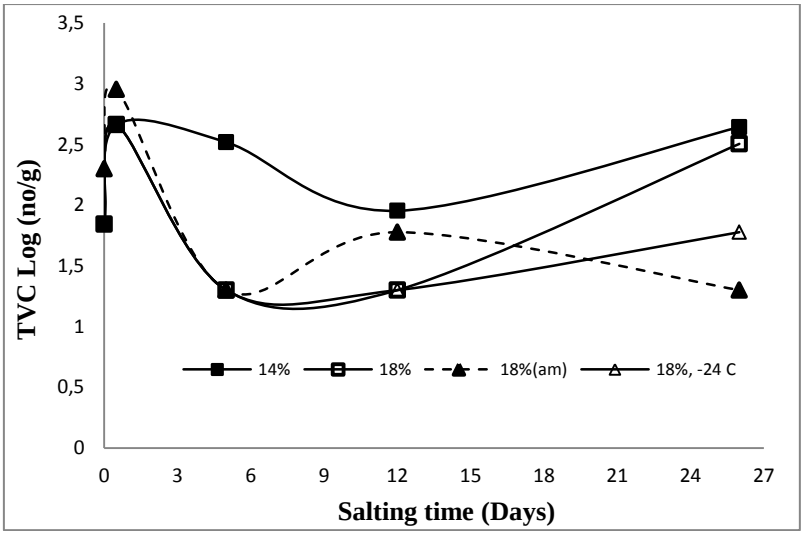

Figure 19: total viable microorganisms in salted herring stored at -8 and -24 °C

4.5 Multivariable data analysis

The table below clarifies the distinctions between brine concentrations by the difference of values in salt and water content, pH, weight yield. The difference between salt contents of brine was significant ($P<0.05$) in weight, pH and weight yield, with no significant difference in salt and water content changes (Table 19).

Table 19: Distinctions between brine concentrations by quality parameters of salted whole herrings

	salt content (%)	water content (%)	Weight change (%)	Weight yield (g)	pH
Brine (14%)	9.02	60.52	1.65	101.65	6.05
Brine (18%)	9.49	61.12	-0.82	99.18	6.04
Prob level	0.06	0.11	<0.001	<0.001	0.12

Table 20 shows the distinctions between brine temperatures by quality parameters. The temperature table means that all factors (salt content, water content etc.) were significantly different in brine samples stored at different temperatures of -2 and -8 °C.

Table 20: Distinctions between storage temperatures by quality parameters of tested samples

	salt content (%)	water content (%)	Weight change (%)	Weight yield (g)	pH
Brining at -2 °C	8.95	60.84	0.21	100.21	6.07
Brining at -8 °C	9.56	60.8	0.63	100.63	6.01
Prob level	<0.001	0.91	0.001	0.001	<0.001

The differences between two groups in 18% brine, in which one group was immersed directly on receiving the raw material and another one was delayed for 16 hours at 20 °C, the ambient temperature. Table 21 shows that the delaying of brining time was significantly different in weight, yield and pH ($P<0.05$).

Table 21: Distinctions between two groups by values of the pH, salt content, water content, weight change and weight yield in tested salted whole herrings

	salt content	water content	weight	yield	pH
Imm	9.31	60.97	0.76	100.76	6.03
Amb	9.37	60.21	-1.5	98.5	6.06
Prob level	0.94	0.36	<0.001	<0.001	0.047

Imm- immediately brined group.
Amb- delayed for 16 more hours at 20 °C.

In order to describe the main characteristics of the samples and to highlight their main differences, a multivariate statistical procedure, principal component analysis, was used on the data obtained (Figure 20). The chemical attributes for quality changes explained most of the variation in the data set, and were closely aligned along a first principal component (PC1). A second PC was explained mostly by pH value, weight and water content changes.

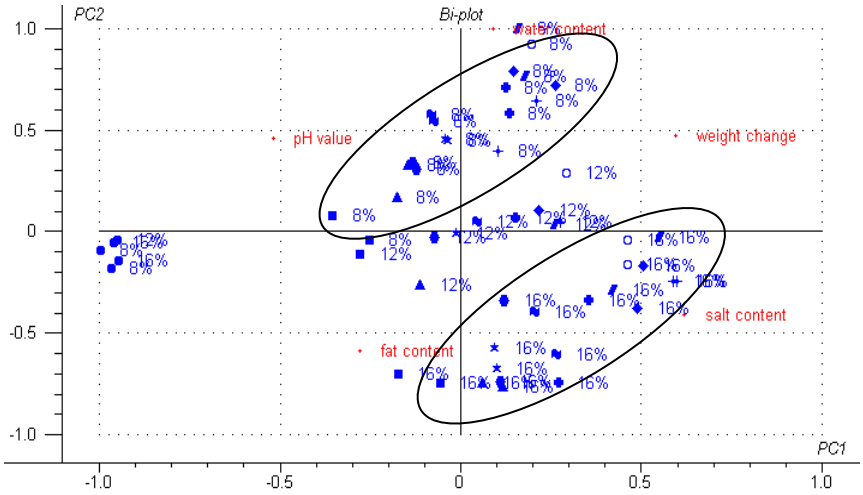

Figure 20: Loading plot for the PCA of the chemical analysis for herring fillet brined in 8%, 12% and 16% at 2, -1 and -4 °C, respectively

Below are two figures that show the score and correlation loadings on the results analysis by PCA. In Figure 20, the numbers indicate salting days and two different colours are 14% (blue) and 18% (red) brine concentrations. On the score plot in Figure 21, the samples are located on the left side of *PC1* characterized by grouping along time. In Figure 21, two distinct groupings emerged based on pH, water content and water yield on the left side of *PC1* whereas grouping were best on the salt content towards the right side of *PC1*.

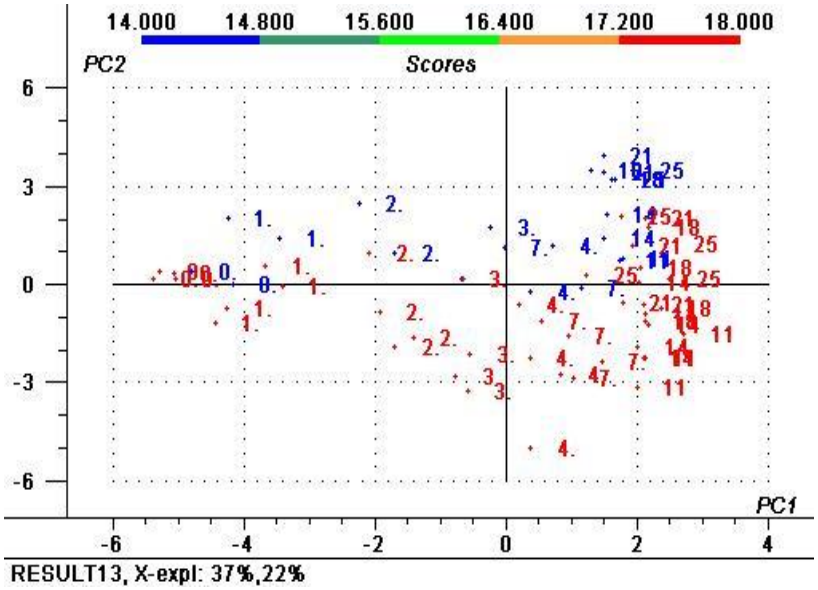

Figure 21: Score plot for the storage time of all samples. Blue=14% brine, Red=18% brine, numbers indicate storage days

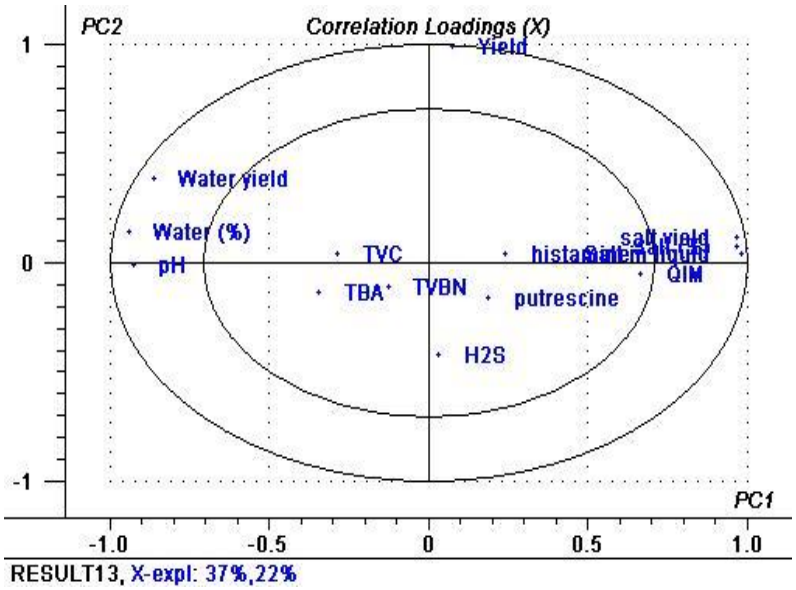

Figure 22: Correlation loading plot for the PCA of the chemical, physical and microbiological changes during storage of all samples

After analysis of all variables some less important factors were extracted and re-analysed in the two figures, 23 and 24 below. The variations of the samples are mainly with regard to water and salt content, pH and QIM score changes along *PC1* explaining 56% difference between the groups. It is also characterized by the variation in yield explaining 37% of the differences along the *PC2* (Figure 21 and 22).

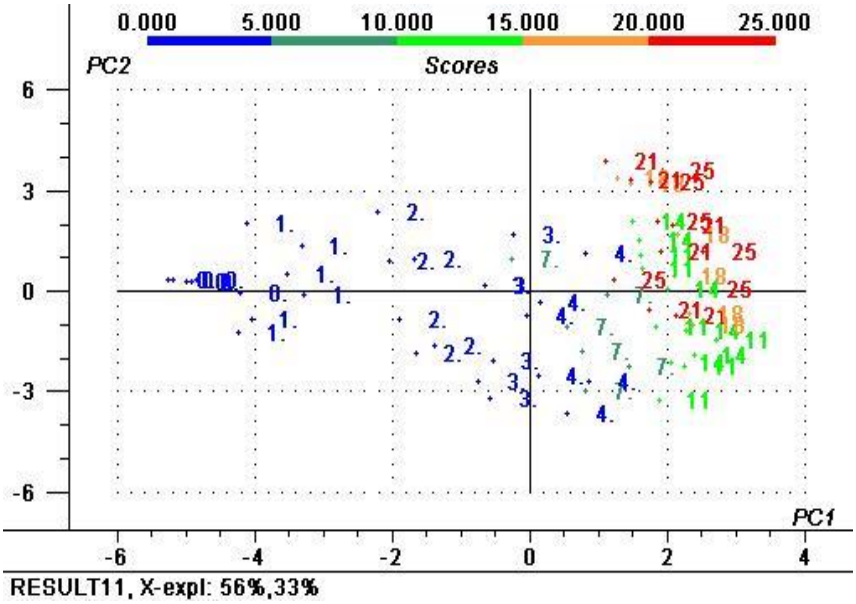

Figure 23: Score plot for the storage time of all samples

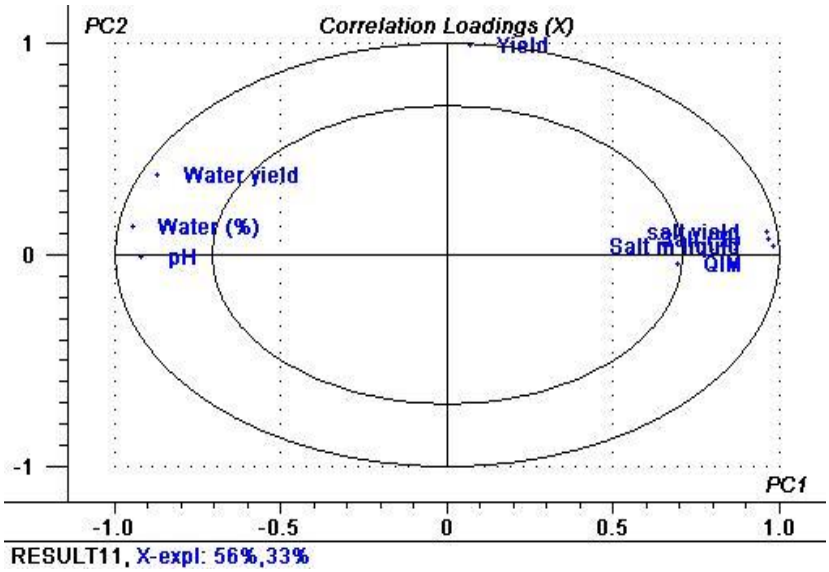

Figure 24: Correlation loading plot for the main varieties by PCA

5 DISCUSSION

The data presented in this paper are based upon experiments carried out by brine salting in different brine salt contents (8%, 12%, 14%, 16% and 18%) and different cooling systems (2 °C, -1 °C, -2 °C, -4 °C, -8 °C and -24 °C) on both whole fish and fillets. Physical, chemical and microbiological analyses have been conducted to confirm the effectiveness of pre-cooling by brine salting.

Sensory evaluation is the most popular way of assessing the freshness of fish. It is fast, simple and provides immediate quality information. The sensory characteristics of fish are clearly visible to the consumer and are essential for consumer satisfaction (Reineccius 1990). In the sensory analyses of this study the softness increased and similar other changes were found too. Although some differences occurred between the three groups of 14%, 18% and 18% (ambient) brines at -2 °C, they were not significant. Similar results were obtained for the

texture attributes of tenderness and watery; both increased during salting. The storage time is concerned to the increase of QIM scores on the appearance. Thus, the negative quality changes of brine salted herring could be retarded and the freshness of herring could be maintained longer at lower temperature.

The changes of herring muscle protein according to the storage temperatures were investigated by determination of TVB-N and TBA. The results were under the maximal permissible level of 35 mg TVB-N/100 g fish flesh specified by the EC guidelines (Commission Decision 1995), all groups of the salted samples were still below this limit by >17 mg/100g, indicating the significant effect of cold brining in the reduction of chemical changes of salted herring. The slight fluctuations in TVB during brining at -2 °C may be initiated by autolytic degradation of nucleotides and free amino acids while the smooth decrease at -8 °C is most likely because of the effects of lower temperatures on autolytic degradation and bacterial growth. Since TVB-N is produced mainly by bacterial decomposition of fish flesh, higher microbial counts were detected during storage in the groups which were kept for longer at 20 °C before brining. The results of this study was similar to that reported for brining and marinating of pacific herring (Sallam *et al*. 2006), brined chub mackerel (Goulas and Kontominas 2005), and brined anchovies (Karacam *et al*. 2002).

The TBA value is a widely used indicator for the assessment of the degree of lipid oxidation. It has been suggested that a maximum TBA value, indicating the good quality of the fish, is 5 mg (MA)/kg, while fish may be consumed up to a TBA value of 8 mg MA/kg (Schormuller 1969). The TBA is an index for measuring secondary stage (last stage) of lipid oxidation; this implies that lipid oxidation has not taken place to any great extent that could cause damage. In this study the TBA value was low and ranged from 0.052 to 0.139 mg MA/kg. The decrease in TBA content after the peak point has been attributed to the interaction between MA and decomposition products of protein to give tertiary degradation products and it is consistent with the reports of Fernandez *et al*. (1997), and Reddy and Setty (1996). The fluctuations in the TBA levels during refrigerated storage of anchovies that were brined in different concentrations of salt (14–26%) have also been reported by Karacam *et al*. (2002), who also concluded that TBA may not be a reliable criterion for anchovies salted under the condition used.

The pH is an important intrinsic factor related to post-mortem changes of fish flesh. Most fish contain only very little carbohydrate (<0.5%) in the muscle tissue and only small amounts of lactic acid are produced post-mortem (Gram and Huss 1996). During the brine salting in this study, the pH value of all groups researched through both of the experiments showed decreasing trends at lower temperature. In case of pH in the fillets, the value fluctuated a little but the range was small (<0.5). Such fluctuations in the pH indicate bacterial growth, loss of quality and possible spoilage. After immersing the fish in the brine, with the migration of salt and soluble matter between brine and flesh the salt content of herring muscle was equilibrated. So the salt content in both the herrings and brine remained constant. The pH values are in agreement with those previously reported by Shiau *et al*. (1998) for commercially salted mackerel.

It is commonly known that there are three types of spoilage for the salting of fish with the 15%-20% brine. The most common type is characterized by the presence of sour, sour/sweet and putrid off-odours and off–flavours, and is caused by growth of a Gram-negative, halophilic and obligate anaerobic rod (up to 10^6-10^7 cfu/g) by the report from Gram and Huss (1996). The brining process in this study did not induce a significant reduction in the initial TVC, the detected number of TVC were all below the permissible limit of 7 $\log_{10}$ CFU/g (ICMSF, 1986).

The average content for each of the four biogenic amines in all samples was lower than 20 ppm and they were under 50 ppm, the allowable limit suggested by the FDA (1992). None of the tested samples even contained cadaverine and tyramine in measurable quantities. Therefore, based on the content of histamine, all groups could be regarded at an acceptable rate as reported by Shiau *et al*. (1998) with their salted mackerel samples. Although the tested mackerel samples did not contain high levels of histamine, the presence of putrescine may synergistically enhance histamine toxicity by inhibiting histamine metabolizing enzymes such as diamine oxidase and histamine methyl transferase (Lehane and olley 2000, Antoine *et al*. 2002)

It is generally accepted that salt migration by diffusion plays an important role in the salting of fish, and the diffusivity of salt is dependent on several factors such as species, temperature, muscle orientation, lipid content, the presence or absence of skin, and fillet thickness and brine concentration (Schwartzberg and Chao 1982, Wang *et al*. 2000). In this study the salt content at most temperatures tested increased more rapidly in fillets than in whole fish becoming stable after about 15 days. In contrast, the salt content increased relatively slowly in the samples which were stored at -24 °C. In the cold brining, there is a slower progression and it takes a long time to reach the equilibrium of salting than in the salting at higher temperature.

An increase of brine concentration increases the equilibrium muscle salt content and decreases the equilibrium muscle water content, thus causing a higher final muscle salt content at higher brine concentrations. A significant effect of brining temperature was detected between 2 °C and -24 °C within the 26 days of the experiments. Similar results were reported by Sveinung *et al*. (2005) on brine salting of herring fillets, and by Thorarinsdottir *et al*. (2004) on the brine curing of cod. Additionally, the presence of skin-on whole fish has been shown to provide an effective barrier against salt penetration (Sakai and Suzuki 1985, Ravesi and Krzynowek 1991), and to some extent, this result is in agreement with information in the literature of Roger *et al*. (1984) on the salting of herring fillets with 14% salt and 7% acetic acid brine.

The water and fat contents at the beginning ranged between 65-68% and 13.4-13.8% respectively, and in comparison with previous research showed similar result (Stroud 2001). Sallam *et al*. (2006) reported that the brining and marinating processes decreased the moisture contents. Similarly the water content of whole herring at -2 and -8°C decreased rapidly during the first three days becoming more stable after 10 days. In contrast, the water content of fillets increased at first, but started decreasing after 7 days, whereas the weight increase stabilized. The fast water and salt content changes in herring fillets in comparison with beheaded herring confirmed earlier results (Magnusson and Ogmundsson 1983). In general, changes observed during salt uptake (weight, salt and water content changes) appeared to follow a typical pattern for salted herring and fillets during the first days of salting (Stefansson 1990). The most obvious reasons for fast salt and water changes observed in fillets were their thinness in comparison with whole herring and the fact that they did not have skin. Moreover the fat layer which lies immediately under the skin was also removed with the skin, but fat slows salt penetration since salt diffusion only occurs in the aqueous phase of tissue (Aitken and Baines 1969; Kiesvaara 1975).

The weight changes both in the fillet and whole herring were measured in relation with the salt content. Generally, the fastest weight gain between days of brining was observed between day 0 and day 1 in fillets both for temperatures. This indicates that equilibrium between the salting medium (brine) and the interior muscle tissue of herring had not been achieved, causing an influx of salt and water gain of fish muscle. An increase in flesh salt content continued even after 7 days. And it indicates that equilibrium conditions were retarded at below 0 °C compared to higher temperatures. Increased brine concentration

reduces a little the weight gain of whole herring during 15 days in brine at both -2 and -8 °C. A significant effect of the presence of skin on the weight gain of herring was also observed by comparison of fillets and whole fish. Whole herring with skin on showed a lower weight gain than skin-off fillets. By comparing the effect of skin between the groups (skin-on herring 14% at -2 °C brine and skin-off fillet 12% at -1 °C brine), the weight gain in the skin-off fillet was around 20% higher than skin-on herring. This result is consistent with the report from Sveinung *et al*. (2005), cured with the 10%, 16.5% and 25.5% brines. Earlier studies showed that maximum solubility and swelling of myofibrils is obtained at 4.6% (0.8 M) salt concentration in the liquid phase of the muscle, but at higher salt concentrations (10-13%) proteins start to denature, which leads to dehydration of the muscle with further increases in salt content (Barat *et al*., 2002).

As mentioned above, there was no sign in the deterioration progress of herring during cold brining for the duration of the experiment (26 days), because the multiplication and proliferation of putrefactive bacteria and the chemical changes by autolytic enzymes or other elements had been controlled or partially prevented by the co-effects of ice and salt. Besides, the salted products became tasty, by delaying the breakdown of muscle protein and could be stored for a longer time.

6 CONCLUSION

The following conclusions can be drawn from the findings of the experiments carried out in this study.

The progress of cold brining involves the following work elements; reception of raw material, cleaning, dripping, pre-cooling, brining with salt and storage.

The 14% brine at -8 °C can be regarded as optimum condition for pre-cooling of catches. The cooling time will take about 20 minutes in the brine, in which the ratio of fish to brine is 1:2.

The cold brining of herring should be done by a mixture of salt and brine. The amount of salt suggested is 10% to the weight of raw fish and some pre-cooling brine should be removed before the addition of the salt, so that the resulting ratio of fish to brine becomes 1: 0.2.

The herring salted by the cold brine can reach the equilibrium of salting more than 20 days later. At this time, the fish muscle could not be frozen because the speed of salting would be preceded with the one of freezing in the fish muscle.

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APPENDIX

Appendix 1: Quality Index Method (QIM) Scheme of herring

Start Date: Sample No. (Stored at)

Quality parameter		Description	Score	Storage time (day)		
Whole fish	Appearance of skin	Very shiny	0			
		Shiny	1			
		Matt	2			
	Blood on gill cover	None	0			
		Very little (10–30%)	1			
		Some (30–50%)	2			
		Much (50–100%)	3			
	Texture on loin	Hard	0			
		Firm	1			
		Yielding	2			
		Soft	3			
	Texture of belly	Firm	0			
		Soft	1			
		Burst	2			
	Odour	Fresh sea odour	0			
		Neutral	1			
		Slight off odour	2			
		Strong off odour	3			
Eyes	Appearance	Bright	0			
		Somewhat lustreless	1			
	Shape	Convex	0			
		Flat	1			
		Sunken	2			
Gills	Colour	Characteristic red	0			
		Somewhat pale, mat, brown	1			
	Odour	Fresh, seaweedy, metallic	0			
		Neutral	1			
		Some off odour	2			
		Strong off odour	3			
Quality Index Sum (0 – 20)						

Appendix 2: Measurement of biogenic amines (histamine, tyramine, putrescine and cadaverine) in fish. Method applied by The Icelandic Fisheries Laboratories.

- Preparation of solvents/buffers

Solvent B (10 % acetonitrile and 90 % sodiumdihydrogenphosphat dihydrate (0.075 M): 23.4 g of the buffer substance were solute and made up to 2 L in a volumetric flask. It was mixed with acetonitrile in ratios 1:9 - acetonitrile: buffer and filtered through 0.4 μm filter before use.
Solvent C (60 % acetonitrile and 40 % sodiumdihydrogenphosphate dihydrate (0.075 M): Phosphatebuffer (0.075M) as previously described was mixed with acetonitrile in ratios 6:4 acetonitrile: buffer and filtered through 0.4 μm filter before use.
Solvent D: 100 % acetonitrile was filtered through 0.4 μm filter.
Boric acid buffer (0.4M pH 10.8): 24.73 g boric acid and 21g potassium hydroxide were solute and pH adjusted to 10.8 with 6 M KOH. Then it was diluted up to 1 L in volumetric flask.
Trichloroacetic acid (TCA) solution (10% w/v): 200 g trichloroacetic acid were dissolved in water and diluted to 2 L in volumetric flask.

- Extraction of biogenic amines

Accurately 25 g of sample weighted into a glass container and homogenized for 45 seconds in ca. 40 ml 10% TCA. The extract is filtered through Whatman 542 filter paper (or similar) under vacuum and made to 100 ml in a volumetric flask. Mixed thoroughly and then a small amount was filtered through a 0.45 μm filter (Millipore).

- Preparation of reagent o-phthaldialdehyde (OPA)

90 mg OPA weighted into a 10 ml volumetric flask. 1 ml methanol were added and solute before 0.2 ml 2-mercaptoethanol was added. They were made to 10 ml with boric acid buffer pH 10.8. It was mixed thoroughly. The solution was made fresh every two days and stored in the refrigerator.

4. Derivation

0.25 ml of sample/standard was added to 0.5 ml OPA reagent in a test tube with a screwed cap. The solution was kept in dark for exactly 3.5 minutes. Then 2 ml ethylacetate was added and vortexes for 1 min. It should be waited until phase separation was completed. Then an aliquot from the top phase was pipette in a vial and exactly 3.5 minutes after addition of ethylacetate the sample/standard was injected for analysis.

5. Standards and quantification

Exactly 100 mg of each standard was weighted and made to 100 ml with 10% TCA in volumetric flasks. A stock solution made by mixing the four amines in volumes which were about 10 mg/100 ml in concentration. Suitable dilutions then were made for a standard curve preparation, for example dilutions of 1/2 - 1/4 -1/8 - 1/16 - 1/32 and 1/64. Quantification of samples was by area measurement determined from a standard area versus concentration plot.

- Calculation

2 or more runs of each standard were made and by linear regression (optimum R^2>0.99) the samples were quantified and expressed as g/kg fish or other units.