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THE EFFECT OF DIFFERENT COOLING SYSTEM ON QUALITY OF PELAGIC SPECIES

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ABSTRACT

Proper handling, rapid chilling and storage of fish on boats ensure the quality of fish. In Sri Lanka, pelagic fatty species such as tuna and various small species sustain great post harvest losses as improper cooling and storage of fish on board boats. Therefore, effective cooling systems are needed to improve the quality of the fish. This study attempts to investigate the quality of a typical pelagic fatty species, herring, stored in three different cooling systems. The cooling systems were designed from ice and sea water (CSW), ice and fresh water (CFW) and pure ice. The chemical and microbiological parameters were monitored as indicators of quality changes of herring. The results of this study show rate of microbiological growth of herring in the CSW is slower than in pure ice. The fat and protein content of herring in the CSW system decreased more than in the pure ice system while the salt content increased steadily in CSW during storage. Herring in CSW system was of better quality during experiment. The CSW cooling system could be introduced for rapid cooling and storage at low temperatures of tuna and small pelagic fatty species in Sri Lanka. The cool sea water (CSW) systems could be prepared by mixing ice and sea water with fresh water to reduce the salt uptake and keep the salt content of fish below 0.7%.

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1. INTRODUCTION

The world fisheries showed a steady increase in landings during the 1980s. Much of the increase can be attributed to three pelagic species, the Peruvian anchovery, the south American and Japanese sardines and the semi-demersal Alaskan pollock (FAO 1989). By-catch is seldom a problem in pelagic fisheries, because of the schooling behaviour of most of the target species.

In recent years fishing vessels have become more specialised and on-board processing has increased. Increased demand for high quality raw materials for all types of products has put a pressure on the fishermen to improve handling and storage of the catch. The special grades of fish meal demand fresh raw materials, which calls for rapid and efficient chilling. The technology and utilisation of refrigerated sea water (RSW) and chilled sea water (CSW) systems has developed considerably over the last few decades in industrial countries. The advantage of RSW and CSW systems has mainly been demonstrated for cold water fish species. However, little information is available on the application of these systems for storage of tropical fish species in developing countries like Sri Lanka.

The fisheries sector in Sri Lanka has undergone a transformation from an entirely artisanal fishery in the 1950s to a more semi-industrial fishery today. At present there are about 10,000 outboard engine boats and 15,000 non-motorised traditional craft operating in coastal fisheries. These boats catch mainly skip jack, seer fish, grouper fish and small pelagic species like herring and some mackerel species. In 1999 there was a total catch of 171,000 metric tons landed from coastal fishery. The fishing fleet in Sri Lanka now consists of about 3,000 inboard engine boats. Most of them are multiday boats, 10 to 15 meters long. They catch around 60,000 metric tons per year, mainly large tuna species and shark (MFARD 2000). In the 1990s, the objective of the Ministry of Fisheries and Aquatic Resources Development (MFARD) was to increase production from deep sea fisheries because fish resources in the coastal zone were reaching their optimal exploitation level. To achieve these objectives the ministry implemented several programmes, such as subsidies for buyers of new boats and equipment.

As a result of mechanisation the fishery has expanded to offshore and deep sea areas. There was a 70% increase in production in the offshore fishery from 1994 to 1998 (Figure 1). Also, the exports of fish increase significantly from 1995 to 1998 because of increased catch from deep sea fishing. However, there was not a corresponding improvement in the quality of the catch.

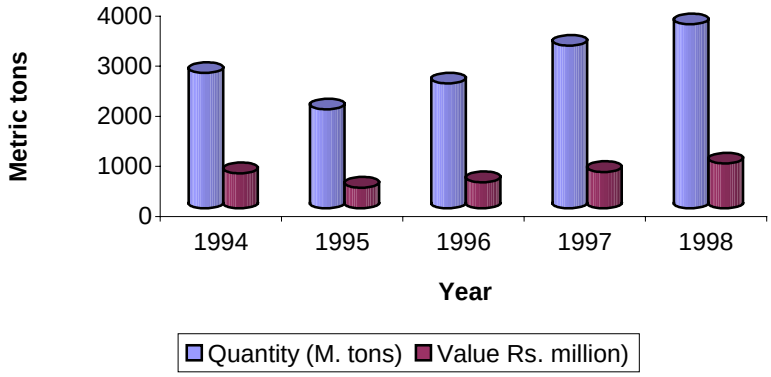

Figure 1: Exports of fish from Sri Lanka year 1994 to 1998.

During the last fifteen years, exports of fishery products from developing countries to more industrialised countries has increased. Increased exports call for stringent quality control measures to be implemented. Rapid cooling and maintenance of low temperatures of the fish on the boats is one of the key factors to ensure the quality and safety of fish products.

A fishing trip of a multiday boat normally takes 10 to 15 days. They mainly use gill nets which are set for 4 or 6 hours before hauling. Normally, three or four crew members are engaged in the hauling which takes an hour or more depending on the catch. The fish is then collected from the net and stored in ice in the fish hold. Often the fish stays on deck for a long time before being stored in ice in the hold. If the fish is caught during the day it gets exposed to the sun and wind leading to an increased core temperature. The ungutted fish is kept in crushed ice to chill. Often, the ice is not distributed evenly in layers. The fish is not submerged as if liquid cooling media is used. Because of delayed cooling, inadequate icing and poor quality ice, such fish is usually of poor quality. The average core temperature of fish landed by multiday boats is normally around 3.8°C. It is estimated that inadequate cooling contributes to post harvest losses of 10 to 15% of the total catch from multiday boats (NARA 1998).

During the herring and mackerel fishing season, small fishing boats operate near the coast and fish is landed within 6 - 8 hours. Fishermen mainly use gill nets or purse seines to catch large quantities of fish which is normally iced after landing. Herring and mackerel are small species with high fat and water content (normally 1-24% fat and 60-81% water) which deteriorate easily if not cooled as soon as they are caught. Proper handling and storage of fish at sea is needed to ensure that the catch stays as fresh as possible until it is landed. A proper handling system is needed on board to cool the fish to a storage temperature of just above freezing point. Alternative systems are necessary, since the traditional method of icing in boxes or fish hold in multiday boats takes too long to cool the fish and causes great decline in quality.

The CSW systems are fast and effective chilling methods. However there are some disadvantage of CSW systems such as salt uptake of the fish because of high salt concentration in the sea water which is used as cooling media (Hansen 1983). RSW systems provide rapid cooling and achieve lower temperatures than ice. RSW systems involve less handling and achieve uniform holding temperatures of pelagic species which is important to reduce oxidative rancidity and textural deterioration.

The pelagic fatty species such as skip jack, tuna and herring are very important for domestic consumption because of the nutritional properties of polyunsaturated fatty

acids. Big tuna species are valuable and in high demand for the export market. Tuna has high proportion of "dark muscle" which is very fatty. The lipid content of the dark muscle is highly seasonally variable.

The lipids in fish muscle are easily oxidised under unfavourable conditions. Pelagic fatty fish tend to be more susceptible than other species because many of the species such as mackerel and herring are small species which are caught in large quantities, rarely eviscerated immediately on capture and chilled inadequately. Under these conditions autolytic enzymes in muscle hydrolysis of protein and lipid as well as other chemical changes that influence the flavour in the post mortem and quality of fish goes down very fast.

It is necessary to introduce an effective cooling system in the fishing industry in Sri Lanka to reduce post harvest losses and improve the quality of fish in the fish processing establishments. The objective of this study is to evaluate three different cooling systems for storing herring.

2. LITERATURE REVIEW

2.1 CSW cooling systems

In CSW systems, sea water surrounds the fish. Initial cooling is rapid and uniform temperature is maintained throughout the system. Studies have shown that the chilling rate for herring can be from 15°C to 0°C within two hours (Kelmann 1977).

The CSW systems ensure a uniform temperature by the application of a so-called “Champagne” method. In this method, heat transfer between fish and cooling media is assisted by agitation with compressed air introduced at the bottom of the tanks. CSW "Champagne" systems can be used in small coastal vessels, e.g. in a fishery for small pelagic fish with vessels of 10-14 m length and carrying capacity from 3 to 10 tons (Roach 1980).

The CSW systems can be used in insulated tanks or containers which are used to store fish on board in order to obtain maximum shelf life of fish. It is important to maintain a homogeneous temperature in the region of -1°C through out the storage period. Effective water circulation to ensure the uniform temperature depends on fish size and forms (Kraus 1992). Small fish species cool quickly if properly handled with a CSW system. Insulated containers of 2 m^3 could be used as CSW tanks. The main advantage of the method is that fish will be undisturbed until processing and are easily unloaded.

Many of the pelagic resources are found near the coast and are easy to catch from small vessels. The facilities for chilling are important, particular when ambient temperatures are high (> 15°C). Bulk storage of fish on board without chilling is possible during cold periods if the landing site is a short distance away. Small vessels can improve handling and increase quality of the catch by using boxes or small insulated containers with CSW. It is important to avoid anaerobic conditions in containers. Air can be pumped through pipes at the bottom of the containers to this end (Jensen and Hansen 1983).

In many countries, canning is the most important utilisation of small pelagic species. The quality of small pelagic fish for canning depends on ambient temperature, the chilling rate on board and on the loading / unloading method. Pelagic fish species are always landed ungutted and are rarely size graded. They are usually landed within 4 or 5 days as a whole batch with ice. Rapid chilling is therefore of utmost importance for such fishery because only a few hours delay at 15 - 20°C will reduce the shelf life of the fish by several days (Hansen *et al*. 1980).

Refrigerated sea water (RSW) systems as a medium for cooling, storing and transporting fish has many advantages such as a more rapid cooling of the fish, significant labour saving and elimination of damage and shrink losses due to excess pressures that often occur when fish is iced. These are also disadvantages such as uptake of water by lean fish species and an increase in total salt content. Controlling the growth of spoilage bacteria in fish stored in RSW also presents a problem.

2.2 Chilling process of fish

Chilling of fish bulk is a process by which the temperature of the fish is lowered to a point near the freezing point of the fish flesh but not below, by means of heat withdrawal. The freezing point for different fish species varies between -0.6 and - 2°C and depends on the concentration of the cell fluids. It is usually taken to be equal to -1°C. Chilling depresses the activity of putrefactive micro-organisms and enzymes which cause deep changes and spoilage of fish (Zaitsev 1965).

The chilling time depends on the properties of both the cooling media and the product, such as thermal indices of the product, specific gravity, temperature of the surrounding media and heat transfer coefficient between the fish and the medium. For some decades, chill storage on board has mainly been done by alternating layers of ice and fish on shelves or in boxes in fish holds. Freshly iced fish cool slowly and unevenly because most of the fish are not in contact with the ice. Cooling is accomplished by contact with other cooler fish and by melt water draining through the catch. Cooling rates also depend on the ratio of surface of fish exposed to ice per unit of weight. Small species such as shrimp, sardines, anchovies and jack mackerels cool fast with ice. When large fish is stored in ice, the temperature equilibrates at 2-3 °C after few hours. Rapid chilling of fish is achieved by the use of a chilling media with high thermal indices, maintenance of lowest possible temperatures of the media during the chilling process and the circulation of the liquid or gaseous media. Chilling is much more rapid in a liquid medium than a gaseous one. The cooling rate of sardines is 190% faster in cold sea water with no circulation, and 420% higher in circulating sea water than the firnly crushed ice (Zaitsev 1965).

The initial quality and microbial load of fresh finfish is affected by the method of harvesting. It is at this point that quality maintenance must begin. Fish caught by hook and line die or are killed relatively quickly and the method minimises stress, an attribute related to deterioration (ICMSF 1998). When trawling, longer tows will generally result in lower quality. During periods of heavy fishing the cod end of the trawl net becomes very full and the resulting catch, which may have been dead for hours, is bruised and crushed from compression. Fish entangled in gill nets struggle, which in turn quickens the onset of rigor and subsequent deterioration (Mayer and Ward 1991). Bacteria may enter the fish through wounds and bruises during the death struggle and multiply rapidly.

Furthermore, if fish is subjected to physical abuse on deck and exposure to ambient temperatures and sunlight, quality may be further affected.

Tuna has a relatively high metabolic rate and some species have the ability to regulate their body temperature. When tuna is captured in a highly stressed state, the build-up of lactic acid in the muscle combined with elevated muscle temperatures results in a serious flesh defect known as burnt flesh (Goodrick1987). The flesh is no longer bright red and the flavour is acidic with a metalic aftertaste. A rapid reduction to low uniform temperature in a CSW system may help to ensure that tuna caught in this state will be accepted for the highly lucrative Japanese sashimi market.

2.3 Microbiological spoilage

The natural bacterial flora resides mainly in the outer layer of the skin, on the gills and viscera of the fish. It may penetrate the muscle and start post mortem spoilage. Bacterial growth depends on intrinsic and extrinsic factors. The initial microflora depends more on the environment in which the fish is caught than on the species, while the total microbial load is subject to seasonal variation (Shewan 1961). 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).

2.3.1 Specific spoilage micro-organisms

The microbial flora on fish depends on many environmental factors, but the same relatively few genera predominate. These are the Gram-negative *Pseudomonas, Alteromonas, Shewenella, Vibrio, Aeromonas* genera and the Gram-positive *Micrococcus* species and the coryneform group. In general the Gram-negative bacteria predominate in fish caught in cold waters and the Gram-positive are more numerous in warmer waters (Liston 1980, Huss 1994).

According to Gormley (1990) not all strains of Gram-negative bacteria produce the spoilage changes. 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.

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 (Precott *et al*. 1996). Spoilage bacteria grow even at low temperatures in RSW

systems in the presence of NaCl because they are adapted to the environment where they are caught (Silva *et al*. 1998).

2.4 Autolytic spoilage

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 involves in particular digestive proteolytic enzymes in the autolytic spoilage. The concentration and activity of digestive enzymes is high in the guts and upon death soon begins to digest the gut walls and surrounding tissues. The enzyme activities and other related reactions do not immediately cease in the fish muscle upon death (Howgate 1982). It caught during a period of heavy feeding the belly of certain fish (e.g. herring, capelin, sparots and mackerel) is very susceptible to tissue degradation and may burst within a few hours of catching (Huss 1988). Autolysis in combination with rough handling results in belly bursting which is very dependent on storage time and temperature (Hansen 1983, Hansen *et al*.1980). Unless properly cooled, herring caught in summer can become unsuitable for smoking in 1 day (Whittle *et al.* 1978).

2.5 Acidity (pH) change during spoilage

The initial post mortem pH varies with species, catching ground and season. Usually pH decreases during anaerobic formation of lactic acids during the first hours after death. During later post mortem changes, pH increases slightly because of the formation of alkaline compounds (Huss 1988). Post mortem pH is low in heavily feeding fish. This may affect autolytic spoilage as connective tissues of fish weaken at low pH (Love 1980). The activity of protease enzymes also changes in relation to pH and salt content. According to Granroth *et al*. (1978) studies have shown the digestive enzymes cathepsin (D) to be quite salt-tolerant. In pyloric caecae in herring some carboxy peptidases have been found which are even more salt-tolerant, i.e. up to 25% NaCl.

Microorganisms frequently change the pH of their own habitat by producing acidic or basic metabolic waste products. Spoilage bacteria on fish make their environment more alkaline by generating ammonia through amino degradation. This leads to proteolysis and the anaerobic breakdown of protein or putrefaction, which releases foul-smelling amine compounds.

2.6 Chemical spoilage (oxidation)

In fatty fish, such as herring, the most important changes taking place in the lipid fraction and 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 the ammonia and sulphite 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 1998). At a low uniform temperature and reduction in available oxygen, the development of oxidation rancidity is retarded. Textural deterioration are also retarded.

2.7 Salt uptake of herring in CSW cooling systems

Salt uptake is probably the most important factor which limits the application of CSW systems. Fish intended for normal processing and marketing can acquire a salty flavour, making them unacceptable. The salt uptake in industrial fish is also critical since it is concentrated during fish meal production. The upper limit is usually equivalent to a NaCl concentration about 0.5% in the raw fish (Graham 1995). In the fish meal industry NaCl concentration is acceptable up to 0.7%.

3. MATERIALS AND METHODS

3.1 Experimental design

The herring used for the experiment was caught off the east coast of Iceland on 19 November 2001 by the local fishing boat “Beitir NK” using a pelagic trawl. The catch had been stored in chilled sea water (CSW) on board until it was landed. At landing on 20 November 2001, a sample of 1050 kg herring was taken for use in the experiment at the fish meal factory in Neskaupstaður. The herring was 2 days old at the beginning of the experiment.

The cooling systems were prepared by using ice, sea water and fresh water in different ratios in three clean insulated tubs of 600 l capacity (Table 1). Ice, sea water and fresh water was obtained from the fish meal factory.

Table 1: Preparation of cooling systems

Cooling system	Ingredients	Ice : liquid ratio
System 1 (CSW)	ice and sea water	70 : 30
System 2 (CFW)	ice and fresh water	70 : 30
System 3	pure ice	

A total of 350 kg of herring was placed into 150 kg of media in each of the three cooling systems (ratio 7:3). The following quality parameters were monitored in each cooling system during storage time.

- Acidity (pH) in herring and cooling liquid
- Salt content in the flesh of herring stored in each cooling system.
- Fat content in herring and cooling liquid
- Protein content in herring and cooling liquid
- Water content in herring and cooling liquid
- TVN in flesh of herring in each cooling system
- Total Plate Count (TPC) in herring and cooling liquid.

One measurement of pH, TPC, fat, water, and TVN content of sample and two measurements of salt and protein content on sample were taken each time.

At the beginning of the experiment, day 0, five herring were randomly selected from the sample group and tested for pH and all the chemical and microbiological parameters listed in Table 2. The same measurements were done for each of the three different cooling systems prior to the storing of herring. The sampling schedule during the experiment is shown in Table 2.

Table 2: The sampling plan for herring and cooling liquid

Sample	**Parameters for analysis**	**Sampling day**							
		0	1	2	3	4	5	6	7
Herring	Protein	x							x
	Fat	x							x
	Water	x		x		x			x
	Salt	x		x		x			x
	TVN	x		x		x			x
	pH	x	x	x	x	x	x	x	x
	Core temp.	x	x	x	x	x	x	x	x
	TPC (22°C)	x			x				x
	Coliform count								
	Total	x							x
	Faecal	x							x
Cooling system	Protein								x
	Fat								x
	Water								x
	Salt	x				x			x
	TVN				x				x
	pH	x	x	x	x	x	x	x	x
	Temp.	x	x	x	x	x	x	x	x
	TPC (22°C)	x			x				x
	Coliform count								
	Total	x							x
	Faecal	x							x

All laboratory analysis was carried out at the Icelandic Fisheries Laboratory (IFL) in Neskaupstaður.

3.2 Physical methods

A probe thermometer was used to measure the core temperature of one randomly selected herring from each of the systems.
The temperature of the cooling liquid was measured in the upper layer, 10 cm below the surface. At the same time the ambient temperature was measured using the same thermometer. The temperature in lower layer of each cooling system was recorded continuously throughout the experimental period, using one thermocouple type T sensor, Optiz Stow Away thermometer (Computer Corporation, Massachusetts, USA) fitted 10 cm from the bottom of each containers.

3.3 Chemical methods

Acidity (pH) was measured using the digital glass calomel electrode pH meter (CG-838, Schott-Gerate, Germany) Glass calomel electrode was dipped to in minced herring sample and cooling liquid samples.

The salt content of the herring and the cooling liquid was determined by the Volhard method (JAOC 1937, AOAC 1990a). A sample of 5.00g of minced fish was placed in a 250 ml centrifuge bottle and 200 ml of distilled water added and shaken for one hour, in a shaker (back and forth or wrist action). A 20 ml of aliquot was pipetted into an Erlenmeyer flask and 5 ml of 0.100N $AgNO_3$ and about 2 ml of indicator added. Titration was done with 0.100N NH_4SCN and $FeNH_4(SO_4)_2$ solution.

The fat content in herring and cooling liquid was determined by the method of A.O.C.S Official Method (AOAC 1990b). A sample of 10.00 g was weighed and mixed with about two table spoons of sodium sulphate (Na_2SO_4) and one tea spoon of sodium chloride (NaCl) and then put into a shaking bottle. 100 ml of petroleum spirit (pet-ether) was added. It was shaken for two hours in a shaker. A 25.0 ml of sample solution was pipetted into a 150 ml Erlenmeyer bottle and placed on a hot plate. Once the ether had evaporated, the flask was dried in a heating oven at 102 - 105°C for 20 minutes. After cooling and it was weighed again. Fat content in the sample was determined by the difference in weight.

The water content in herring and the cooling liquid was determined as the loss of weight during drying at 105°C for 4 hours (ISO 1983). A container with a glass rod was weighed and 5.00 g of the sample weighed in the container. The glass rod was used to spread the sample in the container. The glass rod was left in the container. The container was placed in the oven at 102°- 104°C for 4 hours. Then it was removed from the oven and allowed to cool to ambient temperature in a desiccator and weighed.

Protein content was determined by a version of the original Kjeldahl method (ISO 1979). A 5.00 g sample was mixed with K_2SO_4 and a little of $CUSO_4$ as a catalyst and digested in long necked Kjeldahl bottles with conc. sulphuric acid for approximately 2 hours (One hour after the contents became clear). After that cooling water was added. The long necked Kjeldahl bottles were placed in Kjeltec Auto sampler 1035/30 system where the ammonia was distilled into boric acid and the acid was simultaneously titrated with diluted H_2SO_4.

Total Volatile Nitrogen compounds (TVN) were determined using steam distillation, followed by titration method (AOAC 1990b). A sample of 10.00g was weighed into distillation tube. 3 g of $Ma(OH)_2$ was added and steam distilled with 100 ml of water for 15 minutes. Evaporated NH_3 was collected into 50 ml boric acid solution before titrating with 0.1038 N H_2SO_4 solution.

3.4 Microbiological methods

The basic methodology used at Icelandic Fisheries Laboratory in Neskaupstaður is according to the Compendium of Methods for the Microbiological Examination of Foods published by the American Public Health Association (APHA-1992).
The methods used for individual tests are briefly described below.

A primary dilution was prepared by homogenising 25 g of fish sample with 225 g of buffer using a Seward stomacher 400 lab. system. Decimal dilutions were prepared in buffer and 1 ml of appropriate dilutions poured on a plate with iron agar media with 0.5% NaCl and incubated at 22°C for 72 hrs for detection of psychotrophic bacteria. The conventional "pour-plate" method was used. The number of colonies counted thus constitutes the total plate count (TPC).

Decimal dilutions of cooling liquid were prepared in buffer using liquid sample drawn from the cooling systems and 1 ml of the appropriate dilution poured on plate with iron agar media with 0.5% NaCl and incubated at 22°C for 72 hrs for psychotrophic bacteria. The conventional "pour-plate" method was used. The number of colonies counted thus constitutes the total plate count (TPC).

Using the Most Probable Number (MPN) method fish and cooling liquid samples were tested for total and faecal coliforms. Pre-enrichment was done in LST broth at 35°C for 24 hours and conformation tests were done in BGLB broth for total coliform at 35°C for 48 hours and in EC broth for faecal coliforms at 44°C for 24 hours. The same primary dilution of fish sample was used to prepare decimal dilutions with buffer. Three tubes were used for each dilution. Ten ml of the 1/10 dilution were need to inoculate 10 ml of double strength LST broth, 1 ml in single strength LST and finally 0.1 ml in single strength LST.

The five tube method was used for cooling liquid. A 10 ml of liquid sample was inoculated into each of the 5 tubes of double strength LST in first row, 1 ml into each tube in the next row of single strength LST and 0.1 ml into each tube in the final row. After inoculation the LST tubes were incubated at 35 ± 0.5°C. The presumptive test was considered positive if gas was produced within 48 ± 3 hrs. Positive tubes were then used as confirmation test for total and faecal coliforms. When no gas formation was observed after 48 ± 3 hrs incubation in LST test, the sample was considered negative, i.e. no coliforms were present in the samples.

One loop-full was removed from all LST tubes showing signs of gas formation after 48 hrs (positive sample) and inoculated into 10 ml of BGLB. The BGLB tubes were inoculated at 35 ± 0.5 °C for 48 ± 3 hrs. Gas production confirmed that coliforms were present in the sample.

A test for faecal coliforms was run parallel to the total coliform test. All LST tubes showing sign of gas formation after 48 hrs were inoculated into 10 ml of EC broth with a loop. The EC tubes were incubated at 44.5 ± 0.2 °C in a water bath for 24 ± 2 hrs. Gas production then confirmed that faecal coliforms were present in the sample.

4. RESULTS

4.1 Temperature

The average ambient temperature was 4.9 ± 1.6 °C during the time of the experiment. The initial core temperature of the herring was 3.0°C. The core temperature in the CSW system went down to - 0.5 °C during the first day, and remained at below 0°C for the entire experiment. The temperature of the fresh water systems (CFW and pure ice) was consistently higher, ranging from 0.3 - 1.7 °C, where the higher value was for the CFW system (Figure 2).

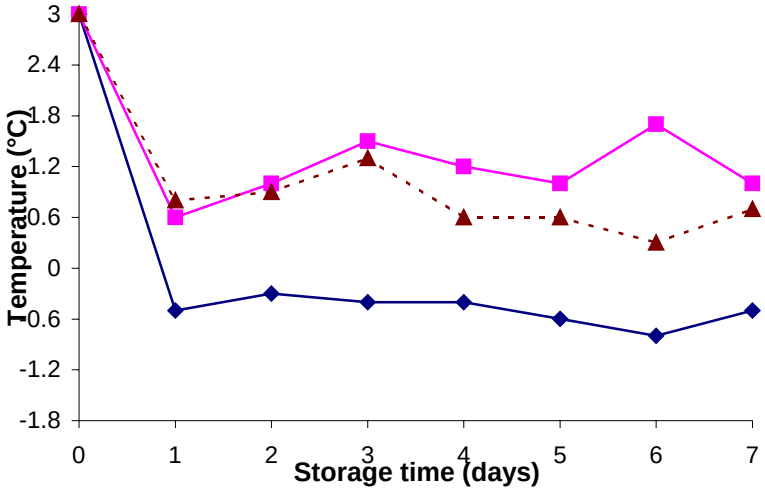

Figure 2: Changes in core temperature in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage time.

The change of temperature in the upper layer of the cooling liquids was - 1.2 to 0 °C in CSW, 0.5 to 1.0°C in CFW and 0.1 to 0.7°C in pure ice from day 0 to day 7 of storage (Figure 3).

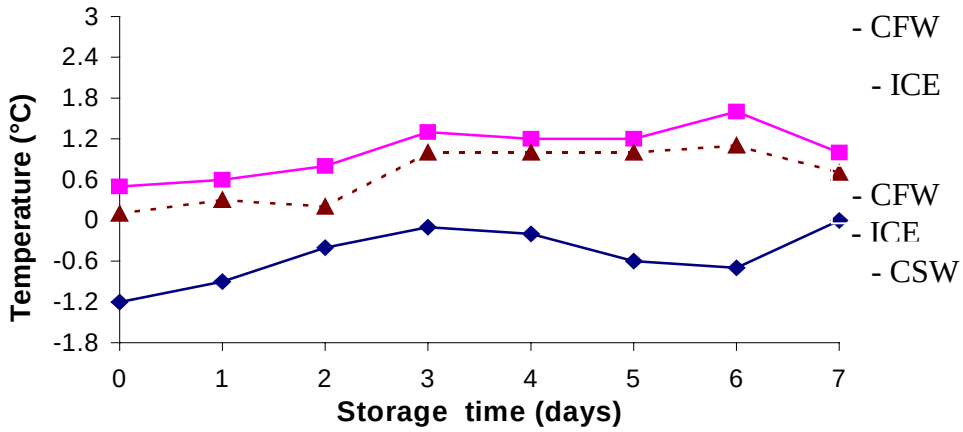

Figure 3: Changes in temperature in upper layer of cooling liquid in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage time.

The temperature in the lower layer of the cooling liquids was -1.6 to -0.3 °C in CSW, 0.0 to 0.4 °C in CFW and 0.3 to 0.7 °C in pure ice during storage.

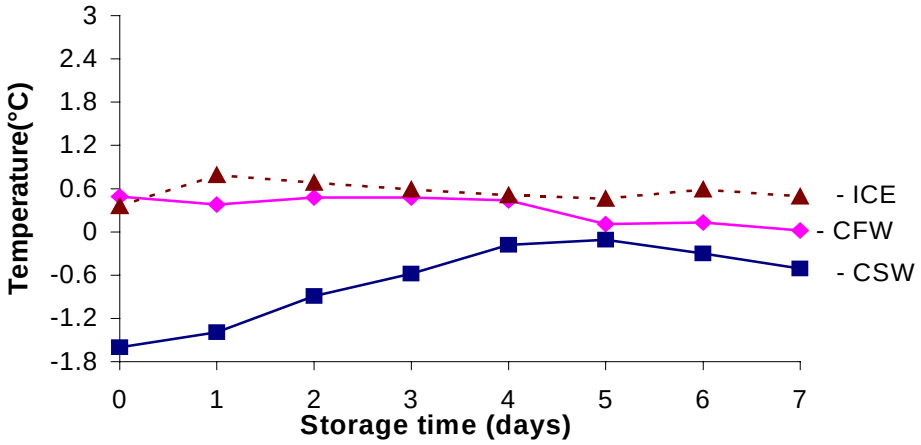

Figure 4: Changes in temperature in lower layer of cooling liquid in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage time.

4.2 pH

The initial pH in the herring was 6.55. During storage time the pH in herring rose in all systems with the greatest fluctuation in the CSW (Figure 5).

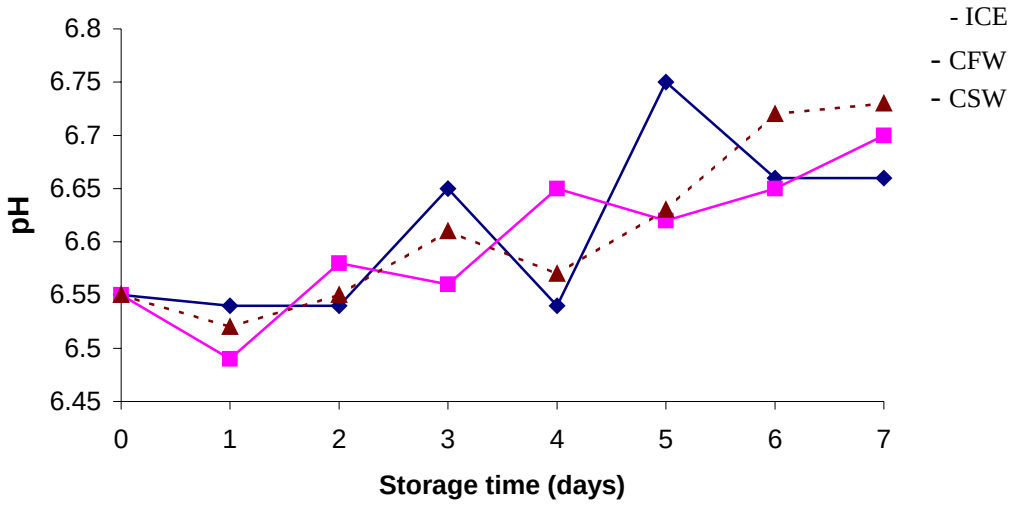

Figure 5: Changes in pH in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage time.

The initial pH in cooling liquid was 6.68 in CSW, 6.23 in CFW and 5.93 in the pure ice system. During storage pH of the cooling liquid increased in all systems. From day 2 to day 5 pH of the cooling liquid in CSW and CFW was higher than in the pure ice system. Acidity (pH) in the cooling liquid in each cooling system reached around 6.5 on the first day of storage (Figure 6).

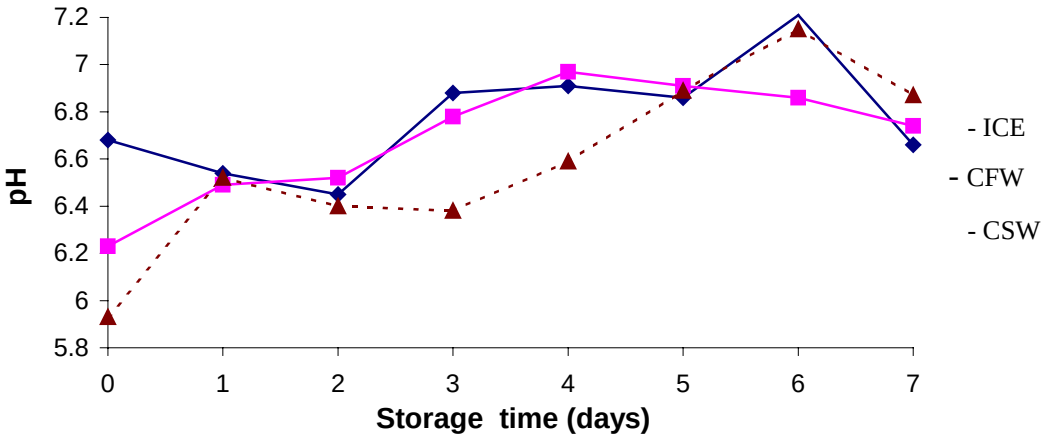

Figure 6: Changes in pH in cooling liquid in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage time.

4.3 Salt content

At the beginning of the experiment, the salt content in herring was 0.625± 0.005%. During the experimental period, the salt content increased in the herring stored in the CSW system while it showed a decreasing trend in the two freshwater systems. On the seventh day of storage, the salt content of the herring was 0.795 ± 0.005 % in CSW, 0.54± 0.02% in CFW and 0.42±0.14 % in the pure ice system (Figure 7).

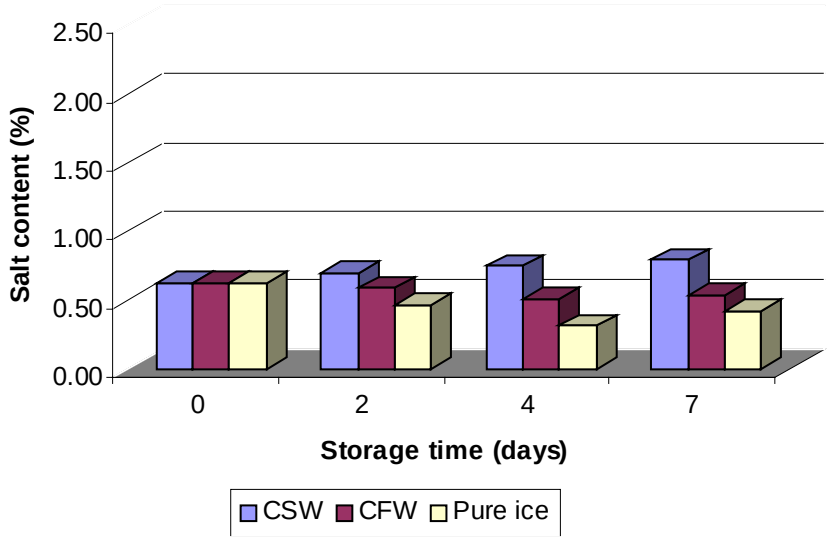

Figure 7: Changes in salt content in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage.

The salt content of the cooling liquid in the CSW system decreased during storage, while it increased from 0 to 0.58% in CFW and 0.51% the pure ice system on the seventh day (Figure 8).

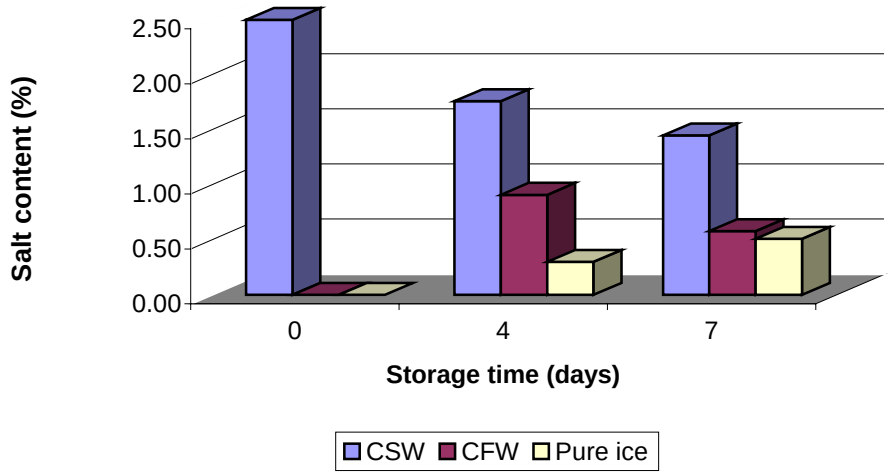

Figure 8: Changes in salt content in cooling liquid in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage.

4.4 Fat content

Fat content in herring was 15.40% before storing in the cooling systems and had decreased to 13.98% in CSW, 14.0% in CFW and 15.12% in the pure ice cooling system by the seventh day (Figure 9). At the end of the experiment, the fat content of herring in CSW system was lower than the fat content of herring in the CFW and the pure ice systems.

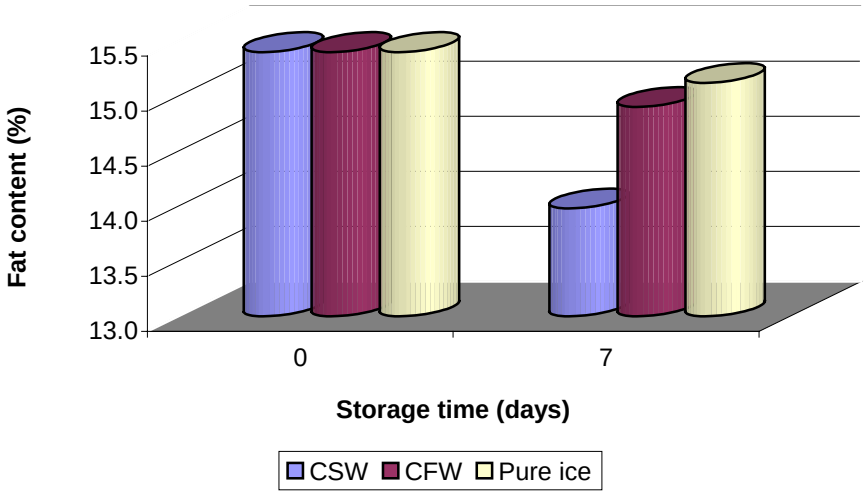

Figure 9: Changes in fat content in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage.

4.5 Water content

The water content of fresh herring was 65.40% at the beginning. It rose in all the systems reaching to 69.69% in CSW, 68.63% in CFW and 67.05% in the pure ice on the seventh day (Figure 10).

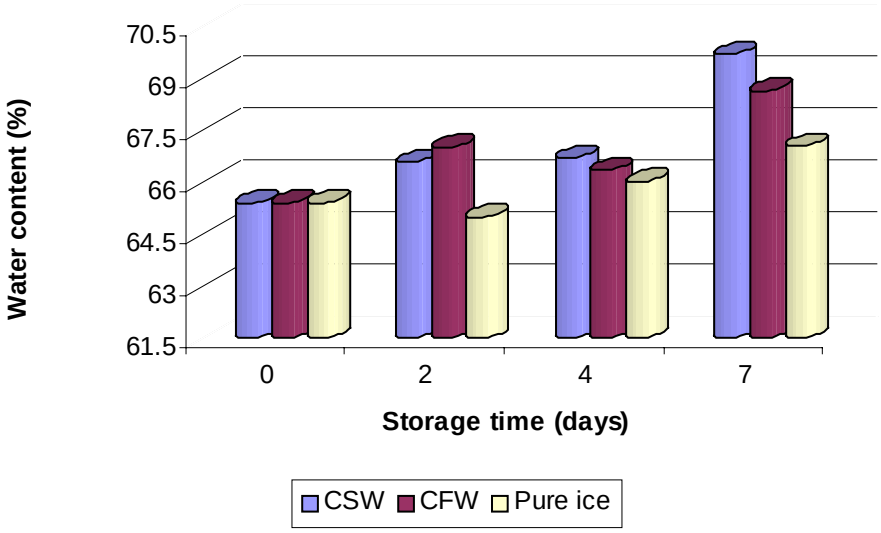

Figure 10: Changes in water content in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage of time.

4.6 Protein content

Protein content of fresh herring was 16.3% and decreased sharply in CSW and CFW during storage (Figure 11). The average protein content of herring stored in pure ice is slightly lower at the end of the experiment.

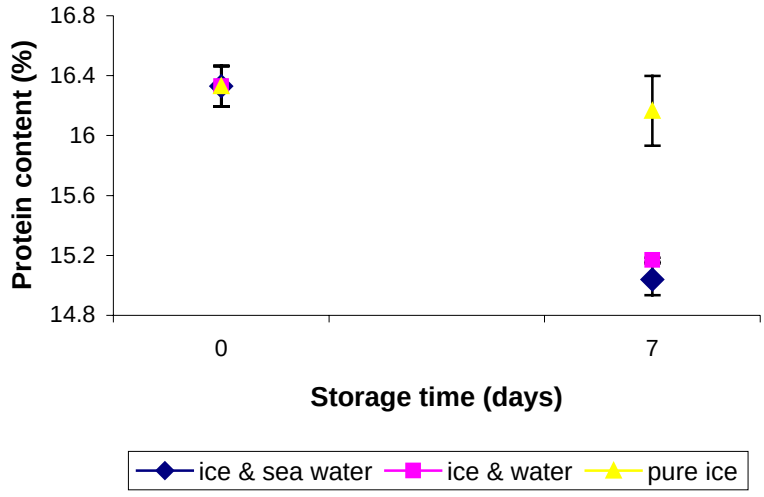

Figure 11: Changes in protein content in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage.

4.7 Total Volatile Nitrogen (TVN)

TVN in fresh herring was 20.1 mg/ 100g before storing in the cooling systems. During storage TVN steadily increased reaching around 50 mg/ 100g, in the herring in all the cooling systems. The rate of increase was highest in the CSW and CFW systems in the last period of the experiment (Figure 12).

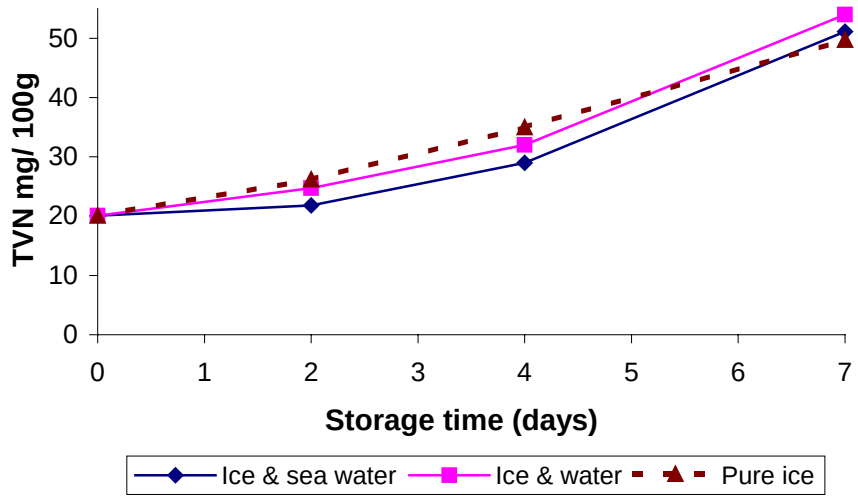

Figure 12: Changes in TVN in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage.

TVN in the cooling liquid in all cooling systems showed an increasing trend with time. After the third day of storage, TVN of the cooling liquid in the pure ice system increased faster than in the other two systems (Figure 13).

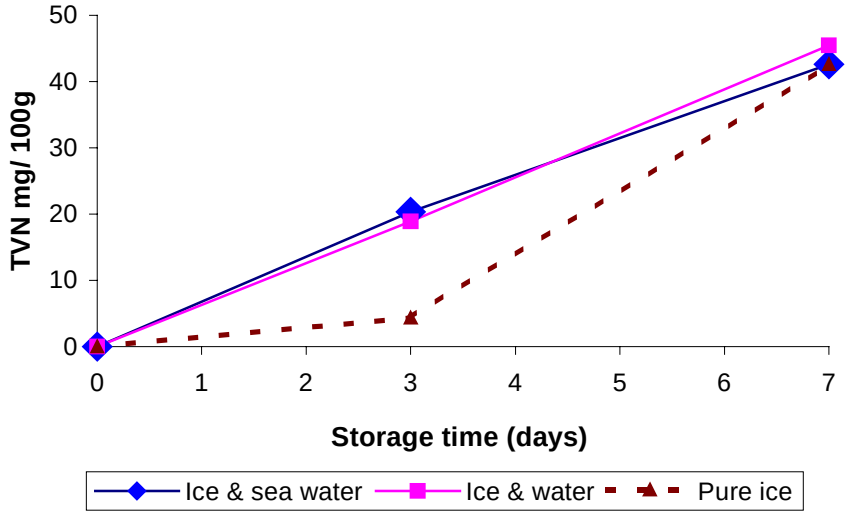

Figure 13: Changes in TVN in cooling liquid in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage.

4.8 Total Plate Count (TPC)

Total Plate Count (TPC) in herring was 2.7 * 10^3 before storing in cooling systems. During the seven days of storage TPC in herring in all cooling systems steadily increased to more than 4.0 * 10^6 on the seventh day of storage. The microbiological growth rate in herring stored in pure ice is higher than in the other two systems during the storage period. On the third day of storage, TPC in herring in the CSW system was higher than in the other two systems (Figure14).

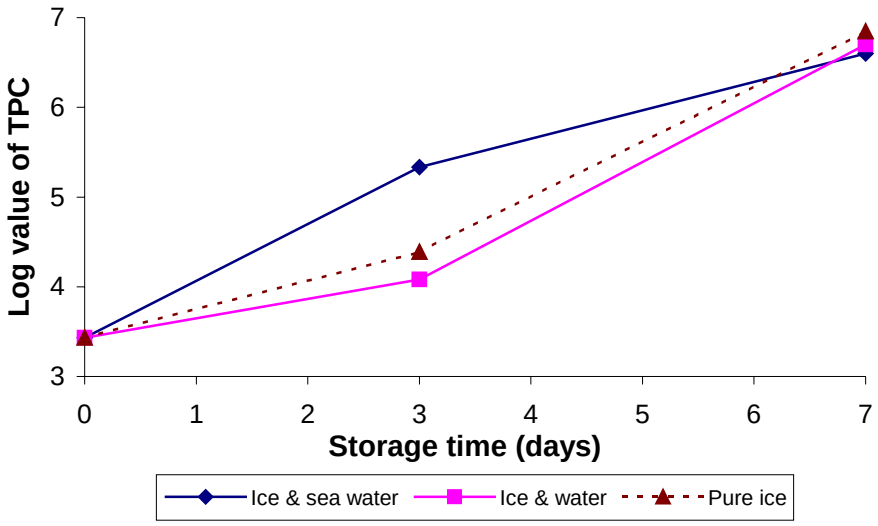

Figure 14: TPC of microbiological colonies in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage time.

TPC of the cooling liquid was 2.6 * 10^2 in CSW, 4.7 * 10^2 in CFW and 1.07 * 10^2 in the pure ice system before adding fish. During the storage period TPC of the cooling liquid in CFW and the pure ice cooling systems steadily increased. The microbiological growth rate in CSW system was less than other two cooling liquids from day 3 to 7. The microbial load (TPC) in cooling liquid in CSW shows higher values than TPC in the other two cooling systems on the third day of storage. Microbiological growth rates in the cooling liquids in CFW and pure ice systems show similar trends during storage (Figure 15).

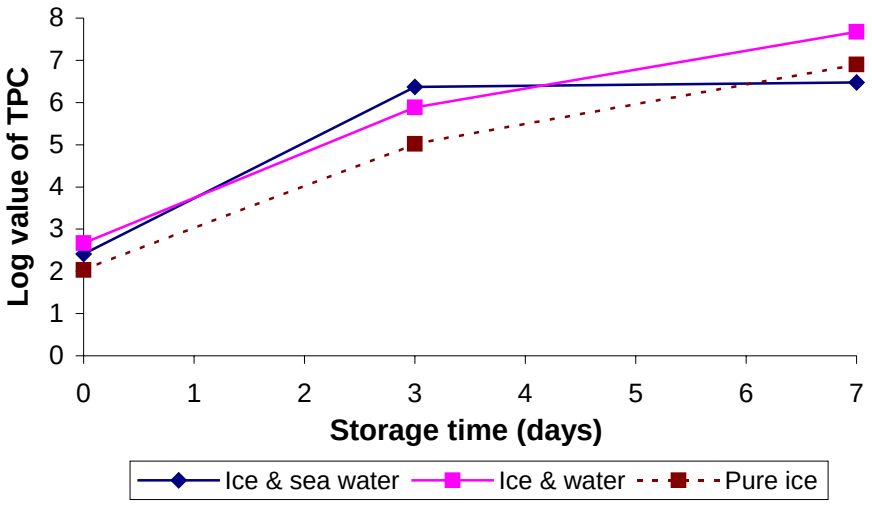

Figure 15: TPC of microbiological colonies in cooling liquid in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage.

5. DISCUSSION

The data presented in this paper are based upon experiment carried out on herring in three different cooling systems. The temperature in the CSW system was below 0°C because of the effect of salt on the freezing point of water. In CFW and pure ice cooling systems where there was no salt to affect the freezing point of water (0°C), the core temperature of herring was above 0°C for the entire experiment. Differences in temperature in the upper and lower layers of the cooling liquid in all systems were small and may not significantly affect the core temperature of herring within each system.

The salt content of the herring increased in the CSW system because of salt uptake from the cooling media which has higher salt concentration than the fish. The salt content of herring stored in the other two storage systems deceased slightly because of leaching of salt into the cooling liquid. During storage the herring in the CSW system absorbed more water because of the high salt content in the herring muscle.

The fat content of herring in the CSW system decreased significantly compared to the fat content of herring in the CFW and pure ice systems. The high salt content in herring in the CSW system (0.79%) may act as a catalyst to the oxidation of fat as has been observed in an earlier study (Castell *et al.* 1965). The peroxide value of sardines increased faster when it was stored in RSW than when it was kept in ice (Perigreen *et al.* 1975).

The protein content decreased in all cooling systems with time. This was most likely due to autolytic breakdown of protein caused by proteolytic enzymes like protease and bacterial spoilage. Such autolytic changes may lead to an increase in pH during storage time. This is in good agreement with Krishnakumar *et al*. (1985) who showed reduction of total nitrogen in fish stored in CSW and ice. The protein loss is greater in the CSW and CFW systems than in pure ice, probably due to an acceleration of the leaching out process because the fish are totally immersed in two systems during storage time.

Increase of TVN in herring in all cooling systems during storage time was most likely caused by autolytic processes which produce volatile amine compounds and bacterial spoilage. TVN in herring mainly contribute volatile nitrogenous compound ammonia which is produced by deamination of protein, peptides and amino acids (Huss 1995). TVN of herring in CSW and CFW increased relatively faster from day 4 to day 7 than before, and it reached up to around 50 mg/ 100g fish on day 7.

After the third day of storage, TVN of the cooling liquid in the pure ice system increased faster than in the other two systems which had reached a higher value by the third day. This may be because of leaching out of total nitrogenous volatile compounds from herring in the CSW and CFW systems into the liquid cooling media which only happened after liquid was formed in the pure ice system.

TPC in herring in all cooling systems increased steadily with time because of microbial growth on the surface of the fish and some bacteria invading the flesh. The microbiological growth rate in herring chilled in pure ice is higher than in the other two systems during the storage period. But at day 3, TPC in herring in CSW system was higher than other two systems because of salt tolerant bacteria can survive. During storage microbiological growth rate in herring in CSW is less than in pure ice. It may be affected by rapid cooling and lower storage temperature (below 0°C) in herring in CSW. Similar microbiological growth rate was seen in cooling liquids in the CSW and CFW system during storage. This is due to the accumulation of blood, dissolved protein and visceral contents in the liquid media.

Quality of fish is influenced by initial microbiological load, storage temperature, chemical composition, and other chemical factors of fish. Comparing the results of microbiological, chemical and physical parameters of herring stored in three cooling systems, that CSW system shows some better results of preserving quality in herring samples. One important factor was the temperature which was lower in the CSW system compared to the other two.

Pure water has a maximum density of 1 g/cm^3 at temperature of 4 °C. The density and freezing point of salt water changes with salinity. With high salinity, lower temperatures are possible but care should be taken to guard against freezing of the fish and uptake of salt. Preparation of the CSW cooling systems should take these factors into account. Although the concentration of NaCl in sea water in the CSW cooling system helps to accelerate cooling and keep the temperature of the fish below 0°C during storage time, salt content in herring in CSW increased up to around 0.8% at day 7 (Figure 7) because of salt uptake. Uptake of salt is probably the most important factor which limits the application of the CSW system. According to Perigreen (1975), NaCl has a catalytic effect on fat oxidation leading to high peroxide values (PV) of fish stored in the CSW cooling system. This observation agrees with the finding of this experiment where fat content in herring stored in a CSW system is significantly lower compared to herring stored in the other two cooling systems. This is a disadvantage in large scale application of CSW systems.

Herring intended for normal processing and marketing can acquire a salty taste which would make it unacceptable. The uptake of salt in herring in the cooling systems depends on storage time and concentration of NaCl in the cooling media. However, this

problem can be controlled by diluting sea water with ice and fresh water, as seen when salt content for CSW and CFW are compared. The salt uptake in herring in the bulk storage on boats with CSW system is also critical since it is concentrated during fish meal processing. Flesland (2000) showed, in a small scale experiment, that it was possible to chill in ice slurry made from sea water and keep the salt concentration below 0.7%, the upper limit specification for quality fish meal. The salt content in the slurry ice can be adjusted by draining off sea water before or during the cooling.

Although many biochemical reactions are temperature dependent, enzymatic reactions involving protease and lipase enzymes are not completely inhibited at low temperatures. The protein content in herring in all cooling systems decreased from day 0 to day 7 because of the autolytic breaking down of protein. It may be caused by digestive enzymes, which are readily available in the ungutted herring, and spoilage bacteria. Microbiological activities were retarded at temperatures below 0°C in the CSW and it is probable that the non-halophilic bacteria, thermophilic and mesophilic bacteria could not survive. However, some Gram-negative psychotrophic or psychophilic and salt tolerant bacteria can survive under such conditions.

During the storage period of herring in the three cooling systems autolytic processes occur even at low temperatures. They may accelerate the growth of spoilage bacteria after day 3 of storage by providing favourable growth environment for such organisms. Showan (1965) showed that TPC was lower in fish stored in CSW than in ice stored fish, and explained his results by faster initial cooling and lower storage temperature during the CSW storage. This is good agreement with the results of the present study.

6. CONCLUSION

Comparison of microbiological, chemical and physical parameters of herring stored in CSW, CFW, and pure ice system showed that the rapid cooling and lower temperature of CSW system gave the best quality herring. Quick and uniform cooling to low temperatures is therefore of great importance in order to obtain optimum quality. The CSW system could be applied for rapid cooling and storage at low temperatures of Skipjack, Yellow fin and Big eye tuna caught by multiday boats, and herring and other pelagic species caught by small fishing boats operated in the coastal fishery in Sri Lanka. As herring and tuna fish are pelagic fatty fish chemical composition of herring and tuna fish show some relationship. Changes of some quality parameters of fish stored in the CSW system show linear. The quality of fish, however, depends on the specie and size of the fish and therefore, it is important to do further studies on tuna stored in a CSW system.

The salt content in herring stored in CSW system increased steadily up to 0.79% at day 7, indicating salt uptake of herring stored in a cooling liquid with high concentration of NaCl. To reduce the salt content and keep below 0.7% which is the limit according to quality specification for fish meal, the cooling system could be prepared by mixing ice and sea water with fresh water or to chill in ice slurry made from sea water. The salt content in the ice slurry can be adjusted by draining off sea water before or during the cooling.

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APPENDIX

Experimental results

Table 3 Changes in temperatures in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice systems during storage time.

Day	Ice & sea water	Ice & water	Pure ice
0	3	3	3
1	-0.5	0.6	0.8
2	-0.3	1	0.9
3	-0.4	1.5	1.3
4	-0.4	1.2	0.6
5	-0.6	1	0.6
6	-0.8	1.7	0.3
7	-0.5	1	0.7

Table 4: Changes in temperatures in upper layer of cooling liquid of ice and sea water (CSW), ice and fresh water (CFW) and pure ice systems during storage time.

Day	CSW	CFW	Pure ice
0	-1.2	0.5	0.1
1	-0.9	0.6	0.3
2	-0.4	0.8	0.2
3	-0.1	1.3	1
4	-0.2	1.2	1
5	-0.6	1.2	1
6	-0.7	1.6	1.1
7	0	1	0.7

Table 5: Changes in temperatures in lower layer of cooling liquid of ice and sea water (CSW), ice and fresh water (CFW) and pure ice systems during storage time.

Day	CFW	CSW	Pure ice
0	0.49	-1.6	0.36
1	0.38	-1.39	0.79
2	0.48	-0.89	0.68
3	0.48	-0.58	0.59
4	0.44	-0.18	0.51
5	0.11	-0.11	0.46
6	0.13	-0.3	0.59
7	0.02	-0.51	0.49

Table 6: Changes in pH in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage tine

Day	CSW	CFW	Pure ice
0	6.55	6.55	6.55
1	6.54	6.49	6.52
2	6.54	6.58	6.55
3	6.65	6.56	6.61
4	6.54	6.65	6.57
5	6.75	6.62	6.63
6	6.66	6.65	6.72
7	6.66	6.7	6.73

Table 7: Changes in pH in cooling liquids in ice and sea water (CSW), ice and fresh water (CFW) and pure ice during storage tine.

Day	CSW	CFW	Pure ice
0	6.68	6.23	5.93
1	6.54	6.49	6.52
2	6.45	6.52	6.4
3	6.88	6.78	6.38
4	6.91	6.97	6.59
5	6.86	6.91	6.89
6	7.21	6.86	7.15
7	6.66	6.74	6.87

Table 8 Changes in salt content (%) in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice cooling systems during storage time.

Day	CSW	CFW	Pure ice
0	0.62	0.62	0.62
2	0.70	0.59	0.46
4	0.75	0.51	0.31
7	0.79	0.54	0.42

Table 9: Changes in salt content in cooling liquid ice and sea water (CSW), ice and fresh water (CFW) and pure ice cooling systems during storage time.

Day	CSW	CFW	Pure ice
0	2.50	0.00	0.00
4	1.76	0.91	0.30
7	1.45	0.58	0.51

Table 10: Water content of herring (%) in ice and sea water (CSW), ice and fresh water (CFW) and pure ice cooling systems during storage time.

Day	CSW	CFW	Pure ice
0	65.40	65.40	65.40
2	66.57	67.00	65.00
4	66.71	66.36	66.02
7	69.69	68.63	67.05

Table 11: Changes in protein content (% of average value and standard deviation) in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice cooling systems during storage time.

Day	Average CSW	Sta.	Average CFW	Sta.	Average Pure ice	Sta.
0	16.33	0.13435	16.33	0.13435	16.33	0.13435
7	15.04	0.106066	15.17	0.014142	16.16	0.233345

Table 12: Total Volatile Nitrogen (mg / 100 g) in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice systems during storage time.

Day	CSW	CFW	Pure ice
0	20.1	20.1	20.1
2	21.8	24.7	26.16
4	29	31.99	34.99
7	51.14	53.98	49.72

Table 13: Total Volatile Nitrogen (mg / 100 g) in cooling liquid in ice and sea water (CSW), ice and fresh water (CFW) and pure ice systems during storage time.

Day	CSW	CFW	Pure ice
0	0	0	0
3	20.35	18.9	4.36
7	42.61	45.45	42.61

Table 14: Log value of Total Plate Count (TPC) in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice systems during storage time.

Day	CSW	CFW	Pure ice
0	3.431363764	3.431363764	3.431364
3	5.334453751	4.08278537	4.389166
7	6.602059991	6.698970004	6.845098

Table 15: Log value of Total Plate Count (TPC) in cooling liquid in ice and sea water (CSW), ice and fresh water (CFW) and pure ice systems during storage time.

Day	CSW	CFW	Pure ice
0	2.414973348	2.672097858	2.029384
3	6.372912003	5.886490725	5.017033
7	6.477121255	7.672097858	6.90309

Table 16: Total coliforms (MPN/g) in herring in ice and sea water (CSW), ice and fresh water (CFW) and pure ice systems during storage time.

Day	CSW	CFW	Pure ice
0	< 0.3	< 0.3	< 0.3
7	<0.3	0.9	0.4

Faecal coliforms MPN/ g = < 0.3 in herring in CSW, CFW and pure ice systems during storage.

Faecal coliforms MPN/ 100 ml = 1.0 in cooling liquids all cooling systems during storage time.

1994	2706	702
1995	1978	413
1996	2497	523
1997	3251	728
1998	3678	898