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METHODS OF PRE-COOLING FOR FRESH COD (*GADUS MORHUA*) AND INFLUENCES ON QUALITY DURING CHILLED STORAGE AT -1.5˚C

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ABSTRACT

In recent years super-chilling and pre-cooling techniques have attracted considerable interest. The aim of this study was to analyse the influences of different pre-cooling methods, i.e slurry ice, CBC (combined blast and contact freezing) and usage of cooling mats, on the quality of fresh cod fillets during further storage at -1.5 ˚C. The objective of this project was to assess the suitability of pre-cooling using CBC as a technique both for whole fish and fillets (or portions). Analysis of variance (ANOVA) was carried out on QDA data in the statistical program NCSS 2000 (NCSS, Utah, USA). PCA (principal component analysis) was performed both for QDA and comparison of measurements in the program Unscrambler (Version 9.5, CAMO Trondheim, Norway). From the CBC pre-cooling experiments an appropriate temperature condition might be concluded as being from -23 ˚C to 20 ˚C for whole cod with an average weight of 1.04 kg when pre-cooled in a CBC freezer for 11 minutes. During the early storage period all of the three different cooling methods slowed down the rate of formation of TVN and TMA spoilage compared to the group without any cooling method applied. The three pre-cooling methods had similar effects on prohibition of growth of bacteria if hygienic handling with slurry ice was performed. The treatment with slurry ice and further chilled storage had an effect on prolonging the process from neutral quality to spoilage with regard to sensory results. Comparison of chemical, microbiological and physical quality parameters of fresh cod showed that TVN, TMA, TVC, pH and H_2S producer have good correlations with the evaluation of the quality of fresh cod.

Keywords: Cod (*Gadus morhua*), pre-cooling, quality, chilled-storage, slurry ice, CBC pre-cooling, cooling mats.

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1 INTRODUCTION

Fish product quality has been studied in recent years because commercial markets require high quality fish as consumers have a strong tendency to select very fresh fish (Luten and Martinsdottir 1997). China's domestic markets show strong demand for aquatic products and the price of aquatic products have shown an upward trend. Trading and consumption of aquatic products were mainly concentrated in Liaoning, Shandong, Jiangsu, Zhejiang and Guangdong and other coastal provinces (FAO 2004) With the development of modern fisheries and the prosperity of the market economic system, there is a high demand for fresh fish. Fresh fish is popular in coastal cities and counties, especially during the early summer-fall period, and is commonly sold for immediate consumption at retail fish stores or at seafood markets. Ice storage comprises the conventional method to preserve fresh fish in these regions.

Even though refrigerated trucks and trains are in use and the capacity of cold storages on land is great, the cold-chain is usually discontinuous resulting in spoiled fish. In spite of the availability of transport facilities, distribution of fresh marine fish especially in inland regions remains a problem. This is due to rapid deterioration of the fish as a result of bacteriological activity leading to loss of quality and subsequent spoilage.

There is a great need to improve the cooling techniques used, especially in the fishery industry. Extending the shelf-life of fish will increase profitability, both because product prices in the fresh market are higher than in the frozen market and by reducing the amount of product becoming unacceptable for sale. Since the shelf-life of fresh fish is lower than that of frozen fish, the need to develop methods for maintaining good post-mortem quality of the fish on its way to the market increases.

It becomes significant to find a simple, economical and feasible method to guarantee freshness of fish. It is necessary to give an example of industrialisation of the cold-chain of fresh fish.

In general, there are three aspects that constrain the provision of fresh fish in the market. Firstly, due to the absence of ice-making machines on medium or small fishing boats, it is very often difficult for fishermen to take cooling or pre-cooling measures on board to keep the low temperature of catch. Poor handling on board results in loss of freshness in the very beginning of the cold chain. Secondly, frozen fish is widely produced in fishery industries using freezing machines or cold storages. As the producers have little knowledge in relation to cooling or pre-cooling techniques it is difficult to introduce cooling equipment to treat the fresh fish. Thirdly, an integrated cold chain system has not come into being, i.e. refrigerated units and equipment have not been applied in the transportation, markets, distribution and circulation of fresh fish. However, ice-makers (flake ice or cube ice), freezing equipment, cold storages, refrigerated trucks and refrigerated trains are used in large scale in the field of food processing, transportation and storage.

In the present study cooling of both whole cod fish and cod fillets in CBC (combined blast and contact freezing) equipment was investigated. Optimal cooling conditions could be found regarding properties such as optimal velocity of blowing air, cooling temperature, cooling duration etc. Quality evaluations on cod fillets during storage

time were carried out. The aim of the project was to study spoilage characteristics of cod under cooling conditions compared with traditional processing (without cooling). Spoilage changes were measured by sensory, microbial, chemical, and physical analysis. From this it could be found which quality indicators are relevant to monitoring quality changes of cod stored under cooling and no-cooling conditions. The results could be applied to optimise the cooling conditions to prolong the shelf-life of cod.

The aim of this work was to analyse the influences of different pre-cooling methods on freshness of cod during storage. The objective of this project was to assess the suitability of pre-cooling and cooling as techniques both for whole fish and fillets (or portions). Furthermore, to compare the various evaluation methods used, to find out the correlation between the methods and how they can be used and to evaluate the quality of fresh fish were also the intentions of this project.

2 LITERATURE REVIEW

2.1 Quality changes and spoilage of raw fish

Consumer's desire to get good quality fresh seafood is increasing. Fish and fish products are now transported between cities or nations and hence the freshness or quality of these products is becoming more and more important. Fresh fish and other fresh seafood products are highly susceptible to spoilage from postmortem microbial growth and enzymatic activity. This spoilage is due to lack of chilling and improper handling during storage, distribution and marketing. The increasing demand for high quality fresh seafood has intensified the search for methods and technologies for better fish preservation. Presently, ice and mechanical refrigeration are the most common means of retarding microbial and biochemical spoilage in freshly caught seafood during distribution and marketing.

2.1.1 Bacteriological changes

The flesh of healthy live or newly caught fish is sterile as the immune system of the fish prevents the bacteria from growing in the flesh (Huss 1997). When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. On the skin surface, the bacteria to a large extent colonise the scale pockets. During storage, they invade the flesh by moving between the muscle fibres. Murray and Shewan (1979) found that only a very limited number of bacteria invaded the flesh during iced storage. Ruskol and Bendsen (1992) showed that bacteria could be detected by microscope in the flesh when the number of organisms on the skin surface increases above 106 cfu/cm^2.

During iced storage, the bacteria grow with a doubling time of approximately one day and will, after two or three weeks, reach a number of 108-109 cfu/g flesh or cm^2 skin. During ambient storage, a slightly lower level of 107-108 cfu/g is reached in 24 hours. The bacteria on fish caught in tropical waters often pass through a lag-phase of one or two weeks if the fish are stored in ice, whereafter exponential growth begins. At spoilage, the bacterial level on tropical fish is similar to the levels found on temperate fish species (Gram 1989, Gram *et al.* 1990).

Volatile sulphur-compounds are typical components of spoiling in fish and most bacteria identified as specific spoilage bacteria produce one or several volatile sulphides. S. putrefaciens and some Vibrionaceae produce H_2S from the sulphur containing amino-acid 1-cysteine (Stenström and Molin 1990), Gram *et al.* 1987).

2.1.2 Biochemical changes induced by bacterial growth during storage and spoilage

In cod and other gadoid fishes, TMA constitutes most of the so-called total volatile bases, TVB (also called total volatile nitrogen, TVN) until spoilage. However, in the spoiled fish where the TMAO supplies are depleted and TMA has reached its maximum level, TVB levels still rise due to the formation of NH_3 and other volatile amines. A little ammonia is also formed in the first weeks of iced storage due to autolysis. In some fish that do not contain TMAO or where spoilage is due to a non-TMAO reducing flora, a slow rise in TVB is seen during storage, probably resulting from the deamination of amino acids (Huss 1997).

2.2 Pre-cooling and cooling techniques

With the aim of reducing loss in freshness, different preservative methods, such as traditional flake ice (Nunes *et al*. 1992), refrigerated sea water (Kraus 1992) and chemical additives (Hwang and Regenstein 1995, Ponce de Leon *et al.* 1993) have been employed.

2.2.1 Slurry ice

Slurry ice, also known as fluid ice, slush ice or liquid ice, has been reported to be a promising technique for the preservation of aquatic food products in an ice–water suspension at subzero temperature (Chapman 1990, Harada 1991). Slurry ice has been shown to provide several advantages over flake ice, such as lower temperature, faster chilling, lower physical damage to products and better heat exchange power.

Although the theoretical advantages of using slurry ice are well known, few empirical data which report the potential practical advantages derived from the use of slurry ice for the storage of marine species are available. However, good results were obtained with slurry ice for the on-board storage of albacore tuna (Price *et al*. 1991), but no significant spoilage differences were obtained between flake and slurry ice when applied to a warm-water fish species (sea bass, *Dicentrarchus labrax*) (Martinsdottir *et al.* 2002). For seabream (*Sparus aurata*), holding in slurry ice was shown to be a good method of sacrificing marine species and it also leads to beneficial stored conditions (Huidobro *et al*. 2001). For crustacean species, practical advantages were obtained in the case of Australian prawns (Chinivasagam *et al.* 1998) and shrimp (Huidobro *et al.* 2002).

2.2.2 Super-chilling

In fish that is not frozen, most storage techniques involve the use of ice on site. In recent years, an additional storage technique, i.e. super-chilling, has attracted considerable interest. Super-chilling employs temperature ranges in which foods remain in a non-frozen condition, despite being kept at subzero temperatures. Storing food at su-

per-chilled temperatures can be advantageous in terms of maintaining food freshness and suppressing harmful microorganisms (Bohnert and Jensen 1996). In this technique, originally introduced on board trawlers in the 1960s (Pearson 1980), the temperature of the fish is reduced to 1°C to 2°C below the initial freezing point, and some ice crystals are formed inside the product (Haard 1992, Sikorski and Sun 1994). How much of the water is frozen is highly temperature dependent (Huss 1995). Freezing points for fishery products vary from about –1°C to –2.5°C, such as in salmon, shrimp, and mackerel at about –2.2°C to carp at about –1.0°C (Rahman and Driscoll 1994) and are usually dependent on the water content in the products (Chang and Tao 1981).

Super-chilling can either be used prior to traditional chilled distribution to store refrigerating capacity in the product (Magnussen *et al*. 1998) or the super-chilling temperature is maintained throughout the storage and distribution. Storage at super-chilled conditions may enhance phospholipid hydrolysis and protein denaturation (Ashie *et al.* 1996), super-chilling also inhibits most autolytic and microbial reactions, and thereby increases shelf-life (Huss 1995, Chang *et al.* 1998).

2.3 Quality measurements for fresh fish

Quality is an arbitrary term and one which causes confusion among consumers, processors and researchers. Fish quality is, therefore, a very complex concept (Bremner 2000, Nielsen *et al.* 2002), which includes nutritional, microbiological, biochemical and physiochemical attributes related to this term.

2.3.1 Microbiological and biochemical measurements

Food spoilage can be considered as any change that renders the product unacceptable for human consumption (Huss 1995). Spoilage of fish and shellfish results from changes caused by oxidation of lipids, reactions caused by activities of the fish's own enzymes, and the metabolic activities of microorganisms (Ashie *et al*. 1996). Fish and shellfish are highly perishable, because of their high water activity (a_w), neutral pH and presence of autolytic enzymes.

The rate of deterioration is highly temperature dependent and can be inhibited by decreasing storage temperature (e.g. fish stored in ice). The spoilage of fresh fish is usually microbial. However, in some cases chemical changes, such as auto-oxidation or enzymatic hydrolysis of the lipid fraction may result in off-odours and –flavours, in other cases, tissue enzyme activity can lead to unacceptable softening of the fish (Huss *et al*. 1997). The degree of processing and preservation, together with storage temperature, will decide whether the fish undergoes microbial spoilage, biochemical spoilage or a combination of both.

2.3.2 Physical measurements

Firmness of fish meat is one of the indicators of the freshness of fish. Fish meat, immediately after death, has strong flexibility and firmness. However, these characteristics are lost during refrigeration; a softening phenomenon occurs within a day. Softened meat is less palatable and less suited for mechanical processing. The collapse of collagen fibrils and softening of meat are promoted without bleeding (Ando *et*

*al.*1991). Proteases in blood that remain in the flesh may play a role. Some matrix metalloproteinase exists in fish flesh and can degrade collagen.

Sensory evaluation is the most important method of freshness measurements nowadays. There has been a trend to standardise sensory evaluation to make it an objective measurement to assess freshness (Olafsdottir *et al.* 1998 and 2006). The Quality Index Method (QIM) is a promising method to measure the freshness of whole fish stored in ice, and is both rapid and reliable (Martinsdottir *et al*. 2001). To evaluate sensory attributes of cooked fish, it is common to evaluate cooked fillets by Torry schemes, which provide scores correlating to storage time (Martinsdottir *et al*. 2001, Huss 1995). In research, Quantitative Descriptive Analysis (QDA) is used for cooked fillets to establish a detailed description and quantify product sensory aspects (Stone and Sidel 1985).

Sensory evaluation performed in a proper way is a rapid and accurate tool providing unique information about food. It offers immediate measurement of perceived attributes and provides useful information for a better understanding of consumer responses (Martinsdottir *et al.* 2001).

The analytical objective test used in quality control can be a discriminative or descriptive test. Discriminative tests are used to determine if a difference exists between samples such as triangle test or ranking test, while descriptive tests are used to determine the nature and intensity of the differences such as Quantitative Descriptive Analysis (QDA). Assessment in quality control must be objective (Huss 1995).

QDA is a technique used to define the sensory attributes of food such as texture, odour and flavour. It provides a detailed description of all attributes both qualitative and quantitative. A trained panel is handed a broad selection of reference samples and uses the samples to create terminology that describes all detectable aspects of the product under the guidance of a panel leader (Huss 1995, Stone and Sidel 1998). The words used to describe the perception are labels without implying any causality (Stone and Sidel 1998). The concepts are listed and used to evaluate the product using an unstructured scale for each concept to quantify the attributes. Panel members are trained to use the scale before performing the sensory analysis (Stone and Sidel 1998). The panel leader is responsible for selecting the product that will be evaluated in each session, facilitate the discussion and assist where there is conflict or disagreement about a particular wording. Choosing people that know the product too well should be avoided (such as producers) as they may provide what is believed to be the expected response, rather than what was perceived (Stone and Sidel 1998).

Sveinsdottir *et al.* (2002) reported that in Quantitative Descriptive Analysis the words used to describe the odour and flavour of the fish could be grouped into “positive sensory parameters” and “negative sensory parameters”, depending on whether they described fresh fish or fish at the end of the storage period.

Furthermore, weight loss in terms of liquid leakage from fish products may represent direct economic losses. The amount of liquid retained in fish is also important for the general appearance and juiciness of the flesh (Hamm 1986, Farmer *et al*. 2000).

3 MATERIALS AND METHODS

3.1 Experimental design

The experiments in this project consisted of two parts due to the aim of the project. One part was trials on CBC (combined blast and contact freezing), since the objective is to obtain cooling curves from this equipment. Both whole cod and cod fillet were chilled in the CBC freezer. The temperature data loggers were inserted into the fish flesh. In this part, optimal cooling conditions regarding cooling temperature, cooling duration etc were to be found. Another part was quality evaluation of cod fillets during storage. Prior to storage the fillets were treated with different pre-cooling methods in the processing plant. Quality indicators and correlations between measurements were summed up from the results of the latter part.

The cod fish used for the storage test was caught by a liner in the southwest of Iceland on 6 January 2008. And the whole fish used for generating cooling graphs was caught on 6 December 2007 and 30 January 2008, respectively. All fish was bled and stored in ice in tubs. On the second day after catch the raw material was transferred to the fish processing company Festi (in Hafnarfjordur, Iceland) and was stored in storage overnight until processing.

The whole gutted fish samples (group A) were directly taken from the tub with flake ice and prepared with tags, temperature loggers and ropes for pre-cooling experiments in CBC (Figure 1). The cod fillets were taken from processing line after they were filleted and trimmed. Groups B, C and D were treated with slurry ice, CBC pre-cooling and cooling mats packaging (Figure 2). Group E was produced the in common way (no pre-cooling methods or treatment were applied). The samples were packaged in Styrofoam (EPS, expanded polystyrene) boxes (593 x 393 x 145 mm) and transported to laboratorial cold storage (at -1.5˚C) on the same day. Each box contained nine fillets (the average weight was 0.66 kg) which were prepared for storage tests on each sampling day until sensory rejection.

Figure 1: Flow graph of whole cod fish processed with CBC pre-cooling. Experiments were done on 8 December 2007 and 30 January 2008.

Figure 2: Flow graph of cod fish processed with different pre-cooling methods and further stored at -1.5˚C. Experiments were done on 8 January 2008.

3.2 Slurry ice preparation

As a pre-cooling or cooling medium, the slurry ice was applied in this study for group B. The slurry ice was prepared with a slurry ice machine made by Skaginn hf. in Iceland (www.skaginn.is). In the machine slurry ice was produced by mixing crushed flake ice with salt and fresh water, resulting in a freezing point below 0.0°C. The ice percentage of this slurry ice was commonly between 15 and 35% and the salt ratio was 2.0 – 3.5%.

3.3 Temperature registering

Temperature data loggers (Stow Away, Onset Computer Corporation, Bourn, Mass., USA) were introduced in the experiments. The loggers for fresh fish during storage time were put in the boxes. The logger for surrounding temperature was located on the top of each box. The interval between temperature measurements was 10 minutes.

3.4 Cooling curves of whole fish in CBC (combined blast and contact freezing)

The whole fish (Figure 1) was treated with CBC (combined blast and contact freezing) (Skaginn, Iceland). The CBC equipment is suitable for fresh fish processing, enabling it to cool the product and leave the skin-on until the final stage of processing for the product to gain strength to better withstand handling. The fish temperature was decreased by moving it through a freezing tunnel on a patented aluminium belt anodised and Teflon coated. The time for fish going through the freezing tunnel was 8.5 minutes. If it was utilised for producing fresh fillet products, the temperature of cold air blowing by fans inside CBC was -10 ˚C.

Before the whole fish went through the freezing tunnel, three small temperature loggers were fixed in the fish flesh. In order to obtain an ideal temperature, all the samples were kept in the tub with flake ice for at least 10 minutes. On 8 December 2007, before the whole fish were put on the belt, one big logger was bound to the surface with string. Otherwise, on 30 January 2008, one big logger was hung up in the CBC at least one hour earlier than the trials stared.

The time was fixed at 11 minutes. Cooling graphs showing temperatures vs. time were obtained.

3.5 Cooling graph of fillet fish in CBC and sample preparation for storage test

As for the fillet fish, groups B, C, D and E (Figure 2) in the factory were gutted, graded and immersed immediately into slurry ice for 15 minutes. The core temperature was about 0.8˚C as it was in ice slurry. After grading it was beheaded. These four groups were then further treated with filleting and trimming. After that they (in total 180 fillets, 45 fillets for each group) were directly moved to Styrofoam boxes (with lids) from the processing line awaiting further processing. Group B was immersed in a tub (250 L) with slurry ice (slurry ice 100 l / fish 30.58 kg, ice concentration 22.8%) containing 2.3% NaCl for 16 minutes until the core temperature of fillets was -1.6˚C. Group C (45 fillets including 8 fillets with loggers for cooling graph) was processed with CBC pre-cooling techniques. Groups D and E were neither put in ice slurry nor treated with CBC.

Before being packaged, four groups were mechanically skinned. Group D was packaged with cooling mats and group E was processed in the conventional method. Groups B, C and E were straight packed in Styrofoam boxes (9 fillets each box) without plastic bags or film except the boxes on the last sampling day plastic films were put in the centre to separate fillets into two layers. Group D was packaged in the Styrofoam boxes with cooling mats on the top and plastic film between the fillets and ice mats. Temperature loggers were inserted into the four boxes (each box for each group) for the last sampling day. Three small loggers were placed underneath the fillets, between the fillets and above the fillets inside the box with a plastic film to separate them, allowing to trace the temperature of the three locations. And one big logger was

fixed outside the box with a rope. The boxes were transported to the laboratory and stored at -1.5˚C the same day until sensory rejection.

A cooling graph was obtained in group C from 8 fillets. One logger was inserted in the flesh of each fillet to record the core temperature before being processed with CBC pre-cooling. One big logger was hung up in the tunnel to follow the temperature of cold air inside.

3.6 Sampling

Sampling was carried out on the second day (one day from processing) after arrival and on 6, 10, 14, and 17 days of storage for evaluation of dripping loss, cooking yield, water content, water holding capacity (WHC), pH, TVB-N, TMN, total viable counts (TVC), counts of H_2S producing bacteria and sensory analysis.

On each sampling day, nine fillets (one box) for each group were used, five fillets for sensory analysis, two fillets for microbiology tests, pH measurement and cooking yield, two fillets for water holding capacity (WHC), TVB-N and TMN. For each sampling day, the fillets were divided in the same way for different analyses in the different laboratories to ensure the fillets from similar locations inside boxes were always used for the respective evaluations. One fillet on the top and one fillet on the bottom were used for microbial analysis running in duplicates. Parts 1, 3 and 4 (Figure 3) of each fillet were minced together for microbial analysis. Two results for bacteria were observed from the top fillet and bottom fillet respectively. Two of part 2 (Figure 3) were prepared for cooking yield assessments running in duplicates. The loins of each fillet were cut into five pieces (part S) (Figure 4) allowing them to be cooked for sensory assessment. Two fillets (one was located on the top and one was located on the bottom of the box) in each group were minced to prepare fillets for water holding capacity (WHC), TVB-N and TMN measurements.

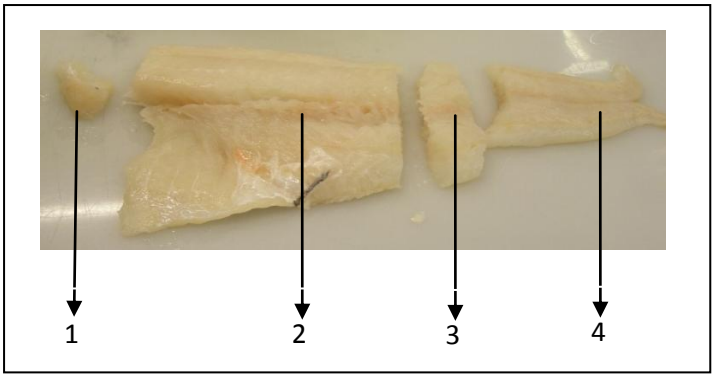

Figure 3: A cod fillet (on day 14 of storage) portions for microbiology tests, pH measurements and cooking yield

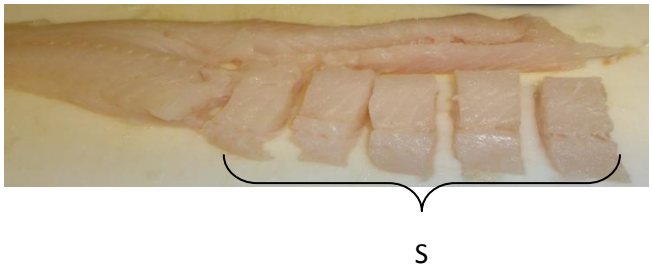

Figure 4: A cod fillet (on day 14 of storage) portions for sensory analysis

3.7 Sensory evaluation

Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (1998), was used to evaluate cooked samples of cod fillets. Four sample groups (during five sampling days) were assessed on sensory evaluation to detect sensory changes with storage time and determine maximum shelf-life.

Thirteen panelists from Matis (Food Research Institute) sensory panel participated in the QDA of the cooked cod fillets. The panel was trained in recognition of sensory characteristics of the samples and describing the intensity of each attribute for a given sample using an unstructured scale (from 0 to 100%) according to international standards (ISO 1993), including detection and recognition of tastes and odour, training in the use of scales, and in the development and use of descriptors. Most of the attributes were defined and described by the sensory panel during other projects (Bonilla *et al*. 2007, Wang *et al*. 2008). The sensory attributes were 30 (Appendix I).

Five fillets for each group were prepared per day. Samples (about 40-50 g) from the loin, were placed in aluminium boxes and cooked in a preheated electric oven (Convotherm Elektrogerate GmbH, Eglfing, Germany) with circulation air and steam at 95-100˚C for 6-7 minutes. The samples were blind coded with three digit numbers. Two sessions were held each day of the sensory evaluation and the samples were evaluated in duplicates, in random order.

Data was treated and collected with a computerised system (FIZZ, version2.0, 1994-2000, Biosystems, Counternon, France).

3.8 Microbial counts

Total viable psychrotrophic 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 17˚C was used instead of 15˚C due to presumptive psychrophiles proliferation at former temperature (Silliker 1980). Plates were surface-plated and incubated for five days. Bacteria forming black colonies on this medium produce H_2S from sodium thiosulphate and/or cysteine. In all experiments cooled maximum recovery dilutent (MRD, Oxoid) was used for dilutions. Samples were analysed in duplicate. All results are presented as an average.

Microbial counts of TVC in slurry ice were conducted using the same method as described above.

3.9 Chemical analysis

The pH was measured in a mixture of 5 g mince and 5 mL deionised water using the Radiometer PHM 80.

The total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined using steam distillation which was performed as described by Malle and Poumeyrol (1989). Briefly, 200 ml of a 7.5% aqueous trichloroacetic acid solution were added to 100 g of fish muscle and homogenised in a Waring blender. The mixture was filtered through Whatman n 3 filter paper.

Steam distillation was performed using a Kjeldahl-type distillator (Struer TVN) (Malle and Poumeyrol 1989). 25 ml of filtrate were transferred into a distillation flask followed by 6 ml of 10% NaOH. A beaker containing 10 ml of 4% boric acid and 0.04 ml of methyl red and bromocresol green indicator was placed under the condensor for the titration of ammonia.

Distillation was started and steam distillation continued until a final volume of 50 ml was obtained in the beaker (40 ml of distillate). The boric acid solution turned green when alkalinised by the distilled TVB-N which was titrated with aqueous 0.0125 M sulphuric acid solution using a 0.05 ml graduated burette. Complete neutralisation was obtained when the colour turned pink on the addition of a further drop of sulphuric acid. The TVB-N content was calculated by the following equation:

$$\mathbf{TVN}\left(\mathbf{^{mgN}/_{100g}}\right) = \frac{\mathbf{^{14mg}/_{mol} \times a \times b \times 2 \times 300}}{\mathbf{25ml}} \left(\mathbf{^{mgN}/_{100g}}\right)$$

Where:

a = ml of sulphuric acid.
b = molarity of sulphuric acid.

To assess TMA the same method was used as for TVB-N but 20 ml of 35% formaldehyde was added to the distillation flask to block the primary and secondary amines, TMA being the only volatile and measurable amine. The TVB-N and TMA content was expressed in mg N/100g cod tissue.

3.10 Water holding capacity (WHC) measurements, dripping loss and cooking yield

Water Holding Capacity (WHC) was determined by a method that was built on a method by Børresen (Eide 1982). The sample glasses were made from plexi-glass and their dimensions are: height 62 mm, inner diameter 19 mm and outer diameter 25 mm. The rotor used was SS-34 for Sorvall centrifuge, type RC-5B (Dupoint, USA). The samples are centrifuged at 1500 rpm for five minutes in special sample glasses. Samples will be prepared by chopping them in a Braun Mixer (Type 4262, Germany) for 10-15 seconds (until homogenous). The sample glass is weighed empty and then 2 g of the sample are weighted into the glass. After centrifugation, the sample glass is weighed again with the sample in it minus the loose bounded water. Each sample is determined in triplicate.

The Water Holding Capacity (WHC) of the sample is then calculated using the following formula:

$$\mathbf{WHC} = \frac{\mathbf{W} - \Delta\mathbf{r}}{\mathbf{W}} \times \mathbf{100\ (\%)}$$

Where:

W is the water content of the sample before centrifugation (%).
Δr is the weight lost by centrifugation (%).

Dripping loss is defined as the amount of liquid that is lost during storage expressed as a percentage of loss based on the initial sample weight. It is determined by the weight of the fillets before and after storage.

Cooking yield is defined with regards to the amount of liquid that was lost during cooking. The cooking yield (CY) is calculated as:

$$CY = \frac{\mathbf{Wcooked}}{\mathbf{Wraw}} \times 100(\%)$$

Where:

W_{cooked} is the weight of cooked sample.
W_{raw} is the weight of raw sample before cooking.

The analyses for dripping loss and cooking yield were run in quadruplicate. The total number of fillets per group at each sampling day is two.

3.11 Data analysis

The data were processed, calculated and plotted against time (using Microsoft Excel 97).

Multivariate analysis of the data was conducted in the statistical program Unscrambler (Version 9.5, CAMO Trondheim, Norway). Principal component analysis (PCA) was utilised to identify on analysing the relationships between measured variables. An average of sample multi-replicates was used for each sample.

QDA data was corrected for level effects (effects caused by level differences between assessors and replicates) by the method of Thybo and Martens 2000. Principal Component Analysis (PCA) on mean level corrected values of sensory attributes and samples was performed. Analysis of variance (ANOVA) was carried out on QDA data corrected for level effects in the statistical program NCSS 2000 (NCSS, Utah, USA). Calculations of multiple comparisons using Duncan's multiple comparison test were implemented in this program. The significance level was set at 5%, if not stated elsewhere.

4 RESULTS AND DISCUSSION

4.1 Cooling graphs

*4.1.1 **Cooling curves of whole fish in CBC***

In total four tests were conducted at different cooling temperatures (air temperature inside the CBC freezer) representing -10˚C, -30˚C, -20˚C and -16˚C respectively (Figures 5, 6, 7 and 8). Before the fish was put on the conveyor belt of the CBC, the temperature of air inside the CBC was stabilised for 10 minutes.

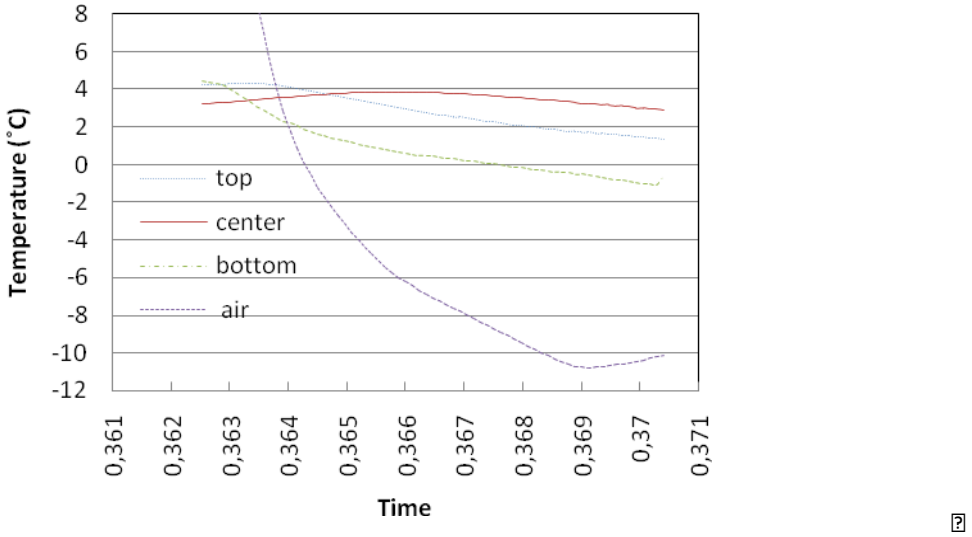

Figure 5: Cooling curves for whole fish (weight: 1.3 kg) when pre-cooled in a CBC freezer. Also shown is the temperature measured close to the fish. The cold air temperature in the CBC freezer was set to -10˚C. The experiments were done on 8 December 2007.

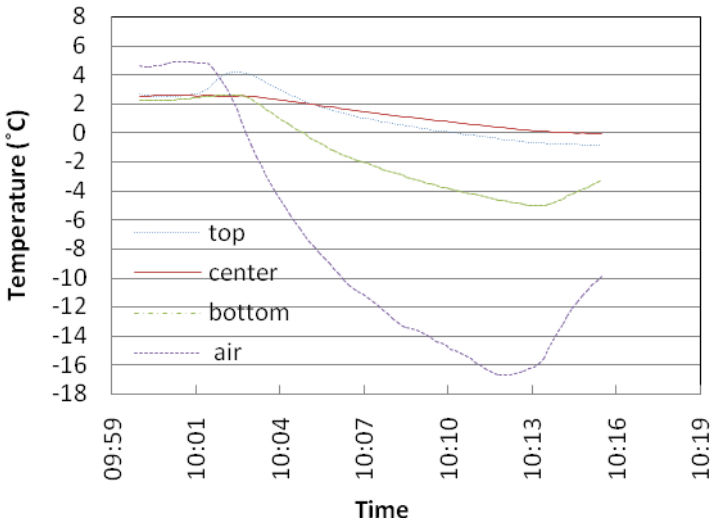

Figure 6: Cooling curves for whole fish (average weight of four specimen: 1.03 kg) when pre-cooled in a CBC freezer. Also shown is the temperature measured close to the fish. The cold air temperature in the CBC freezer was set to -30˚C. The experiments were done on 8 December 2007.

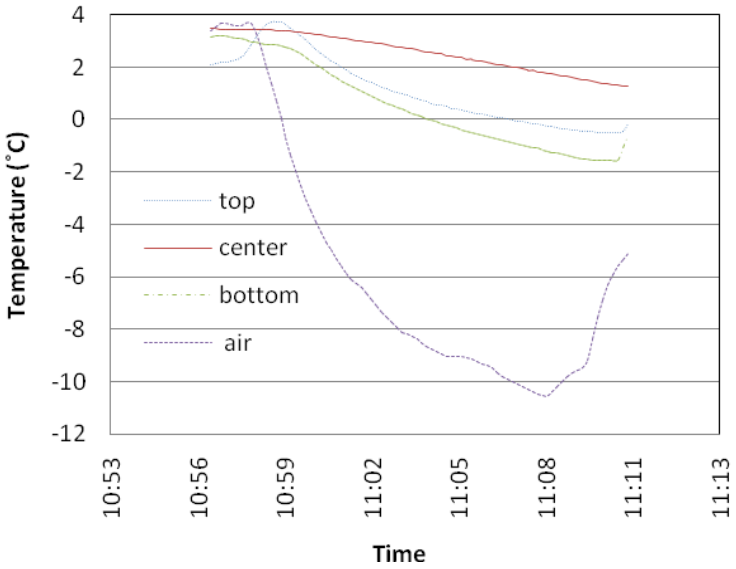

Figure 7: Cooling curves for whole fish (average weight of four specimens: 1.03 kg) when pre-cooled in a CBC freezer. Also shown is the temperature measured close to the fish. The cold air temperature in the CBC freezer was set to -20˚C. The experiments were done on 8 December 2007.

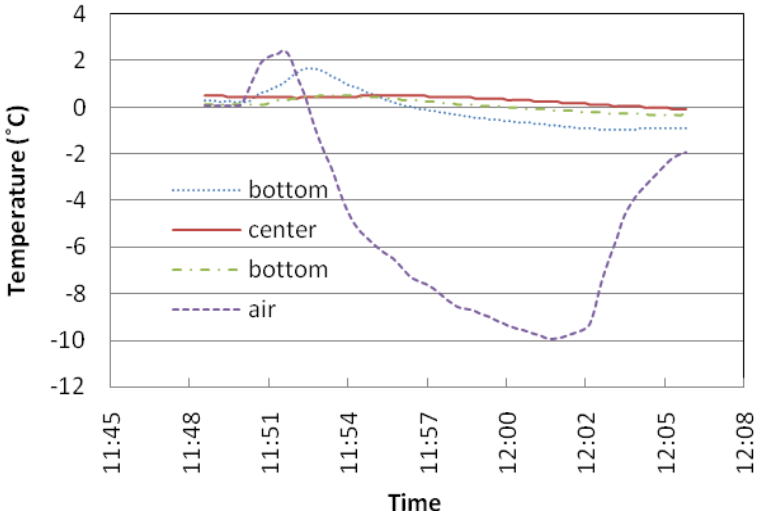

Figure 8: Cooling curves for whole fish (weight: 1.01 kg) when pre-cooled in a CBC freezer. Also shown is the temperature measured close to the fish. The cold air temperature in the CBC freezer was set to -16˚C. The experiments were done on 8 December 2007.

For each fish/specimen four temperature data loggers (Figure 9) were applied. One of them was put in the central thickest part of the flesh (close to the spine), and the other two were put under the skin of both sides. The fourth one was attached to the top of the fish/upside of the fish by a rope representing cold air temperature in the CBC.

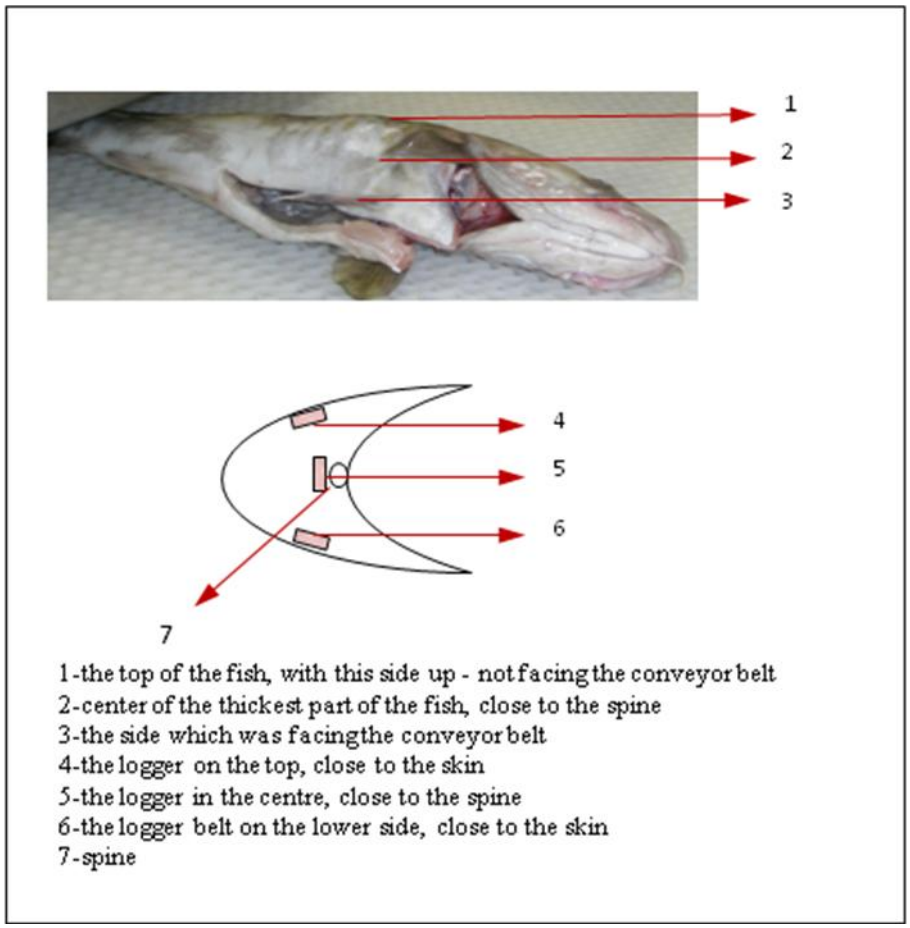

Figure 9: Location of the three loggers of each whole cod fish

The cooling graphs for whole fish from 30 January are shown in Figures 10, 11, 12 and 13. The weight of each individual fish conducted in these trials was between 1.06 kg and 1.02 kg.

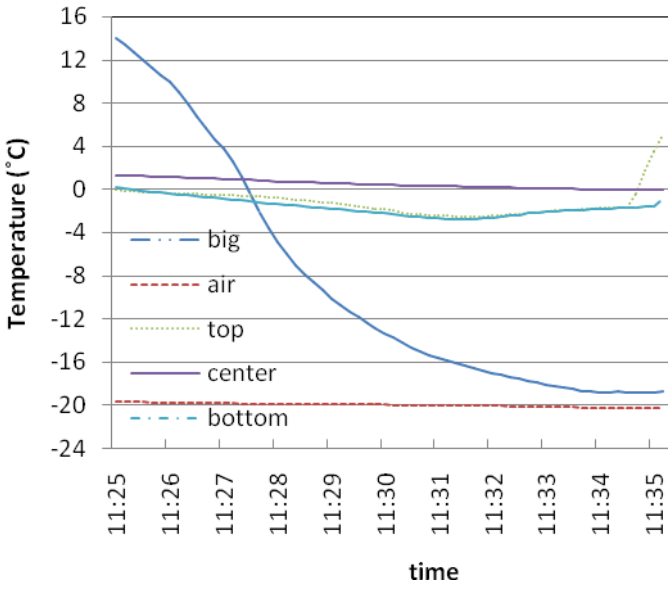

Figure 10: Cooling curves for whole cod fish (weight: 1.03 kg) when pre-cooled in a CBC freezer. Also shown is the temperature measured close to the fish (represented by "big") and the cold air temperature in the CBC away from the conveyor belt (represented by "air"). The temperature of the CBC freezer was set to -18˚C. The experiments were done on 30 January 2008.

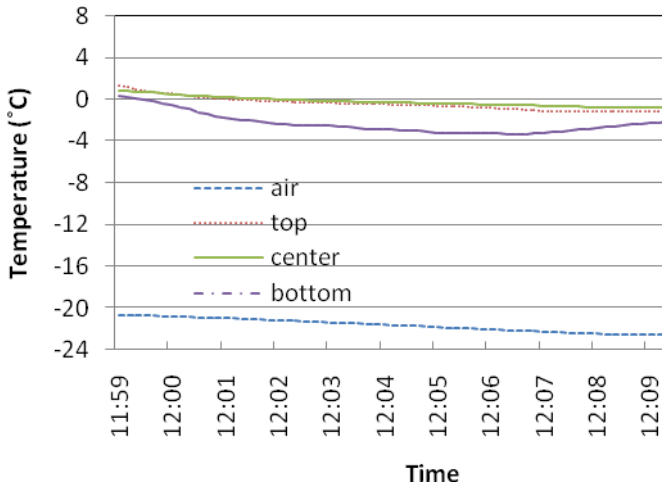

Figure 11: Cooling curves for whole cod fish (weight: 1.06 kg) when pre-cooled in a CBC freezer. Also shown is the cold air temperature in the CBC away from the conveyor belt (represented by "air"). The temperature of the CBC freezer was set to -20˚C. The experiments were done on 30 January 2008.

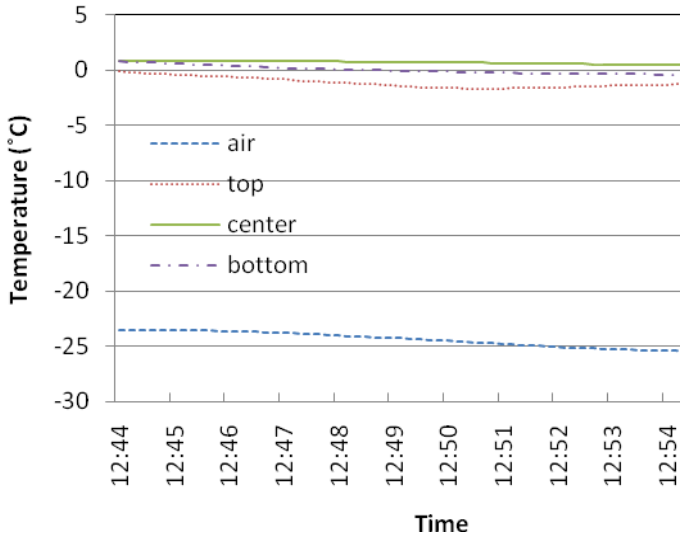

Figure 12: Cooling curves for whole cod fish (weight: 1.04 kg) when pre-cooled in a CBC freezer. Also shown is the cold air temperature in the CBC away from the conveyor belt (represented by "air"). The temperature of the CBC freezer was set to -23˚C. The experiments were done on 30 January 2008.

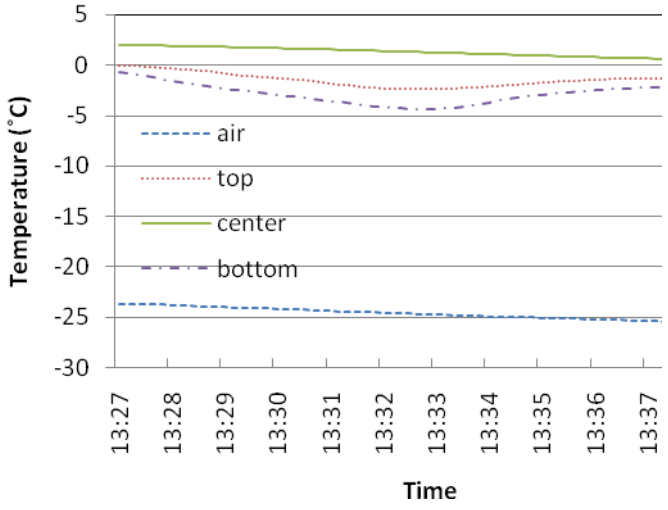

Figure 13: Cooling curves for whole cod fish (weight: 1.02 kg) when pre-cooled in a CBC freezer. Also shown is the cold air temperature in the CBC away from the conveyor belt (represented by "air"). The temperature of CBC freezer was set to -24˚C. The experiments were done on 30 January 2008.

4.1.2 Cooling curves of cod fillet in CBC

A big logger was hung up in the cold air inside CBC registering the environmental temperature (Figure 14). Eight tagged fillets containing loggers were placed scattered with other fillets in group C (37 fillets were selected without loggers) on the conveyer belt. The average temperature in the centre decreased from 6˚C to 1˚C with an environmental record of -10˚C.

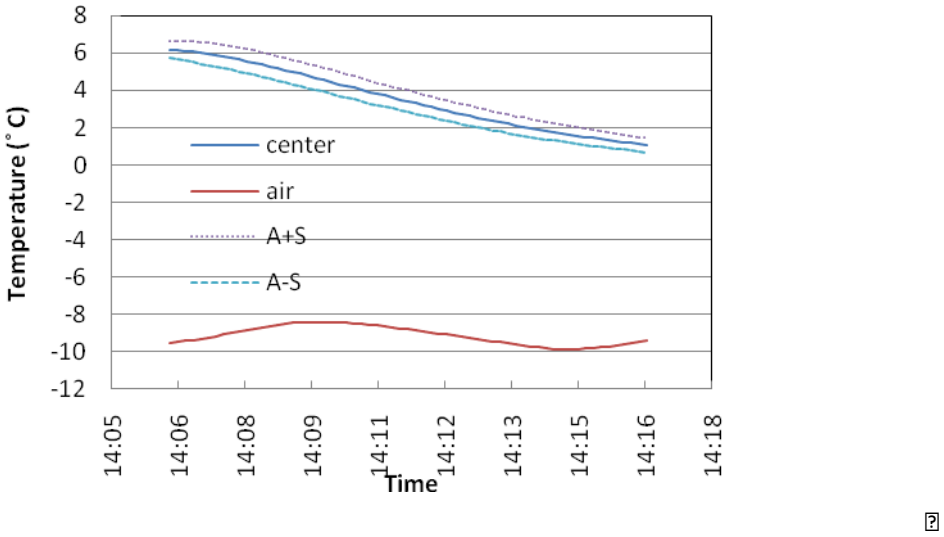

Figure 14: Cooling curves for cod fillets (average weight of the eight fillets: 0.59 kg) when pre-cooled in a CBC freezer. The temperature of the CBC freezer was set to -10˚C. The experiments were done on 8 January 2008. Air–the cold air temperature in the CBC away from the conveyor belt. A – the average of the central temperature of eight fillets. S –standard deviation.

4.2 Temperature profiles during chilling storage

The core temperatures inside each box throughout the storage period of groups B and C are shown in Figure 15. The top temperature inside the box of group C is shown in Figure 16. The cold air temperature inside cold storage (outside the box) is shown in Figure 17.

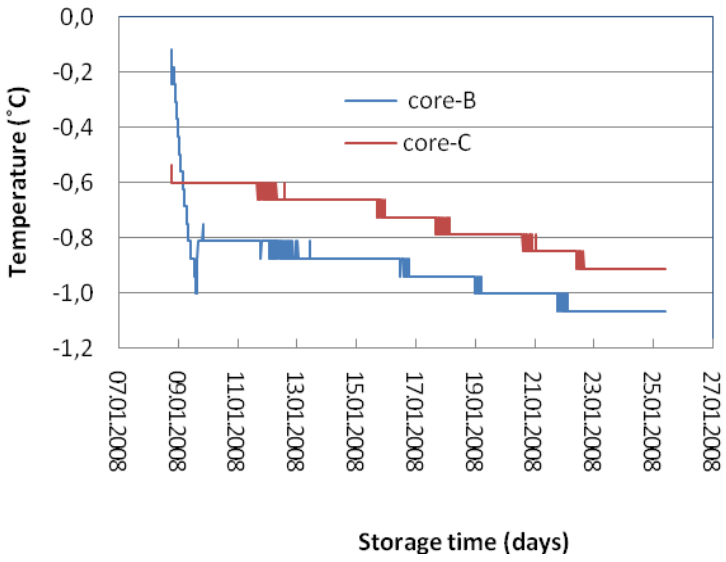

Figure 15: Temperature evolution in the centre of packaged boxes of groups B and C during storage in a cooling chamber from 8-25 January 2008. The temperature in the cooling chamber was set to -1.5˚C. B-slurry ice, C- CBC

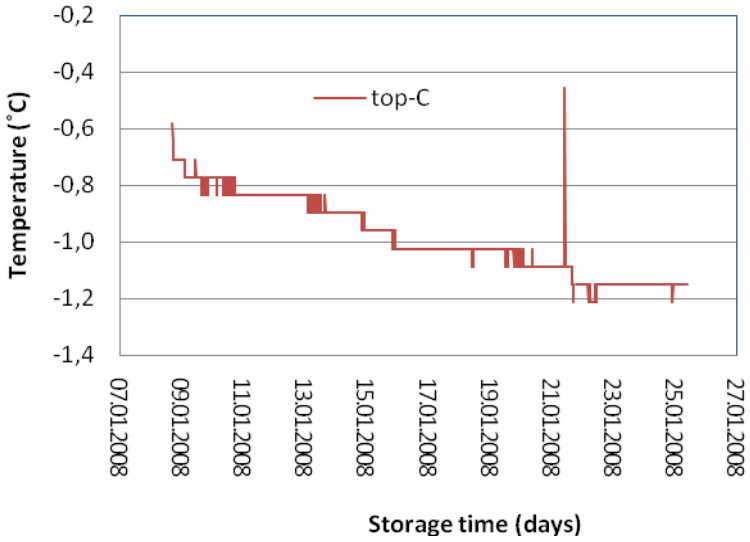

Figure 16: Temperature evolution on the top of the packaged box for group C processed with CBC during storage in a cooling chamber from 8-25 January 2008. The temperature in the cooling chamber was set to -1.5˚C.

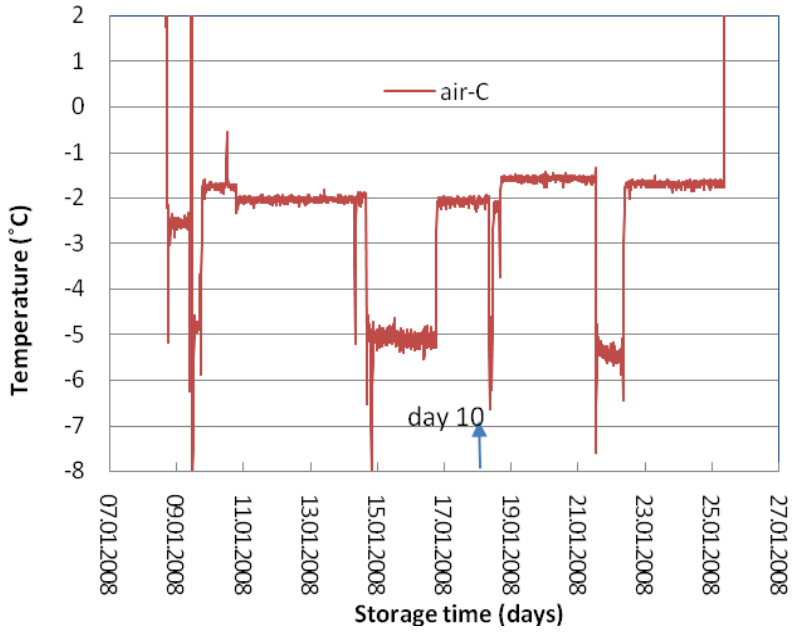

Figure 17: Temperature evolution outside of packaged box for group C processed with CBC during storage in a cooling chamber from 8-25 January 2008. The temperature in the cooling chamber was set to -1.5 ˚C.

4.3 Sensory evaluation

In order to describe the main characteristics of samples and highlight their main difference, a multivariate statistical procedure, principal component analysis (PCA) was performed on the data obtained. The results concerning both variables (correlation loadings) and samples (score plot) are presented in Figures 18 and 19. The first two principal components (PC1 and PC2) explained 68% of the variances in the dataset. Significant differences between samples are shown in Table 1.

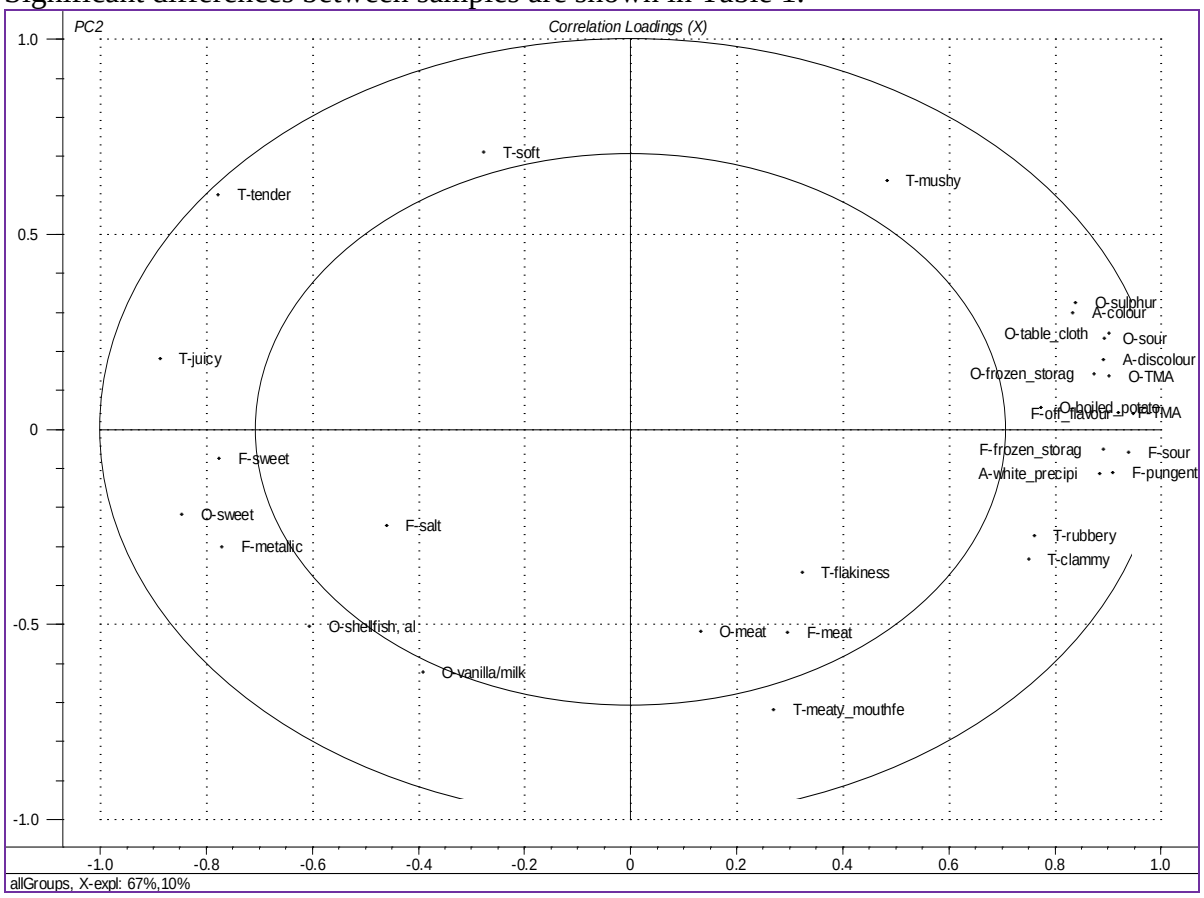

Figure 18: PCA describing sensory quality of correlation loadings for the products as evaluated by a trained sensory panel. PC1 vs. PC2 (X-expl.: 67%, 10%). Ellipses mark the 50% and 100% explained variance limits. O - odour, A - appearance, F - flavour, T - texture, B-slurry ice, C-CBC, D-cooling mat, E- traditional method (neither pre-cooled nor cooling mat).

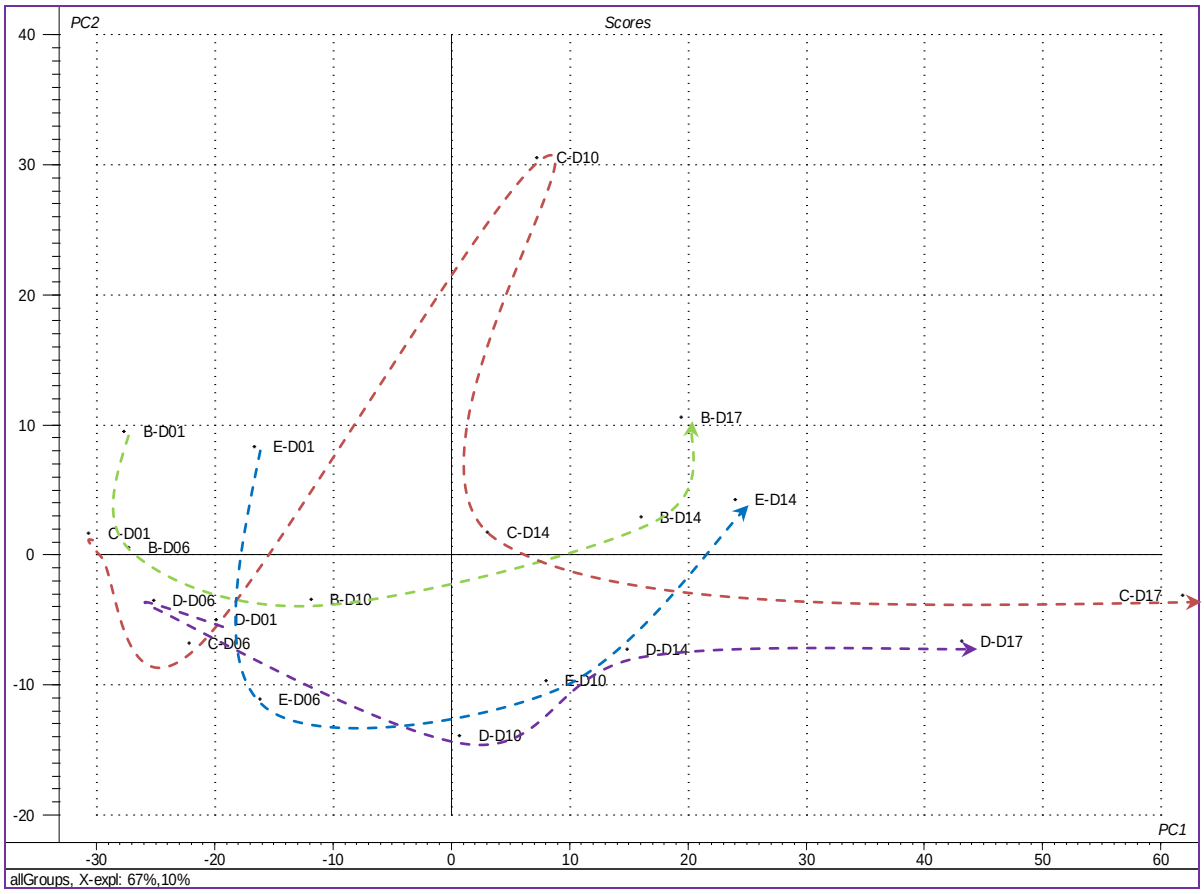

Figure 19: PCA describing sensory quality of scores for the products as evaluated by a trained sensory panel. PC1 vs. PC2 (X-expl.: 67%, 10%). Ellipses mark the 50% and 100% explained variance limits. O - odour, A - appearance, F - flavour, T - texture, B- slurry ice, C-CBC, D-cooling mat, E- traditional method (neither pre-cooled nor cooling mat).

Table 1: Average sensory scores of QDA attributes of cooked cod loins

A		Odour									
Storage time (d)	Group	Sweet	Shell	Meat	Vani	Boil	Froz	Table	TMA	Sour	Sulph
Significance		***	ms	*		***	*	***	***	***	***
1	B	43^{ab}	31	19	21	10^{cd}	5	6^{ce}	5^{bcdf}	5^{bc}	3
	C	40	34	16^{b}	21	11^{bc}	5	3^{ef}	5^{ce}	5^{bc}	2
	D	38	33	17	21	15	5	5^{ef}	4^{ef}	3^{cd}	3
	E	36	32	21	21	17	7	6^{ce}	4^{ef}	5	4
6	B	43	37	24	25	21	4	4^{ef}	3^{ef}	2^{cd}	1
	C	38	35	20	21	18	5	5^{ce}	4^{ce}	2^{cd}	2
	D	41^{a}	35	22	24	18	4	4^{ef}	2^{ef}	2^{cd}	2
	E	39^{ab}	34	23	24	17	4	5^{ce}	5^{bcdf}	2^{cd}	1
10	B	42	38	24	25	20	6	6^{bcdf}	5^{bcdf}	6	2
	C	32	30	19	16	18^{a}	6	14^{ac}	7	9	5
	D	40	39	28^{a}	26	19	5	10	7	6	4
	E	36	33	20	22	18	5	7^{bcdf}	4^{bcdf}	4^{bc}	2
14	B	26^{bc}	30	22	18	25^{a}	8	13	12	8	5
	C	29	27	19	19	20	6	8^{bcdf}	6^{bcdf}	5^{bc}	3
	D	29	31	23	20	24^{a}	7	8^{bcdf}	8	7	2
	E	24^{cd}	28	17	17	21^{a}	8	20^{a}	14^{ac}	14^{ab}	5
17	B	27	24	17	17	19^{a}	10	15^{ab}	17^{a}	13	5
	C	23^{bc}	21	19	18	24^{a}	10	18^{a}	17^{a}	16^{a}	6
	D	21^{bc}	23	19	18	21^{a}	9	17^{a}	15^{ab}	12	5

B		Appearance					Flavour				
Storage time (d)	Group	Colo	Disc	White	Salt	Men	Sweet	Meat	Froz	Pung	Sour
Significance		***	***	***	***	***	***		***	***	***
1	B	26^{bcd}	23^{cde}	23^{cdf}	16	27	31^{a}	14	3^{def}	9^{b}	5^{cd}
	C	18^{df}	24^{bd}	26^{bce}	17	34^{ab}	29^{ab}	15	6^{bcd}	8^{b}	4de
	D	20^{ce}	23^{cde}	24^{cdf}	18	33^{ac}	29^{ab}	16	6^{bcd}	15^{b}	7^{cd}
	E	23^{bcd}	25^{bd}	19^{efh}	14	35^{a}	23	16	9	16^{b}	8^{cd}
6	B	21^{ce}	23^{bd}	28^{cdf}	17	26	23	12	4^{ce}	7^{b}	3de
	C	17^{ef}	20^{df}	27^{cdf}	17	32^{ac}	24^{ac}	16	5^{bcd}	9^{b}	7^{cd}
	D	20^{ce}	19^{df}	23^{defg}	16	26	19	13	3^{ce}	8^{b}	4^{cd}
	E	17^{df}	19^{df}	31^{bce}	15	25	17	14	6	12^{b}	6^{cd}
10	B	23^{bcd}	26^{bd}	30^{bce}	20	25	22	16	3^{ce}	10^{b}	5^{cd}
	C	32	38^{bc}	31^{bcd}	16	15^{df}	14^{bcd}	13	6	12^{b}	8^{cd}
	D	20^{ce}	27^{bd}	33^{bcd}	17	25	20	14	8	13^{b}	10^{cd}
	E	32	40^{b}	40^{b}	20	27	20	19	6	13^{b}	7^{cd}
14	B	36^{ab}	38^{b}	40^{bc}	21^{a}	19	17	11	9	14^{b}	10^{cd}
	C	28	30^{bd}	38^{bcd}	10^{b}	18^{bd}	13^{ce}	13	6	14^{b}	7^{cd}
	D	29	34^{bd}	41^{bc}	10^{b}	21	13^{ce}	11	7	14^{b}	9^{cd}
	E	26^{bcd}	31^{bd}	38^{bc}	10	16^{df}	9d^{ef}	12	12^{ab}	17^{b}	16^{bc}
17	B	36^{ac}	38^{b}	31^{bce}	11^{b}	14^{cde}	11^{bcd}	12	10	18^{b}	16^{cd}
	C	42^{a}	51^{a}	57^{a}	12	14^{df}	10^{ce}	14	13^{a}	32^{a}	32^{a}
	D	32	34^{bd}	40^{bc}	11^{b}	14^{bd}	10^{bcd}	16	12^{ac}	29^{a}	25^{b}

C		Flavour			Texture						
Storage time (d)	Group	TMA	OffFl	Flak	Soft	Juicy	Tender	Mushy	Meat	Clam	Rub
Significance		***	***		*	***	***	**	**	***	***
1	B	3^{ce}	3^{df}	38	51	55^{a}	57^{ab}	35^{b}	20^{b}	13^{bc}	5^{cd}
	C	2^{ce}	3^{df}	37	42^{b}	57^{a}	56^{ab}	34^{b}	25	9^{cd}	6^{bc}
	D	9^{bcd}	5^{df}	44	43^{b}	52^{a}	51	33^{b}	31	16	11^{bc}
	E	7^{bcd}	12^{bd}	40	49^{b}	51^{ab}	56^{ab}	37^{b}	19^{b}	9^{cd}	13^{bc}
6	B	2^{ce}	2^{df}	42	54	53^{a}	60^{ab}	37^{b}	29	19	7^{bc}
	C	4^{ce}	4^{df}	44	46^{b}	47^{ac}	54^{ac}	38^{b}	29	18	10^{bc}
	D	3^{ce}	3^{df}	42	55^{b}	45	55^{ac}	35^{b}	32	16^{bc}	10^{bc}
	E	6^{ce}	7^{cde}	40	48^{b}	48^{ac}	49	32^{b}	31	23	11^{bc}
10	B	4^{ce}	5^{cde}	45	53^{b}	52^{ac}	54	38^{b}	28	13	12^{bc}
	C	8^{bcd}	12^{bd}	35	72^{a}	48	64^{a}	59^{a}	21	16	15^{bc}
	D	6^{bcd}	10^{bd}	42	52^{b}	47	45^{bcd}	37^{b}	34^{a}	20	16
	E	6^{bcd}	7^{bd}	42	52^{b}	41^{cde}	46	35^{b}	33	20	20^{ab}
14	B	10^{bcd}	13^{bd}	45	47^{b}	43	47	46^{b}	28	26	12^{bc}
	C	6^{bcd}	9^{bd}	45	47^{b}	38^{bcd}	51	42^{b}	26	17	9^{bc}
	D	10^{c}	14^{bd}	45	48^{b}	38^{bcd}	44^{bcd}	40^{b}	29	30^{ab}	18
	E	17^{ab}	21^{bc}	46	52^{b}	38^{bcd}	49	39^{b}	26	26	14^{bc}
17	B	23^{a}	24^{b}	38	48^{b}	40	52	46^{b}	29	22	14^{bc}
	C	27^{a}	44^{a}	48	41^{b}	28^{df}	35^{def}	44^{b}	27	28	21
	D	24^{a}	35^{a}	39	45^{b}	31^{cde}	38^{ce}	49^{b}	30	38^{a}	32^{a}

#: 1. Different letters within a row indicate significances between groups.
2. ms = marginal significance (p = 0.05-0.10); * p < 0.05; ** p < 0.01; *** p < 0.001
3. B-slurry ice, C-CBC, D-cooling mat, E- traditional method (neither pre-cooled nor cool- ing mat)

4.4 Chemical measurements

Changes in pH, TVN and TMA with different processing types during storage are shown in Figures 20, 21 and 22.

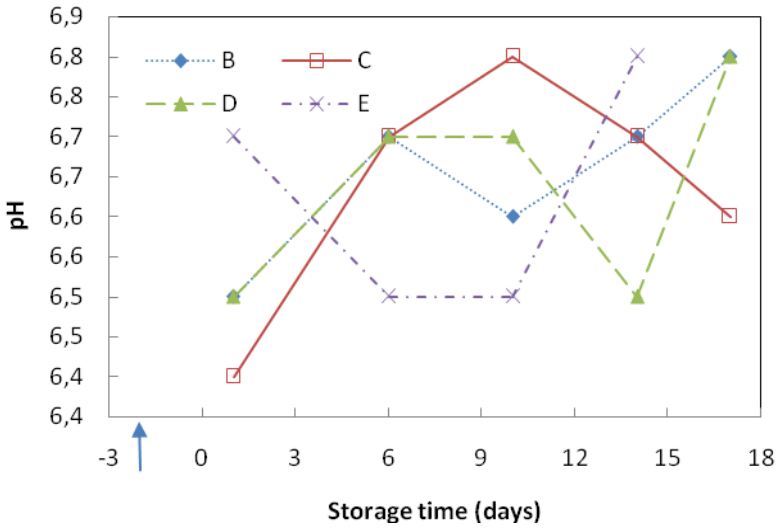

Figure 20: pH of processed cod fillets stored at -1.5˚C (B-slurry ice, C-CBC, D-cooling mat, E-traditional method (neither pre-cooled nor cooling mat))

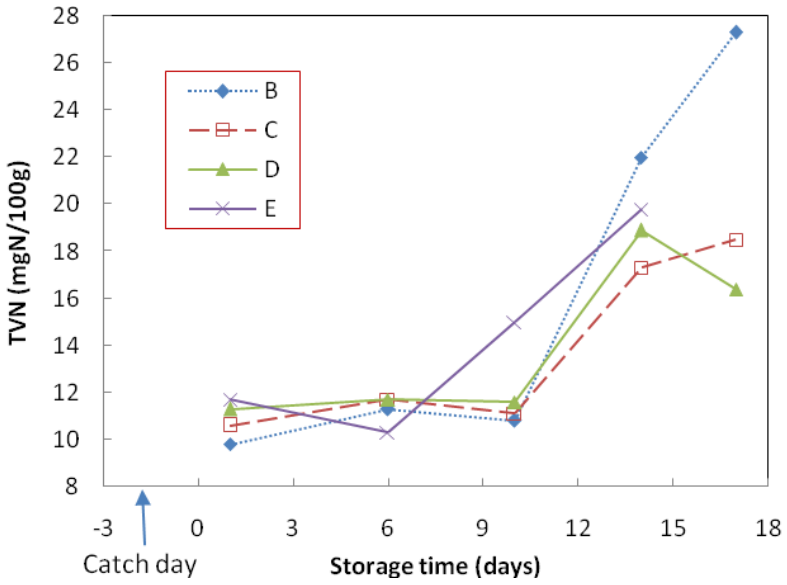

Figure 21: TVN in processed cod fillets stored at -1.5˚C (B-slurry ice, C-CBC, D-cooling mat, E-traditional method (neither pre-cooled nor cooling mat))

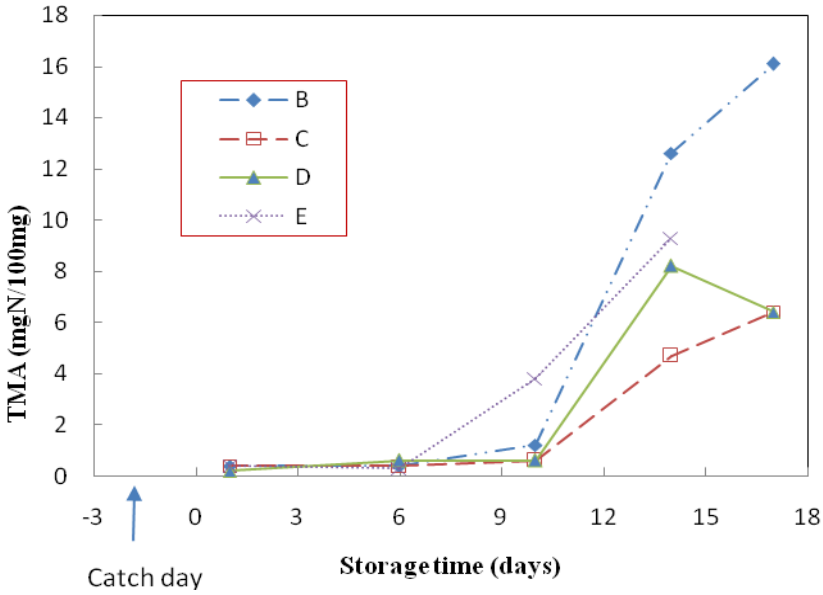

Figure 22: TMA in processed cod fillets stored at -1.5˚C (B-slurry ice, C-CBC, D-cooling mat, E-traditional method (neither pre-cooled nor cooling mat))

4.5 Microbial analysis

The total viable counts (TVC) and counts of H_2S producers are shown in Figures 23 and 24.

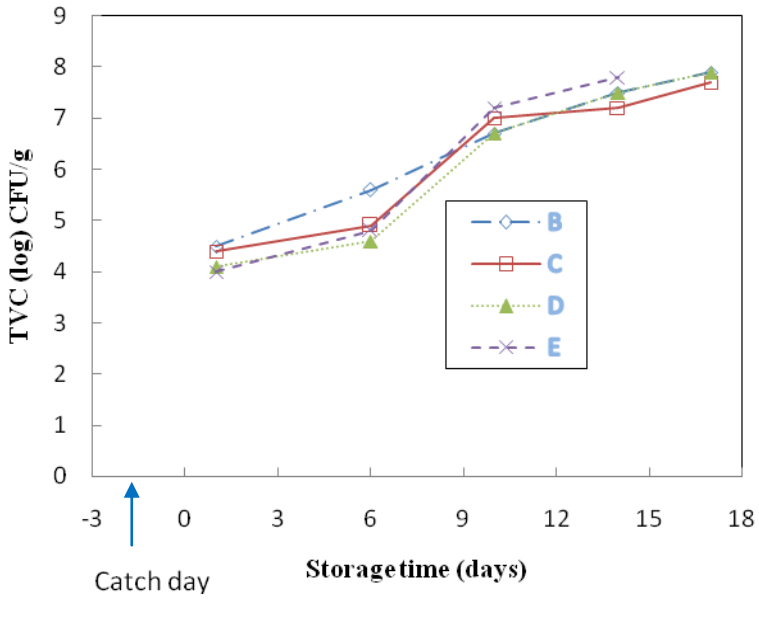

Figure 23: Total viable counts of bacteria on Iron Agar at 17˚C in processed cod fillets stored at -1.5˚C (B-slurry ice, C-CBC, D-cooling mat, E-traditional method (neither pre-cooled nor cooling mat))

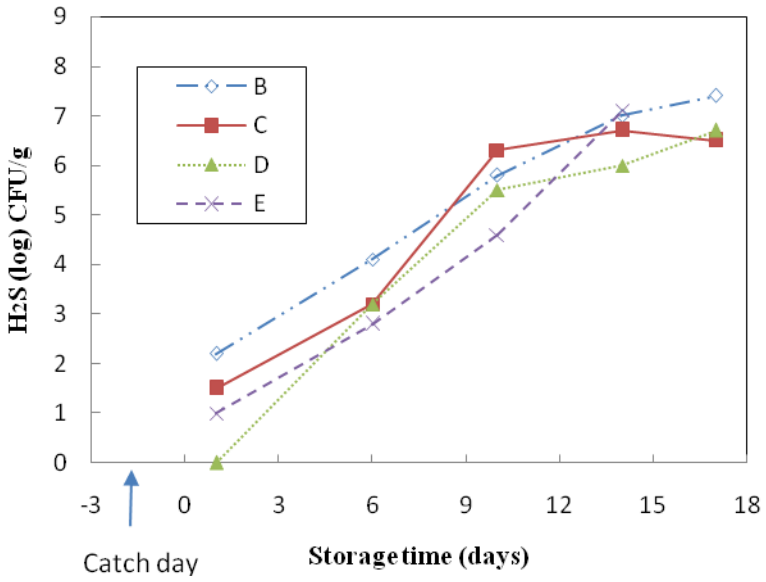

Figure 24: Counts of H_2S producing bacteria on Iron Agar at 17˚C in processed cod fillets stored at -1.5˚C (B-slurry ice, C-CBC, D-cooling mat, E-traditional method (neither pre-cooled nor cooling mat))

4.6 WHC, dripping loss and cooking yield measurements

The results of water holding capacity (WHC) are shown in Figures 25, 26 and 27.

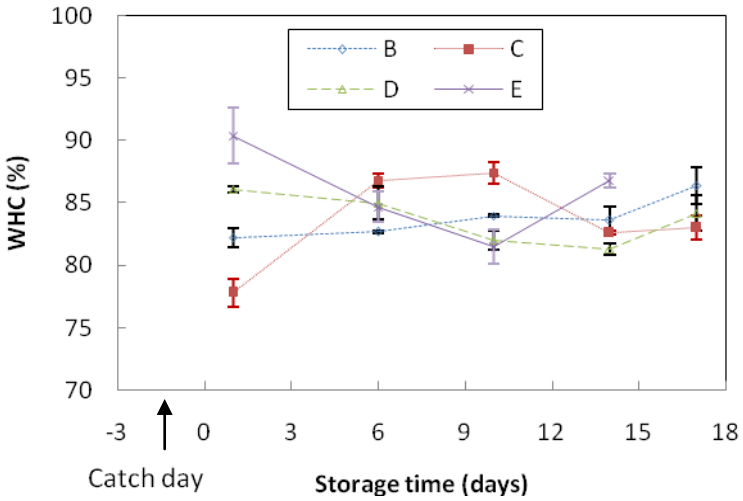

Figure 25: Water holding capacity (WHC) in processed cod fillets stored at -1.5˚C (B-slurry ice, C-CBC, D-cooling mat, E-traditional method). Vertical bars show standard deviation.

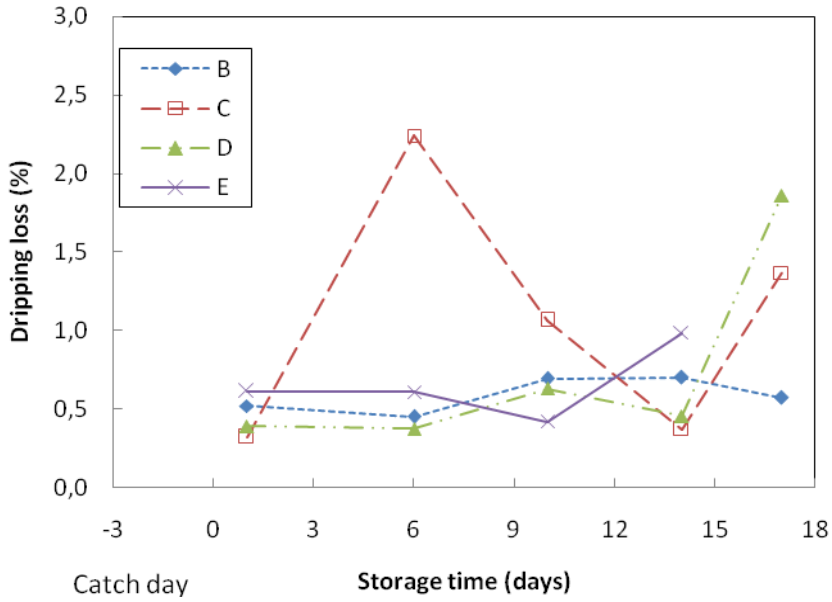

Figure 26: Dripping loss in processed cod fillets stored at -1.5˚C (B-slurry ice, C-CBC, D-cooling mat, E-traditional method).

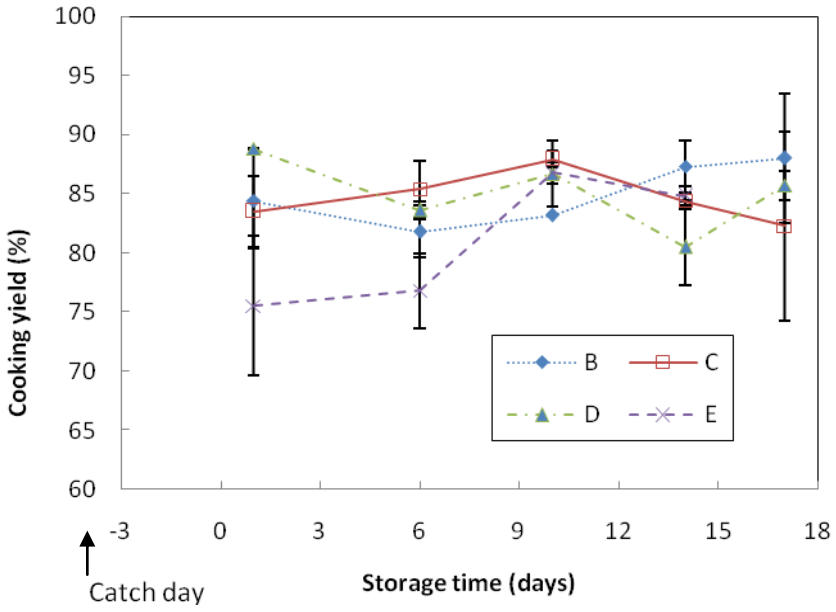

Figure 27: Cooking yield (CY) in processed cod fillets stored at -1.5˚C (B-slurry ice, C-CBC, D-cooling mat, E-traditional method). Vertical bars show standard deviation.

4.7 Comparison of chemical, microbiological and physical quality parameters

The principal component analysis (PCA) transforms the original variables (pH, TVN, TMA, TVC, H_2S, WHC, dripping loss and cooking yield measurements) into a bi-plot. The results concerning both variables (correlation loadings) and objects (score plot) are presented in Figure 28. The first two principal components (PCs) described 71% of measured variables and 15% of measured variables respectively.

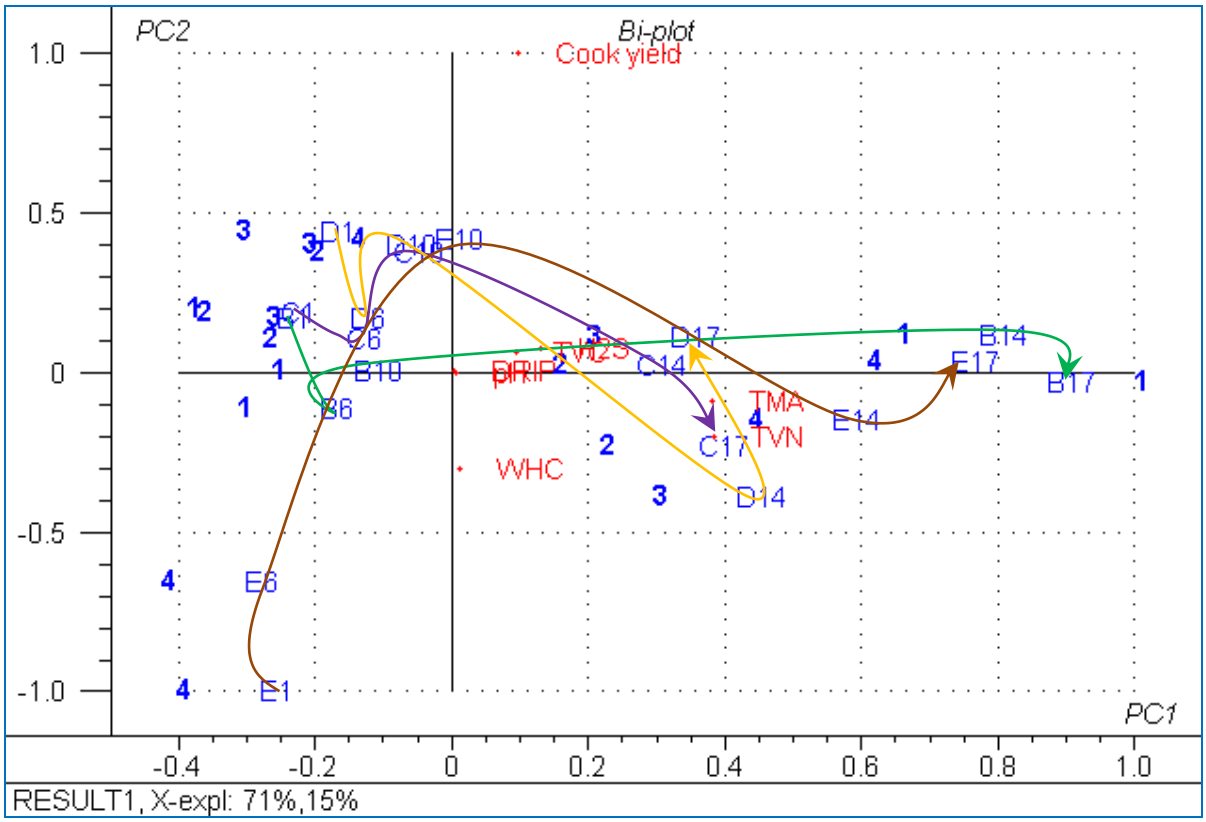

Figure 28: Bi-plot of scores (samples or groups) and loadings (different measurements: pH, TVN, TMA, TVC, H_2S, WHC, dripping loss and cooking yield) PC1 vs. PC2 (X-expl.: 71%, 15%). B-slurry ice, C-CBC, D-cooling mat, E-traditional method.

5 DISCUSSION

5.1 Cooling graphs

The results from the first trial of cooling whole fish in the CBC freezer at air temperature -10 ˚C (Figure 5) showed that the core temperature was higher than expected. Even the average temperature of the top loggers was above 0˚C. It was therefore obvious that -10˚C was not cold enough to chill the whole fish. Unfortunately, the temperature of the bottom obtained from the second test at air temperature -30˚C (Figure 6) was lower than expected, with the lowest level of -5˚C when coming out of the tunnel of the CBC freezer. The fish was evidently frozen rather than fresh. The data on the third trial at -20˚C (Figure 7) on the same day was not ideal because the bottom temperature was close to -1.8˚C at the end of pre-cooling processing. On the other hand, the data at -16˚C (Figure 8) showed that the bottom of the fish was not cooled enough.

In conclusion, valuable recommendations in practical points were generated and were useful for further experiments on cooling techniques in CBC.

Considering the temperature decrease rate of the big logger on top of each specimen was very slow, a temperature probe should be fixed inside the CBC with a display

outside in order to make sure that the temperature inside the CBC is stable before the test is done.

Due to the fact that the central temperature was difficult to decrease down to -1˚C, a longer cooling time should be tried.

From these experiments the appropriate air temperature in CBC should be in the range between -10˚C and -20˚C.

Since changing the speed of the belt was not possible in this plant, the pre-cooling duration was kept at 11 minutes. Adequately referring to the previous suggestions, an amendment was carried out in further experiments done on 30 January 2008. Instead of using a big logger bound to each fish, a big logger was initially hung up in the air inside the CBC (close to the belt) at least one hour before the tests started. This was done to ensure that this particular logger was actually measuring the air temperature inside CBC. Regarding the fact that different locations of the belt had different temperatures, one fish was prepared for each trial and was always put on the same location on the belt (in the centre of the width of the belt).

From Figures 10, 11, 12 and 13, it can be seen that for the air temperature -18˚C the core of the thickest part of the fish was not cooled enough with the core temperature above 0˚C. When the temperature of the CBC was set to -20˚C and -23˚C respectively, a relatively ideal state of temperature and appearance was observed. The thickest part of the whole cod was cooled enough and the surface (skin) was stiff. The core temperature was close to 0˚C. Interestingly, when the temperature of the CBC was set to -24˚C, the lower half (the side which was laid on the conveyor) of cod was totally frozen with a lowest temperature (on the bottom) of -4˚C.

For these experiments an appropriate temperature condition might be concluded as being from -20˚C to 23˚C for whole cod of an average weight of 1.04 kg when pre-cooled in a CBC freezer for 11 minutes. The suitable temperature range would actually be different if the chilling time was different but this was not investigated in present study.

5.2 Temperature profiles during chilling storage

It should be noted that a lower temperature was recorded before storage in group C compared with group B, -0.6°C in the former group and -0.2°C in the latter (Figure 15). The relatively lower temperature, which was observed in group B (processed with slurry ice) compared with the other groups, might be demonstrating the penetration of extra water into the fish flesh resulting in a higher conductive coefficient (W / (m k)).

A temperature lag was recorded for both groups D and E, representing around two days delay in the former group and one day delay in the latter group. Unfortunately, a lower environmental temperature registered by the big loggers outside the boxes was obtained compared with the other two groups (D and E) (data not shown). In detail, from one day to two days of storage, the lowest air temperature was in group D (the average level was about -1.8˚C) and second lowest air temperature in group E (the average level was about -1.7˚C). During the storage period of day 2 to day 3 the low-

est air temperature in group D was observed at -2˚C and a similar value was recorded in the other three groups (at -1.8˚C). It could be pointed out that the temperature inside the boxes decreased more slowly in groups D and E compared with the other groups at the beginning of storage. This might be explained by the higher initial temperature (before storage) for groups D and E (5.2˚C and 5.6˚C) since it must take some time to chill the products in groups D and E down to the initial temperatures in groups B and C.

5.3 Sensory evaluation

The predominant difference between the samples was freshness, mainly sweet odour and flavour and metallic flavour to the left, but the storage characteristics to the right, such as sour odour and flavour, and TMA odour and flavour (Figure 18). There were also a difference observed between samples with regard to texture attributes, such as softness, mush, tenderness and flakiness.

There was not much difference detected in sensory attributes related to freshness between groups during the early storage period such as on day 1 and day 6. However, as storage time progressed, groups C and D show much greater spoilage characteristics such as TMA odour, TMA flavour, sour flavour, frozen storage flavour etc, than groups B and E. Within day 14 and day 17 of storage, the deterioration rate of groups C and D was much faster than of group B, as seen by the location on day 14 close to neutral attributes and on day 17 close to spoilage attributes. Even at day 17 of storage group B was still at neutral related by neutral attributes such as meat odour and meat flavour. The spoilage signs such as sour flavour and pungent flavour in group B did not have much significance ($P < 0.001$) (Table 1) difference compared to the other groups. This might be pointed out that treatment with slurry ice and further chilled storage had an effect on prolonging the process from neutral quality to spoilage with regard to sensory results.

The locations of sampling days for the four groups (Figure 19) were mainly on the lower part of the score plot except the point of group C on day 10 of storage. Group B was more described by soft texture at the end of the storage time compared to the other groups. However, groups C and D were more described by flakiness in texture at the end of storage time. Texture attributes in group B did not change much with storage time. However, groups C, D and E appeared to be significantly less juicy and tender with storage time.

Group C on day 10 had a significant difference (Table 1) in texture from the other samples, as it was more soft ($p < 0.05$), tender ($p < 0.001$) and mushy ($p < 0.01$). This phenomenon recalls the temperature conditions of the cold storage. From Figures 15, 16 and 17, temperature fluctuations (outside box) in the cold storage occurred three times during storage resulting in the temperature difference inside the box of 0.2˚C (the temperature abused outside of box was in the range of about -1˚C to -5˚C), representing the temperature range inside the box about –0.8˚C to -1.0˚C. This might be illustrated as follows:

Huss (1995) reported that at the beginning of the spoilage stage the off-flavour might be slightly sour, fruity and slightly bitter. During the later stages sickly sweet, cabbage-like, ammoniacal, sulphurous and rancid smells developed. The texture became

either soft and watery or tough and dry. In this study, more tender, soft and mushy texture ($p<0.001$) was observed in group C at day 10 (Table 1).

As fish is exposed to the abusive temperature range stated above, many catabolic reactions in the muscle proceed faster than in the unfrozen tissue (Sikorski and Kolakowska 1994). Nowlan and Dwyer (1969) also found that as the time tissue spends at between – 0.8˚C and – 5˚C (the critical temperature zone) increases, so does tissue metabolism.

Van den Thillard *et al.* (1990) illustrated that the probable reasons for this included the ultrastructure damage caused by ice crystals piercing organelle membranes, thus activating enzymes through the release of calcium ions from the sarcoplasmic reticulum. As water ice crystals formed, the concentration of enzymes, ions and effectors in the intracellular fluid increased. It is probable that temperature fluctuation caused the tissue to pass through the critical freezing zone at a relatively slow rate during freezing and thawing. The maceration of the tissue facilitated intracellular fluid mixing, thereby increasing enzymatic activity.

It had not been confirmed that there were other possible reasons except those mentioned above corresponding to the changes in texture in group B at day 10, such as aspects of raw material (before processing) and the processing before pre-cooling in factory, etc.

For groups B and C the colour became darker and more discoloured with storage time, and these two groups were in some cases darker and more discoloured compared to groups D and E.

5.4 Chemical measurements

Changes in pH (Figure 20) showed that on the first sampling day (one day after processing) groups B, C and D had a pH of 6.4 and 6.5 but group E had a pH of 6.7. Similar increasing trends in pH for groups B, C and D were observed during the 10 days of storage, except for group B where pH decreased slightly on day 10 (10 days storage, 10 days after processing). The pH value in group E declined before day 10 and went up again after day 10, reaching 6.9 at day 17. Accordingly, the pH in the four groups was always below 7, in agreement with the results of cod loins stored at -1.0˚C (Wang *et al*. 2008).

In general, increases both in TVN and TMA were obtained throughout storage time in the four groups (Figures 21 and 22). There was not much difference in TVN and TMA observed at first day 6 in all groups. The TMA from day 1 to day 6 recorded an almost horizontal line. After 10 days of storage there was a much faster increase in the four groups than before day 10 both in TVN and TMA. The formation of TVN and TMA was delayed during the 10 days of storage in groups B, C and D compared to group E. Slurry ice, CBC pre-cooling and cooling mats had a similar influence on the production of TMA and TVN from one day of storage up to day 10 of storage. It could be pointed out that these three different cooling methods slow down the rate of spoilage compared to group E without cooling any method during the early storage period before 10 days of storage.

The highest TVN and TMA values were measured at 27.3 mg N/100g and 16.1mg N/100g for group B on day –17 of storage. A more rapid increase both in TVN and TMA in group B after day 10 of storage was obtained compared to the other groups. This demonstrates that cross-contamination in the processing with slurry ice played an important role in the production of TVN and TMA in the later stages of storage time. This might be explained as relatively more microorganisms leaked from the slurry ice into the fish flesh resulting in a reduction of Trimethylarnine Oxide (TMAO) (Zeng *et al.* 2005).

5.5 Microbial analysis

The total viable counts (TVC) were similar in all experimental groups over the storage period (Figure 23). On day 6, the highest counts of TVC were obtained in group B. However, on day 14, the highest counts were observed in group E. No definite conclusions could be drawn from the records of TVC.

No H_2S producing bacteria were found in group D (Figure 24) at the beginning of storage. During the 17 days of storage, the highest levels of H_2S producing bacteria were in group B (where slurry ice was used) except on day 10 when the highest counts of H_2S producer were in group C. This is in good agreement with the results of highest value in TMA for group B after day 10. This might suggest that some H_2S producing bacteria that could reduce TMAO to TMA played an important role in the spoilage of fresh cod such as in group B.

Interestingly, the H_2S producing bacteria count taken on the first sampling day for slurry ice was about 17 times higher than in cod fillets for group B at the same day, or 3.4 log [CFU]/g compared to 1.5 log [CFU]/g in the fillets. This indicates that some contamination might have taken place from the slurry ice used in pre-cooling processing in plant. Although temperature evolution in the centre of the packaged box during storage (Figure 15) in group B was slightly lower than the other groups, still the highest numbers of bacteria was observed. It should be pointed out that changing slurry ice could decrease the initial number of bacteria contaminated by slurry ice in fresh fish.

As storage time progressed the number of H_2S producing bacteria increased. However, no distinct difference was found between the experimental groups although the highest counts were recorded in group B at day 17 of storage.

From microbiological analysis it could be concluded that the three pre-cooling methods had similar effects on prohibition of growth of bacteria if hygienic handling with slurry ice was performed.

5.6 WHC, dripping loss and cooking yield measurements

A big difference in initial levels of WHC was observed after one day of storage, as seen by group E on the highest level and group C on the lowest level (Figure 25). A possible reason might be pre-cooling methods such as chilling in CBC destroyed cells resulting in loss of water. After six days of storage, similar levels of WHC were obtained in groups B, D and E. Between day 6 and day 10, the highest value in WHC for group C was detected compared to the other three groups.

Not much difference in dripping loss (Figure 26) was observed at the beginning of storage (after one day of storage) between all groups. Similar values of dripping loss were detected in groups B, D and E between day 6 and day 14 of storage. A distinct higher level of dripping loss was found in group C at day 6 of storage compared to the other groups.

No obvious difference in cooking yield (Figure 27) for all groups was found during the storage period between the four groups.

5.7 Comparison of chemical, microbiological and physical quality parameters

The chemical and microbial parameters TMA, TVN, H_2S producer and TVC (Figure 28) which contribute to the spoilage mechanism, explained most of the variation in the dataset. The first PC is defined by storage time of the four groups, as seen by location of samples at the beginning of storage time to the left, while the location of samples at end of storage to the right was along PC1. The second PC corresponding to physical parameters was presented as cooking yield, dripping loss and WHC.

The chemical parameters, microbiological characteristics, dripping loss and WHC are placed to the right and close together and, therefore, positively correlated. On the other hand, cooking yield had no obvious correlations with the above variables. Between days 14 and 17, the samples appeared to be located to the right of the plot, which shows that the four groups had high values of TVN and TMA during this storage period. This result has a good agreement with chemical and microbial evaluation. The spoilage bacteria corresponding to the quality of fresh cod might be TMA producing bacteria as mentioned before. A valuable recommendation for further study might be identifying the specific spoilage bacteria for fresh cod performing the pre-cooling methods.

All the samples located on the left of the plot have lower values of TVC, H_2S producer, TVN and TMN before day 10. The samples of early storage clustered except group E at day 1 and day 6 of storage away from the cluster. Firstly, this presents that the samples of four groups from day 1 to day 10 of storage had relatively fresh quality. Secondly, lower cooking yield levels were obtained in group E at day 1 and day 6 of storage compared to the other groups between day 1 and day 10 of storage.

This graph also expresses the trends of quality changes of samples corresponding to storage time. In detail, group B and group E had a higher tendency related to bad qualities at the end of the storage time compared to the other two groups, which is in good agreement with the records of TVC, H_2S producer, TVN and TMA.

In general, comparison of chemical, microbiological and physical quality parameters of fresh cod showed that during the early storage period the fresh quality in the four groups did not differ much. Cooling mats and CBC pre-cooling methods delayed the growth of bacteria and decreased the formation of TVN and TMA. Group B treated with slurry ice at the end of storage showed a high correlation to TVN and TMA. TVN, TMA, TVC, pH and H_2S producer have a good correlation for evaluation of the quality of fresh cod.

6 CONCLUSIONS

From CBC pre-cooling experiments an appropriate temperature condition might be concluded as being from -20˚C to 23˚C for whole cod of an average weight of 1.04 kg when pre-cooled in a CBC freezer for 11 minutes. The suitable temperature range would actually be different if the chilling time is different but this was not investigated in present study.

It could be highlighted that these three different cooling methods slow down the rate of formation of TVN and TMA spoilage compared to group E without the cooling method during the early storage period, i.e. before 10 days of storage. From microbiological analysis it could be concluded that the three pre-cooling methods had similar effects on the prohibition of growth of bacteria if hygienic handling with slurry ice was performed. It might be pointed out that treatment with slurry ice and further chilled storage had an effect on prolonging the process from neutral quality to spoilage with regard to sensory results.

Comparison of chemical, microbiological and physical quality parameters of fresh cod showed that TVN, TMA, TVC, pH and H_2S producer have a good correlation with evaluation of quality of fresh cod. A valuable recommendation for further study might be identifying the specific spoilage bacteria for fresh cod performed with the pre-cooling methods.

The three different pre-cooling methods studied for fresh cod are very promising techniques to effectively ensure the high quality of fresh fish products.

The good results obtained in the microbiological, sensory, and chemical analyses performed clearly confirm the practical advantages of using slurry ice for the refrigeration and moderately prolonged chilled storage of lean fish species of relevant commercial value, such as cod. Slurry ice as a treatment of pre-cooling is effective in the inhibition of growth of bacteria if hygienic aspects of slurry ice are adequately taken into account in application. It should be pointed out that changing slurry ice could decrease the initial number of bacteria contaminated by slurry ice in fresh fish.

Results from this study show that CBC pre-cooling is a good method for delaying quality deterioration of fresh cod from the point of view of chemical and microbiological assessments. Very careful temperature monitoring in further chilled storage also plays an important role in guaranteeing the quality of fresh fish.

Results from the present study give a valuable contribution to future studies aimed at defining the optimal combination of CBC pre-cooling and chilled storage temperatures for cod fish. To be able to put forward recommendations on optimal pre-cooling conditions and chilled storage conditions such as temperatures, future studies should aim at separately elucidating the underlying mechanisms determining changes in physical, mechanical and rheological properties of cod muscle upon pre-cooling and chilled storage at different temperatures and durations.

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Appendix 1: Sensory vocabulary of QDA for cooked samples of cod *(Gadus morhua)*

Sensory attribute	Description of attribute
Odour	
sweet	Sweet odour
shellfish, algae	Characteristic, fresh
meat	Reminds of boiled meat or halibut
vanilla/warm milk	Vanilla, warm milk
boiled potatoes	Odour reminds of boiled potatoes
frozen storage	Refrigerator, freezer storage odour
table cloth	Reminds of a table cloth (damp cloth to clean kitchen table, left for 36 h)
TMA	TMA odour, reminds of dried salted fish, amine
sour	Sour odour, sour milk, spoilage sour, acetic acid
sulphur	Sulphur, matchstick
Appearance	
colour	Left end: light, white colour. Right end: dark, yellowish, brownish, grey
appearance	Left end: homogenous, even colour. Right end: discoloured, heterogeneous, stains
white precipitation	White precipitation on the fish surface
Flavour	
salt	Salty taste on tongue
metallic	Characteristic metallic flavour of fresh cod
sweet	Sweet flavour
meat	Reminds of boiled meat, meat-sour
frozen storage	Refrigerator, freezer storage flavour
pungent	Pungent taste on tongue
sour	Sour taste, spoilage sour
TMA	TMA flavour, reminds of dried salted fish, amine
off-flavour	Intensity of off-flavour
Texture	
flakiness	The fish portion slides into flakes when pressed with the fork
soft	Left end: firm. Right end: soft. Evaluate how firm or soft the fish is during the first bite
juicy	Left end: dry. Right end: Juicy. Evaluated after chewing several times: dry - pulls juice from the mouth
tender	Left end: tough. Right end: tender. Evaluated after chewing several times
mushy	mushy, porridge
meaty mouthfeel	reminds of meat texture, rough fibers
clammy	Clammy texture, tannin (dry redwine)
rubbery	Rubbery texture, chewing gum