THE EFFECTS OF TEMPERATURE ABUSE AT THE BEGINNING OF STORAGE ON THE QUALITY AND SHELF LIFE OF FRESH WATER ARCTIC CHARR (*SALVELINUS ALPINUS*)

Cyprian Ogombe Odoli
Kenya Marine and Fisheries Research Institute
P.O.Box. 81651, Mombasa 80100, KENYA
codoli@kmfri.co.ke

Supervisors
Emilia Martinsdottir
Icelandic Fisheries Laboratories/MATÍS
emilia.martinsdottir@matis.is
and
Kolbrun Sveinsdottir
Icelandic Fisheries Laboratories/MATÍS
kolbrun.sveinsdottir@matis.is

ABSTRACT

The main aim of this project was to study the influence of temperature abuse during the early stages of storage on the shelf life of Arctic charr (*Salvelinus alpinus*). A shelf life study was performed with farmed Arctic charr stored under two different conditions: a) iced and stored at 1^oC (well handled lot) and b) iced, but stored at 18^oC for 24 hours (resulting in a temperature increase from 3-12°C) on the second day of storage prior to re-icing and storage at 1^oC (temperature abused lot). Approximately every third day during storage, samples were collected for sensory analysis using the Quality Index Method (QIM) and Quantitative Descriptive Analysis (QDA) and microbiological counts (Total Viable Counts (TVC) and H_2S producing bacteria). The maximum shelf life of well handled and temperature abused Arctic charr was 17 and 15 days respectively according to sensory evaluation of cooked fillets (QDA). Sensory evaluation showed a high linear correlation between Quality Index (QI) and storage time for the well handled lot. At the end of shelf life the QI was 17 for both groups. The TVC at the consumption limits were 10^5-10^6 CFU/g of flesh for both lots. H_2S producing bacteria constituted a higher proportion of TVC in the abused than in the well handled fish at the end of the storage time (study). High correlation between QI scores, TVC and H_2S producing bacteria was found.

Key words: Arctic charr, sensory evaluation, microbial counts, storage temperature and shelf life.

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1   INTRODUCTION

Seafood has traditionally been a popular part of people's diet in many parts of the world. In Kenya it has even constituted the main supply of animal protein for low and middle level income groups (Government of Kenya-FD 2004). Kenya's fisheries resources contribute to the national economy through employment creation, foreign exchange earnings and food security support. It has been calculated that the fisheries sector constitutes almost 5% of the gross domestic product (agriculture and tourism contribute the most). The artisanal sector, in spite of its low technological development, remains the backbone of fish production in Kenya. Fisheries Department statistics indicate that artisanal fisheries contribute approximately 90% of the average 170,000 metric tonnes landed annually (Government of Kenya-FD 1982-2004). The other 10% is derived from commercial prawn and yellow fin tuna trawlers operating in the inshore and off shore waters of the Kenyan coast respectively.

Artisanal fisheries in Kenya are often undertaken by small boats, cataracts and planked pirogues open at the top. Only about 20% of the fishing crafts are motorised (McClanahan 1997) and fish is generally not iced. This is because ice is too expensive for the low-income groups and is not readily available in the fishing communities. On arrival at landing sites, fish have been exposed to high ambient temperatures, which may lead to rapid quality deterioration. Therefore, the problem of post harvest losses on board in the artisanal fisheries sector cannot be overemphasised. Year after year many artisanal fishermen and dealers experience losses attributable to poor handling and preservation augmented by ignorance and lack of capital.
Conversely, in commercial (industrialised) fisheries, precautionary measures are taken to freeze the catch or maintain low temperature.

In Kenya, landed fish appear to be held over a long period between catching and landing in open boats often without ice where plant fibre, woven baskets and gunny bags are thought to prevent direct heating of the fish by the sun, thereby diminishing the rate of bacterial decomposition. These treatments of the fish after catch may result in cross contamination of microbes from the holding materials to the fish since it does not conform to simple hygienic and good fish handling practice. Thus it is necessary to improve handling conditions by implementing national regulations and fish inspections.

It is surprising that there is tremendous effort in the processing plants, fish auctions and transporting tracks to ensure fish quality while little attention is being paid to the quality of raw materials on board the fishing vessels. Understanding the mechanisms of fish spoilage and ways of controlling it to minimise losses during handling is therefore important. Recognising the contribution made by fisheries to food security and the incomes of millions in Africa, the New Partnership for Africa Development (NEPAD) endorsed the *Fisheries Action plan* in the NEPAD-*Fish for All* summit (NEPAD-Abuja 2005). This was aimed at enhancing productivity by minimising post harvest losses and spoilage prior to fish landing and the successful processing and distribution stages.

Kenyan fisheries are controlled by the Fisheries Department (FD) and the Kenya Marine and Fisheries Research Institute (KMFRI), both departments of the Ministry of Livestock and Fisheries Development. The former (FD) is responsible for management and law enforcement as regards fisheries, while the KMFRI has the mandate to conduct research in aquatic resources and give appropriate information and advice to the FD.

The main aim of the present project is to study the influence of temperature abuse during early stages of storage on the shelf life of Arctic charr. The study was done on farmed Arctic charr because handling of farmed fish is more easily controlled from slaughter than handling of wild fish and it is easier to exclude factors influencing quality other than those being tested. Fish handled under ambient temperature of about 18°C for 24 hours prior to re-icing may be assumed to reflect handling conditions in Kenya and spoilage at this temperature in Iceland is comparable to 27°C in Kenya. The knowledge acquired in this study is a base for running similar projects in Kenya, the results from which will be proposed to the FD for implementation to address key factors in fish post harvest losses prior to landing, hence improving the overall freshness of fish landed and in return increase revenue to the fisher folk.

More specifically, this study has the following objectives:
- To study quality changes in Arctic charr stored in ice (well handled) and temperature abused for 24 hours at 18^oC before re-icing;
- To observe sensory changes (sensory evaluation: Quality Index Method (QIM) and Quantitative Descriptive Analysis (QDA));
- To test the previously developed QIM scheme for Arctic charr;
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- To observe bacterial growth (microbiological analysis: TVC and H_2S producing bacteria);
- To note changes in pH (chemical analysis);
- To record temperature changes during storage in ice (temperature data logger);
- To estimate the shelf life of Arctic charr under the experimented conditions;
- To analyse and interpret the results in comparison to the tested conditions;
- To compare the various evaluation methods used.

The main research questions to be answered are:
- What quality changes occur in Arctic charr stored in ice and temperature abused for 24 hours prior to re-icing?
- Are there correlations among the results of the sensory, chemical (pH) and microbiological analyses carried out?
- Is the shelf life of Arctic charr stored in ice and of Arctic charr under the experimented conditions different? if so, how and how large is the difference?
- How well does the QIM scheme for Arctic charr function when the fish has been stored under ambient temperature for part of the storage time?

2   LITERATURE REVIEW

2.1   Quality and changes in raw fish

Fish is one of the most perishable types of food. The success of any effort in the food industry, including fisheries, is highly dependent on getting the product to the consumer in an acceptable condition. Consequently, efforts are required to maintain the quality and acceptability of the fish and fisheries products. 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).

The term "quality" with reference to food can have different meanings. It can refer to the sensory characteristics of a product, such as its appearance, flavour, odour and texture, but it can also indicate nutritional value, safety and other characteristics. Before buying a food product for the first time, the consumer evaluates its quality based on these characteristics (Jones and Disney 1996).

When fish dies, the muscle is totally relaxed and the elastic texture persists for some hours and then the muscles contract. When the whole body becomes inflexible, the fish is said to be in *rigor mortis*. This condition usually lasts for a day or more and then the muscle relaxes again and recovers its flexibility, but not elasticity (Huss 1995).

Death initiates a series of deteriorative changes resulting in spoilage. The deteriorative changes responsible for spoilage are due to a combination of microbiological, chemical and autolytic phenomena (Huss 1994). These changes can be accelerated or retarded by physical conditions like temperature, physical damage to fish, pollution and contamination by bacterial flora. Of all the physical and chemical factors influencing spoilage, temperature is the most significant. Chemical reactions, enzymatic activity and bacterial multiplication require an optimal temperature range (Lima 1981). Fish kept directly exposed to the sun's heat spoils faster than those kept in the shade and fish kept in ice has a longer shelf life than those kept in shade. Fish frozen and stored in cold rooms stay preserved for extended periods of time (FAO 1996).

Loss of freshness and spoilage of fish are complicated processes and various factors such as species and different storage conditions influence the spoilage pattern. Therefore, it has been suggested that no single spoilage or freshness indicator for fish can be used, but rather a combination of selected indicators that represent the different changes occurring during spoilage (Olafsdottir *et al*. 1997). Sensory changes occur in appearance, odour, taste and texture during storage of fish. Sensory assessment of the outer appearance of fish and /or the sensory assessment of the sample in the cooked state is the most convenient and successful method for fish freshness determination (Olafsdottir *et al*. 1997). Spoilage of fish is not clearly defined although obvious signs of spoilage include the formation of off-odours and off-flavours, slime formation, gas production and changes in texture (Reay and Shewan 1949). The shelf life of fish stored under ambient tropical conditions which is generally noted to be less than a day, will depend on factors like handling conditions, species, quality of fishing ground, season, sexual and nutrition status (Jones and Disney 1996).

The quality changes during storage of fresh water Nile perch (*Lates niloticus*) investigated during the FAO project in Kenya (Gram *et al*. 1990), showed that Nile perch stored at ambient temperature (20-30°C) spoiled rapidly and was unacceptable for human consumption after 11-17 hours, whereas icing ensured a storage life of 4 weeks. Fish caught in temperate waters

rarely keeps more than 2-3 weeks in ice and the storage trials with Nile perch confirmed that fish caught in warm waters often have extended iced storage lives as compared to temperate species (Gram *et al*. 1990). Several proposals have been made to explain the long iced storage lives of warm water fish such as suggesting a lower initial number of psychrotrophilic bacteria (Shewan 1977), a different flora or a non bacterial spoilage (Gram *et al*. 1990).

2.1.1   *Microbiological spoilage*

On live and newly caught fish, the microorganisms are found on the skin, gills and in the intestines. The total number of organisms varies enormously depending on the environment and on the fish species. Fish caught in very cold, clean waters carry lower numbers compared with fish caught in warm waters. The flesh of healthy live or newly caught fish is sterile. When a fish dies, the bacteria are allowed to proliferate in the beginning on the skin and during storage, they invade the flesh (Huss 1988).

Microbial spoilage of foods may take diverse forms, but all of them are the consequence of microbial growth, which are manifested as changes in sensorial characteristics as shown in Table1.

Table 1: Microbiological spoilage of foods (Gram and Huss 1996).

Microbiological activity	Sensory manifestation
Breakdown of food components	Production of off-odours and flavours
Production of extra cellular polysaccharide material	Slime formation
Growth of moulds, bacteria, yeasts	Large visible pigmented or non-pigmented colonies
CO_2 – form carbohydrate or amino acids	Production of gas
Production of diffusible pigments	Discolouration

The total number of bacteria on fish rarely indicates sensorial quality or expected characteristics. However, it is well recognised that certain Gram- negative bacteria are the main cause of spoilage (Huss *et al*. 1974).

Initial loss of quality of fish, chilled or not chilled, is caused by autolytic changes, while spoilage is mainly due to the action of bacteria. One example is the reduction of trimethylamine oxide (TMAO) in chilled marine fish by a bacterial process with the formation of trimethylamine (TMA). The initial flora of the fish is very diverse, although normally it is dominated by Gram-negative psychrotrophic bacteria. In tropical areas, fish may have a slightly higher load of Gram-positive organisms and enteric bacteria. During storage a characteristic flora develops, but only a part of this flora contributes to spoilage (Huss 1994).

The spoilage of marine temperate-water fish is characterised sensorial by the development of offensive, fishy, rotten, H_2S off-odours and off-flavours. This behaviour is different from some tropical and fresh water fish, where fruity, sulphydryl off-odours and off–flavours are more typical (Gram and Huss 1996).

2.1.2 Chemical spoilage

The chemical composition is an important aspect of fish quality, and influences both the keeping quality and the sensory characteristics of the fish (Huss 1988).

The most important chemical spoilage processes are changes taking place in the lipid fraction of the fish. The auto-oxidation process is a reaction involving only oxygen and unsaturated lipids. The first step of the oxidation process leads to formation of hydro-peroxides, which are tasteless but can cause brown and yellow discolouration of the fish tissue. The degradation of hydro-peroxides gives rise to the formation of aldehydes and ketones. These compounds have a strong rancid flavour (Huss 1994). Factors such as heat, light, and several organic and inorganic substances like copper or iron, can initiate and accelerate oxidation (Huss 1994).

The pH of muscle tissue of live fish is close to neutrality. The initial post mortem pH varies with species, catching ground and season. Due to post-mortem anaerobic formation of lactic acid, pH decreases usually within the first day of death. During the later post-mortem changes, pH is more or less constant or slightly increased due to the formation of basic compounds (Shewan 1977, Love 1980, Huss, 1988). Even though the changes in pH are generally rather small, they have great technological importance. The post-mortem pH is, according to Huss (1988), the most significant factor influencing the texture of the meat and the degree of "gaping", i.e. the rupture of the connective tissue. One of the reasons for this is that even minor changes in pH drastically affect the properties of the connective tissue.

2.1.3 Autolytic spoilage

The autolytic changes are responsible for the initial loss of quality in fresh fish, but contribute very little to spoilage of chilled fish and fish products. However, in frozen fish, the autolytic changes have great importance. Bacterial action is inhibited and TMAO is broken down by autolytic enzymes to dimethylamine (DMA) and formaldehyde (FA). The FA formed causes increased on denaturation of fish tissue, changes in texture and loss of water retention capacity (Huss 1994).

2.2 Methods for measuring fish freshness

The methods for evaluating wet fish quality may conveniently be divided into two categories: sensory and instrumental. Since the consumer is the ultimate judge of quality, most chemical or microbiological methods must be correlated with sensory evaluation before being used in the laboratory. However, sensory methods must be performed scientifically under carefully controlled conditions so that the effects of the test environment and personal bias may be reduced (Connell 1986).

Sensory evaluation is a scientific discipline used to evoke, measure, analyse and interpret reactions to characteristics of food as perceived through the sense of sight, smell, taste, touch and hearing. Most sensory characteristics can only be measured meaningfully by humans. Scientifically, the process can be divided into three steps; detection of a stimulus by human sense organs, evaluation and interpretation by a mental process, and then the response of the assessor to the stimuli (FAO 1996). Variations among individuals in the response to the same level of stimuli are sometimes found and can contribute to a non-conclusive answer of the test. In most cases sensory methods are useful for identifying products of very good or poor quality. Thus, biochemical and chemical methods may best be used in resolving issues regarding

products of marginal quality. Such objective methods should however correlate with sensory quality evaluation and the chemical compound to be measured to increase or decrease with the level of microbial spoilage or autolysis (Connell 1986).

2.2.1 Sensory evaluation methods

Sensory evaluation is the most important method today for freshness evaluation in the fish sector, because the consumer is the ultimate judge of quality (Connell 1986). There are several methods used in sensory evaluation of seafood e.g. Quality Index Method, Torry scale, raw fillets grading method and Quantitative Descriptive Analysis.

Quality Index Method (QIM)

QIM was developed by the Tasmanian Food Research unit in Australia (Bremner 1985). QIM is based on characteristic changes that occur in raw fish. A score from 0-1, 0-2, or 0-3 demerit points is given for changes occurring in odour, texture and in the external appearance of the eyes, skin and gills. It has been used by many research laboratories in Europe and has partly been implemented in the industry. When compared to the EU scheme, the main advantages of the QIM method is that it is specific for each species and confusion about attributes is minimised (Martinsdottir *et al*. 2001, Sveinsdottir *et al*. 2003). Each fish species has its own characteristic sensory attributes of flavour, appearance, odour, and texture which change with time and temperature after harvest (Olafsdottir and Fleurence 1997).

QIM is based on significant, well-defined characteristic changes of appearance attributes that occur in raw fish such as eyes, skin, gills and changes that occur in odour and texture with storage time (Luten and Martinsdottir 1997, Hyldig and Nielsen 2004). A score from 0 to 3 demerit (index) points is given for each quality parameter according to the specific parameter descriptions. The scores are summarised to give an overall sensory score referred to as the Quality Index (QI). If the total length of shelf life of the species in ice is known, the total number of index points can also be used to estimate the past and remaining shelf life as the QI increases linearly with the storage time in ice. The shelf life can be determined with sensory evaluation of cooked fish (Luten and Martinsdottir 1997, Martinsdottir *et al*. 2001). In addition, in shelf life studies, it is useful to conduct microbiological and chemical analyses in parallel to the sensory evaluation to have supporting information about the spoilage of fish (Chytiri *et al*. 2004).

QIM schemes have been developed for several fish species. QIM Eurofish published a manual (Martinsdottir *et al*. 2001), available in 11 languages. It contains QIM schemes for 12 fish species and information about how to use the QIM schemes (QIM-Eurofish 2004). Some of the advantages of QIM are that it requires only short training, is rapid and easy to perform, non-destructive to the sample and can be used as a tool in production planning and quality assurance work (Hyldig and Nielsen 1997).

Considering food safety, it is important to maintain the high quality of fish in each link of the chain from catch to consumer. Sensory evaluation is one of the most often used methods for assessing freshness and quality in the fish sector and in fish inspection services. The QIM may also be a useful tool for fishermen and thus affect handling of the catch on board. Also, it can be a part of labelling and identification of the catch (Hyldig and Nielsen 2004).

Several authors have applied QIM in their studies. It is a rapid, reliable and generally accepted method to assess freshness in practical circumstances of auctions and processing sites (Martinsdottir 2002). The QIM chain project, in which eight European institutions were involved, showed that some fish auctions in the Netherlands, Belgium and UK have started to use the QIM method on a daily basis (QIM-Eurofish 2004). A project initiated by the Sydney Fish market has been underway at the Department of Primary Industries and Fisheries in Australia to develop schemes according to the QIM methodology. The QIM is specific for each species and various QIM schemes have been developed for different fish species. QIM Schemes have been developed for species such as cod (*Gadus morhua*) (Larsen *et al.,* 1992), herring (*Clupea harengus*) (Jonsdottir 1992), Atlantic mackerel (Scomber scombrus), horse mackerel (*Trachurus trachurus*), and European sardine (*Sardina pilchardus*) (Andrade *et al.,* 1997). A draft scheme for Arctic charr stored in ice has been developed (Milanes 2004).

Evaluation of cooked fillets (Torry and QDA)

For sensory evaluation of fish fillets, it is common to cook the fillets and evaluate quality attributes such as odour, flavour, appearance and texture. The Torry scale is the most used scale in the fish industry for evaluating the freshness of cooked fish (Martinsdottir *et al*. 2001), but sensory descriptive analysis is also used in research laboratories in Europe (Hyldig and Nielsen 1997). The Torry scale is a grading scheme from 10 (very fresh) to 3 (spoiled), through 5.5 which is often used as limit for consumption (Martinsdottir *et al*. 2001). The scheme was developed at the Torry Research Station and has been developed for lean, medium fat and fat fish species.

The Torry scale provides limited information about how the individual characteristics of cooked fish change through the storage time, but by using Quantitative Descriptive Analysis (QDA), much more detailed information can be gained (Stone and Sidel 1985). Quantitative descriptive analysis (QDA) is a method used at the Icelandic Fisheries Laboratories by a sensory panel to evaluate cooked fish (Sveinsdottir *et al.* 2002). This method uses a linear scale, which has anchored in their ends words or terms that describe the intensity or character of attributes evaluated. The intensity increases from left to right, for example, little to much sweat flavour, none to much rancid odour and so on (Stone and Sidel 1985).

Descriptive analysis provides a complete word description of all sensory properties of a product. The success of a descriptive test depends largely on the sensory language describing the attributes of the products to be evaluated (Stone and Sidel 1985). The QDA method relies heavily on statistical analysis to determine the appropriate terms and procedures. QDA panellists evaluate products one at time in separate booths to reduce distraction and panellist interaction. Panellists enter the data into the computer, or on score sheets that are collected individually from the panellist as they are completed. Data is entered for computation usually with a digitizer or card reader directly from the score sheets. Panellists do not discuss data, terminology or samples after each taste session. The results of a QDA test are analysed statistically (Meilgaard *et al*. 1999).

2.2.2   *Chemical and physical methods*

The pH of fish meat may give some valuable information about its condition. Measurements are carried out using a pH meter by placing the electrodes (glass-calome) either directly into the flesh or into a suspension of fish flesh in distilled water (Huss 1988).

According to Doyle (1995), "seafood shelf life is a function of temperature". The temperature will control the rate of bacterial spoilage, enzyme activity and oxidation reaction. At low temperatures, the spoilage rate of fish is reduced and the products remain edible longer. Temperature during shelf life studies can be determined using thermometers such as thermometer data loggers.

2.2.3   *Microbiological methods*

Microbiological examination of fish evaluates the possible presence of bacteria or organisms of public health significance and gives an impression of the hygiene and quality of the fish including temperature abuse and hygiene during handling. The number of specific spoilage bacteria will give information on the remaining shelf life which can be estimated from such numbers. When such microbiological measurements are needed it is recommended to use the numbers of specific spoilage organisms (SSO) or total viable counts (TVC) measurements (Olafsdottir *et al*. 1997).

3   MATERIALS AND METHODS

3.1   Experimental fish (Arctic charr)

Fish used for this study was purchased from the Arctic charr breeding programme at the Holar Agricultural College in northern Iceland. In total 86 fish of an average weight of 650 g was selected for the study, slaughtered and gutted on 30/11/2006. Fish was bled for 20-30 minutes in chilled water and iced prior to transportation to the Icelandic Fisheries Laboratory.

On arriving at the laboratory one day after slaughtering, Arctic charr body temperature was about 3°C (Appendix 1), they were randomly divided into two lots of 35 and 44 fish for different treatments. The remaining seven fish were immediately evaluated in the laboratory using sensory and microbiological techniques described later.

In the first lot (temperature abused), the fish (35) were put in closed styropore boxes with a thermometer logger, under a controlled ambient temperature of 18ºC for 24 hours. They were iced thereafter and treated like the second sample lot.

The second lot (iced), fish (44) were re-iced immediately on arrival in styropore boxes with holes for drainage to allow the escape of melted ice and stored in a cold room (cabinet) at 1ºC, during the study. Fish body surface temperature was kept at 0°C throughout the study period. Melted ice was replenished every second day. In both lots iced fish were arranged not to be in contact with one another e.g. one part of ice to two parts of fish was used at the time of icing.

Fish were sampled approximately every 3 days until the shelf life (rejection point) was reached in both lots. Each day of sampling, seven fish were taken randomly from each lot; five for sensory evaluation with QIM and thereafter three fish from each set evaluated taken for microbiological and pH analysis. The remaining two fish from the seven taken per lot were used for sensory evaluation of the cooked fillets (QDA) carried out parallel to QIM evaluation. The expected maximum storage time of iced Arctic charr was 14-17 days according to a prior experiment by Milanes (2004), and therefore the experimental plan assumed sampling for up to 18 days of storage. Table 2 shows the sampling plan developed for the study.

Table 2: Sampling plan for the shelf life studies of lot 1 and 2.

Measurements	Days from slaughter					
	1	6*	8*	11*	14*	18*
QIM	5	5	5	5	5	5
Microbiology, pH**	3	3	3	3	3	3
QDA	3	2	2	2	2	2

* Numbers in columns refer to amount of samples from each group (lot 1 and lot 2)
** Samples collected from samples previously used for QIM assessment

3.2   Sensory evaluation of whole/raw fish (QIM)

This method involved evaluation of the appearance of skin, eyes, gills and abdomen, odour and texture using the Quality Index Method (QIM) based on the significant sensory parameters for raw fish and a score system from 0 to 3 demerit points. Prior to the experiment, 11 panellists were trained during one session in the use of the QIM for sensory evaluation. They were members of the Icelandic Fisheries Laboratory sensory panel, all trained according to international standards (ISO 1993); including detection and recognition of tastes and odours, training in the use of scales, and in the development and use of descriptors. The members of the panel were familiar with the QIM method and experienced in sensory analysis of seafood.

The panellists were introduced to the evaluation procedure using the modified QIM scheme from Milanes (2004) developed for Arctic charr stored in ice (Table 3).

Table 3: The QIM scheme for farmed Arctic charr- modified from Milanes (2004).

Quality parameter		Description	Score	Sample codes					
Skin	Colour/ appearance	Pearl-shiny all over	0						
		Less pear-shiny	1						
		Yellowish, mainly near the abdomen	2						
	Mucus	Clear, no clotting	0						
		Milky, clotted	1						
		Yellow and clotted	2						
	Odour	Fresh sea weed, neutral	0						
		Metal, cucumber, grass	1						
		Hey, sour	2						
		Rotten, dish cloth	3						
	Texture	In rigor	0						
		Finger mark disappears	1						
		Leaves mark over 3 sec.	2						
Eyes	Pupils	Clear & back, metal shiny	0						
		Dark grey	1						
		Mat, grey	2						
	Form	Convex	0						
		Flat	1						
		Sunken	2						
Gills	Colour/ appearance	Red/ fresh blood	0						
		Pale red, pink/light brown	1						
		Grey-brown, brown, grey, green	2						
	Mucus	Transparent	0						
		Milky, clotted	1						
		Brown, clotted	2						
	Odour	Fresh, metal	0						
		Metal, cucumber, grass	1						
		Sour, mouldy	2						
		Rotten	3						
Abdomen	Blood in abdomen	Blood red/ not present	0						
		Blood brown, yellowish	1						
	Odour	Neutral	0						
		Cucumber, melon	1						
		Sour, reminds of fermentation	2						
		Rotten, rotten cabbage	3						
Quality index (0-24)									

Five whole gutted fish from each lot were placed randomly on a clean table at room temperature and under white fluorescent light with as little distraction as possible. Each fish was coded with a number consisting of three digits that did not indicate the storage conditions of the fish. The panellists individually evaluated changes in skin, eyes, gills and abdomen in accordance with the QIM scheme for Arctic charr stored in ice presented in Table 3.

3.3   Sensory evaluation of cooked fillets (QDA)

Evaluation of the cooked Arctic charr was performed parallel to the QIM evaluation to determine the sensory characteristics changes with storage time of cooked Arctic charr. The panel was trained in describing the intensity of each attribute for a given sample using an unstructured scale (from 0% to 100%), for the QDA attributes previously used for Arctic charr presented in Appendix 6 (Milanes 2004). During preparation of cooked fillets, Arctic charr were filleted and the loins cut into pieces about 2-2.5 cm long and 2-3 cm wide. Pieces were placed accordingly in aluminium boxes coded randomly with three digit numbers and cooked in a preheated electric oven Convostar (Convotherm-German) with circulation air and steam at 95-100°C for 7 minutes. Each panellist evaluated duplicates of samples in random order for each storage condition. The 15 attributes evaluated were related to odour (characteristic, metallic, oily, earthy, sour, rancid), flavour (characteristic, metallic, oily, earthy, sour, rancid) and texture (soft, juicy, tender). A computerised system (FIZZ, Version 2.0, 1994-200, Biosystemes) was used for data recording.

3.4   Microbiological analysis and pH measurements

Fish samples for the microbial analysis were collected and labelled after QIM evaluation for both experimental storage conditions (lots). The samples were taken from the flesh of the anterior-dorsal region of each of the three fish per lot, observing strict hygiene to obtain a low bacterial count. This was done by cleaning the skin side thoroughly with 70% ethanol, removing the surface slime. Thereafter skin was aseptically removed and the underlying flesh cut off the skeleton using sterile scalpels and forceps. Samples of mince flesh, weighing 25 g each, were placed in stomacher bag containing 225 Butterfield's Buffer solution to obtain a 10-fold dilution. Blending was done in stomacher for 1 minute. Aliquots were plated in triplicate in Iron Agar as described by Gram *et al.* (1987) with the exception that 1% NaCl was used instead of 0.5%. Enumeration of total viable counts (TVC) and counts of H_2S -producing bacteria was performed after 4 days incubation at 15°C. Black colonies were recorded as sulphide producers. The pH of each sample mince for bacterial enumeration was determined (5 g mince + 5 ml buffer) using the digital glass calomel electrode pH meter PHM 80, dipped in minced Arctic charr samples. The values for three samples in each lot were averaged for every storage day evaluated.

3.5   Data analysis

The mean values of QI, QDA attributes scores, TVC and counts of H_2S-producing bacteria were plotted separately against storage time for both lots. To observe if the two groups were different within a storage day, t-Test Paired Two Sample for Means was performed on QI, bacterial counts and pH results using Microsoft Excel. QDA data were corrected for level effects (effects caused by level differences between assessors and replicates) using the method of Thybo and Martens (2000). Multivariate comparison of different sensory attributes and samples were performed with Principal Component Analysis (PCA) on mean level corrected sensory attribute values using full cross validation. Multivariate Analysis was performed using the statistical program Unscrambler ® (Version 8.0, CAMO, Trondheim, Norway).Analysis of variance (ANOVA) was carried out on the QDA results in the statistical program NCSS 2000 (NCSS, Utah, USA). The program calculates multiple comparisons using Duncan's test to determine if sample groups are different. Significance of difference was defined at the 5% level.

4   RESULTS

4.1   Temperature changes during storage

The average temperature in styropore boxes with Arctic charr was 3°C on arrival in the laboratory. During storage of the temperature abused fish at 18°C for 24 hours, the temperature in the boxes rose from 3-12.6°C. Thereafter on re-icing and storage at 1°C, the temperature dropped to about 0°C which was maintained throughout the study. Well handled fish re-iced immediately on arrival and stored at 1°C recorded temperature of 0°C in the boxes after re-icing and throughout the storage time (Appendix 2).

4.2   Sensory evaluation of whole/raw fish (QIM)

4.2.1   Individual attributes/descriptors

Whole gutted well handled and temperature abused Arctic charr stored for 6 and 18 days is shown in Figures 1 and 2. On the sixth day, the iced Arctic charr had pearl-shiny appearance (Figure 1a) with convex eyes (Figure 1b) and red gills (Figure 1c) while the temperature abused fish were less pearl-shiny (Figure 1d) with sunken eyes though black (Figure 1e) and less red gills (Figure 1f). Like day 6, each lot appeared to have distinct characteristics on day 18 of storage, although in general both groups had dull yellowish skin with thick mucus (Figures 2a and 2d), brown and greyish-brown gills with milky clotted mucus for well handled and abused fish respectively (Figures 2c and 2f) and sunken dark grey pupils in both lots (Figures 2b and 2e).
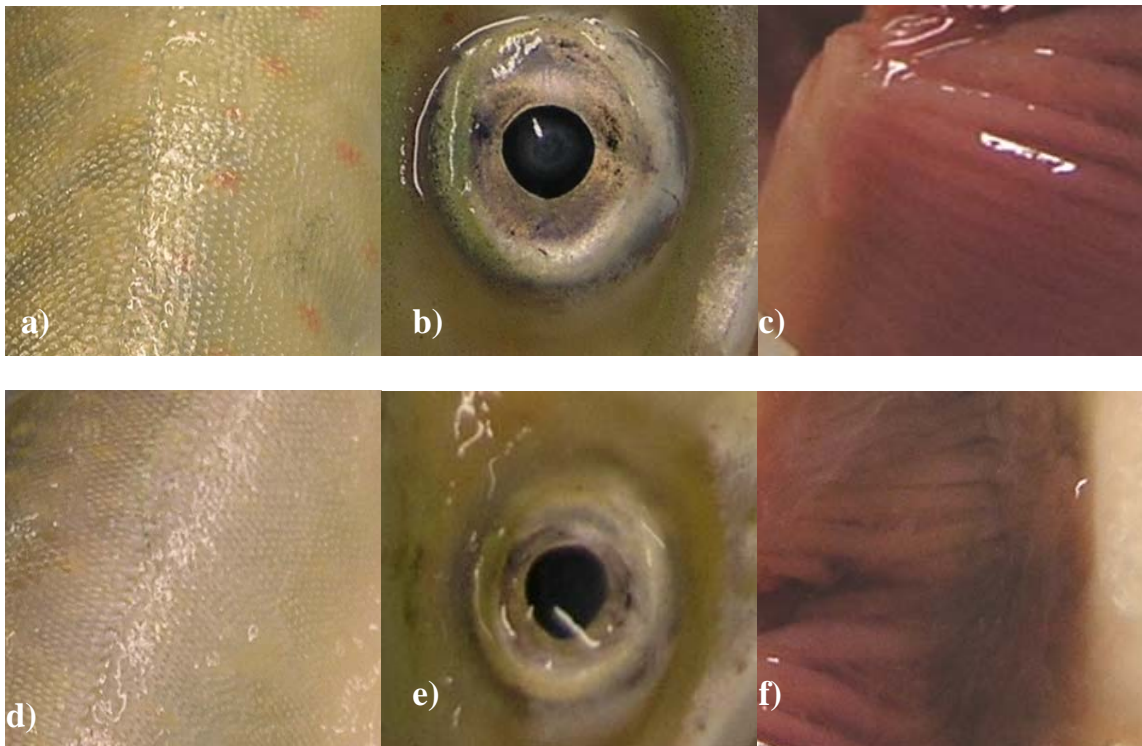
Figure 1: Appearance of well handled (a, b and c) and temperature abused (d, e and f) Arctic charr stored for 6 days in ice.
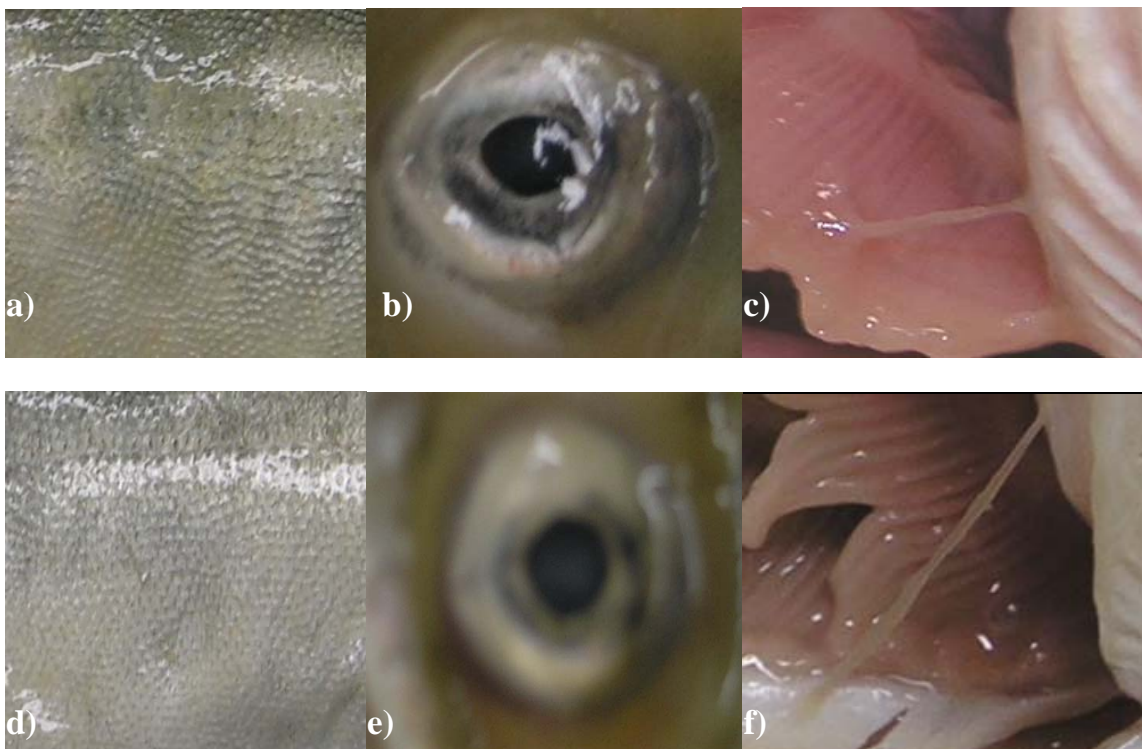

Figure 2: Appearance of well handled (a, b and c) and temperature abused (d, e and f) Arctic charr stored for 18 days in ice.

Individual sensory descriptors evaluated using QIM varied considerably within the lots (treatments) and by storage days as shown in Figure 3. The average skin colour score increased from 0 (pearl-shiny all over) for the iced lot on day 1 to 1.4 and 1.6 on day 18 of storage for well handled and temperature abused fish respectively. Changes in skin colour increased gradually from day 6 to day 18 (yellowish, mainly near the abdomen) when fish under both treatments attained a maximum score.

The skin mucus and odour changes had the same trend after day 6 in both lots for the remaining part of storage, with a sharp increase between days 14 and 18 of storage. However, temperature abused fish appeared to have higher scores for both descriptors. Texture changes increased linearly for both lots throughout the storage time from 0.7 (finger marks disappears) on day 1 for iced fish to 1.4 and 1.6 (leaves mark over three seconds) on day 18 of storage for well handled and temperature abused fish accordingly.

Eye pupils and form descriptor scores increased steadily up to day 11 of storage for both lots with abused fish appearing to have higher scores. Thereafter, changes in eye form had the same scores for both lots while pupils' scores alternated between the lots from day 14 to day 18 of storage.

Scores for gill colour, mucus and odour increased sharply for iced fish from day 1 to day 6 of storage. The rate reduced in preceding days (11, 14 and 18) for colour and mucus, but increased considerably for odour from day 14 to 18 of storage for both lots. The same trend in skin odour was observed with gill odour, abused fish appearing to score higher than well handled.

The abdomen odour was described at first as neutral with an average score of 0.1, reaching a score of 2 and 2.5 for well handled and abused fish accordingly at end of the evaluation, described as sour or reminds of fermentation and rotten or rotten cabbage respectively. The average scores for abdomen blood increased linearly to a score of 1 for both lots at the end of storage time. On day 18 most of the panellists gave the maximum demerit point score to this attribute in both lots.
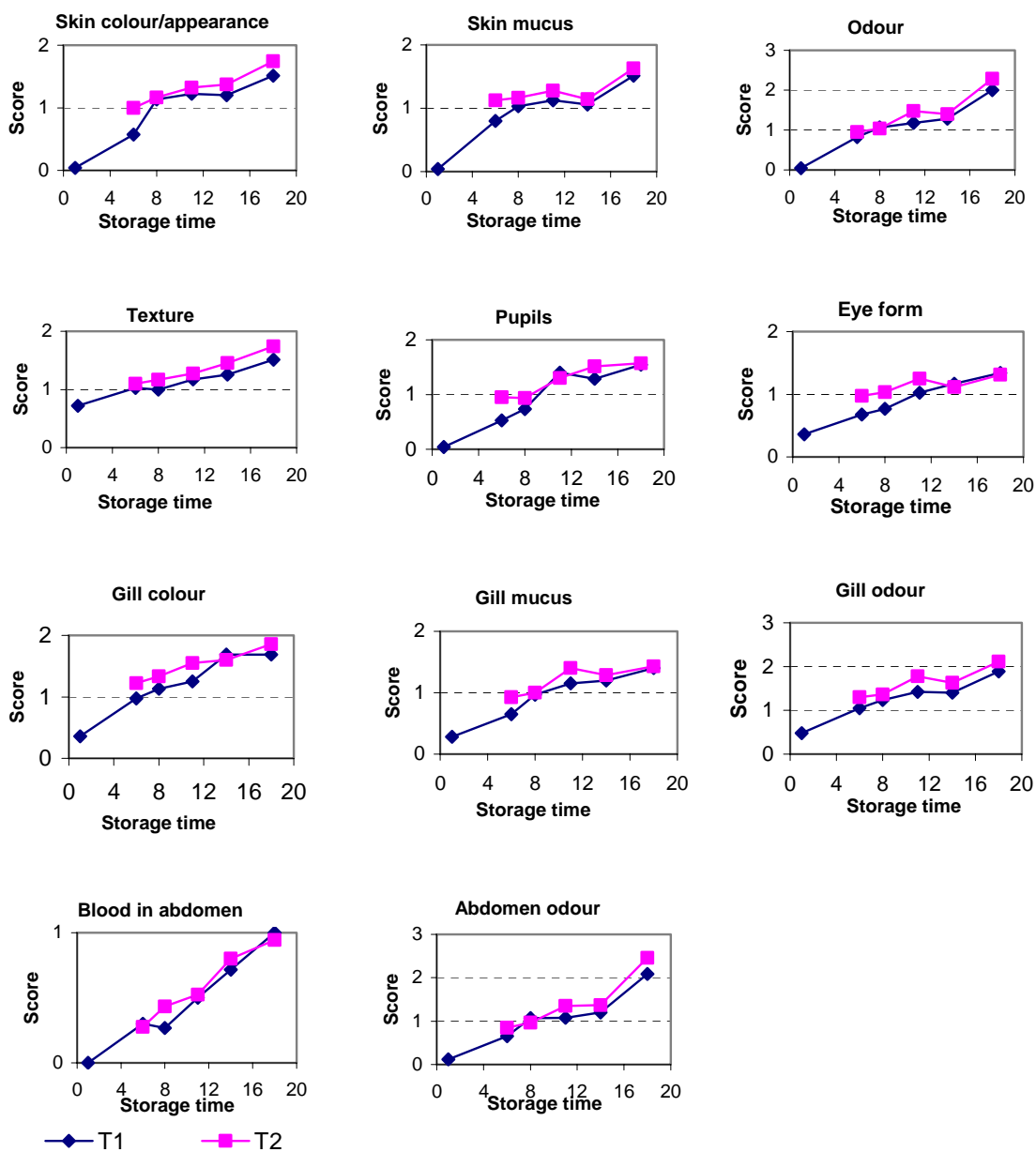
Figure 3: Average scores of individual descriptors in the QIM scheme for Arctic charr (iced (T1) and temperature abused (T2)) with days of storage in ice.

4.2.2   Quality Index

The sum of the individual descriptor (attribute) scores evaluated according to the QIM scheme (Table 3) was presented as the Quality Index (QI). Quality index calculated for six different storage days (1, 6, 8, 11, 14 and 18) based on the average of five samples in the iced lot, formed a linear relationship with the storage time (Figure 4). The iced lot (T1) showed a strong correlation of $R^2 = 0.9727$ between QI and storage time. After the abuse, there was on average 1.7 score difference in QI between the lots for every successful evaluation (days 6, 8, 11, 14 and 18) as shown in Figure 4. The QI was different between lots on days 6, 11 and 18 of storage, having $P < 0.05$. Variations were higher within samples in the abused lot (T2) compared to the iced lot throughout the storage time.
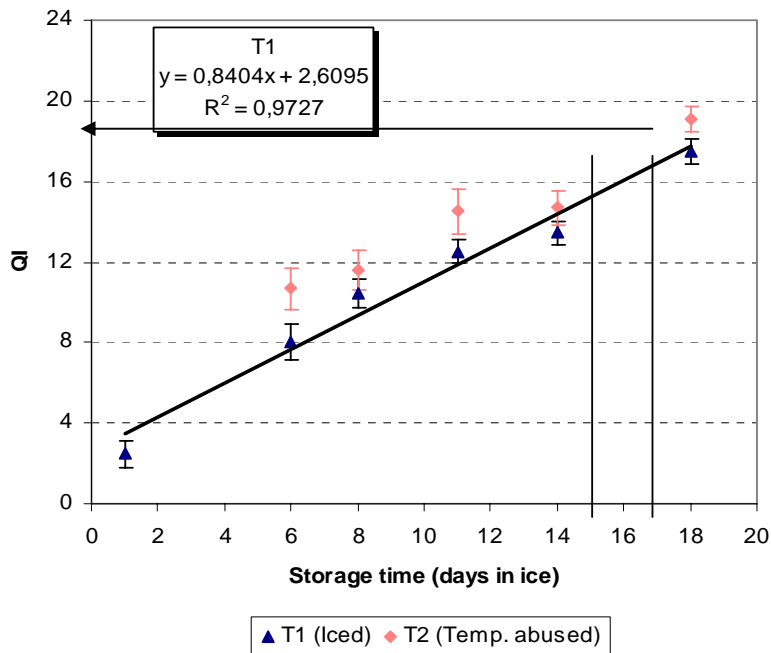

Figure 4: Average Quality Index (QI) for Arctic charr (iced and temperature abused) against storage days in ice.

4.2.3   Panellists

Most of the panellists used in this study were experienced in evaluating fish using the QIM scheme and participated in developing the QIM scheme for Arctic charr stored in ice (Milanes 2004). Each day of evaluation, five to eight panellists were used, but the same persons did not attend each session. Figure 5 shows in general an increase in QI with storage time as given by individual panellists for both lots. Although there were inconsistencies due to absenteeism, the results showed narrow variation in QI between panellists during the evaluation days as shown in Figure 5 (also Appendix 5). Whereas the inconsistencies were similar in both lots as they were evaluated simultaneously, variations were different between the lots. There was fairly high variation between panellists' scores in abused fish on day 11 of storage with a deviation of 2.4 compared to well handled fish which had the highest deviation of 1.9 on day 6 (Appendix 5). Panellist 10 (P 10) was excluded during data analysis since he/she was inexperienced in using the QIM scheme, which was reflected in his/her results. The QI scores given by this panellist were very inconsistent and did not give any indication of correct use of the QIM scheme during the three sessions he/she attended for both lots.
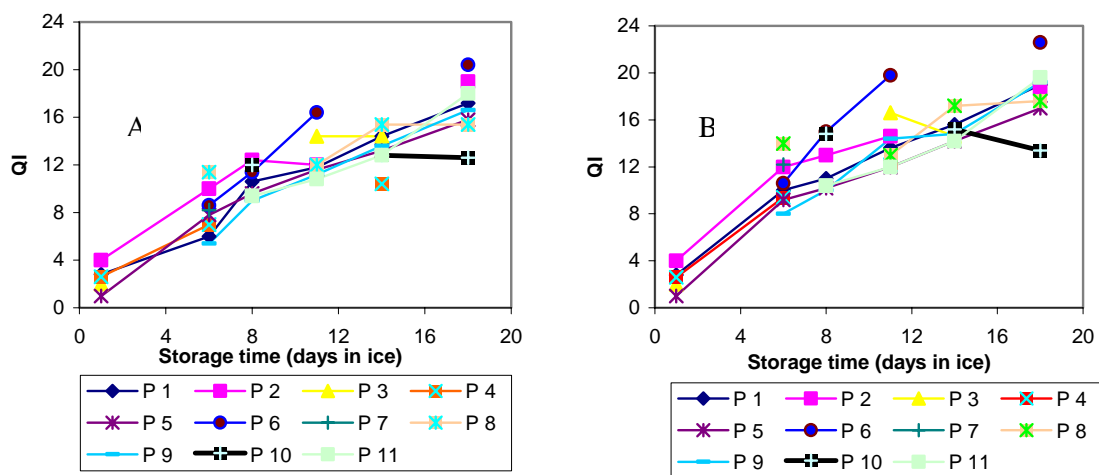

Figure 5: Quality Index (QI) for each evaluation day given by individual panellists during evaluation of (A) and temperature abused (B) Arctic charr iced.

4.3   Sensory evaluation of cooked samples (QDA)

The panellists evaluated the attributes of odour, flavour and texture of the cooked samples using the list of attributes from the QDA developed by Milanes (2004). The attributes detected at the beginning of shelf life along the first principal component (PC1) shown in Figure 6 were considered to be positive attributes (characteristic, metallic and oily). Consequently, the attributes detected closer to the end of shelf life describing spoilage (earthy, sour and rancid), were considered to be negative attributes. Figure 6 shows how the two different sample groups (iced (T1) and abused (T2)) of Arctic charr were described by the sensory attributes. The samples varied mainly with regard to differences in odour and flavour attributes along the first principal component (PC1), explaining 88% of the variation between the samples. The main difference occurred with the storage time, as the sample groups are located to the left side at the beginning of storage but on the right side after longer storage.

Samples also varied with regard to differences in texture attributes along the second principal component (PC2), explaining 7% of the variation between the samples. This was mainly because the sample evaluated on storage day 1 (d01) had more tender texture.

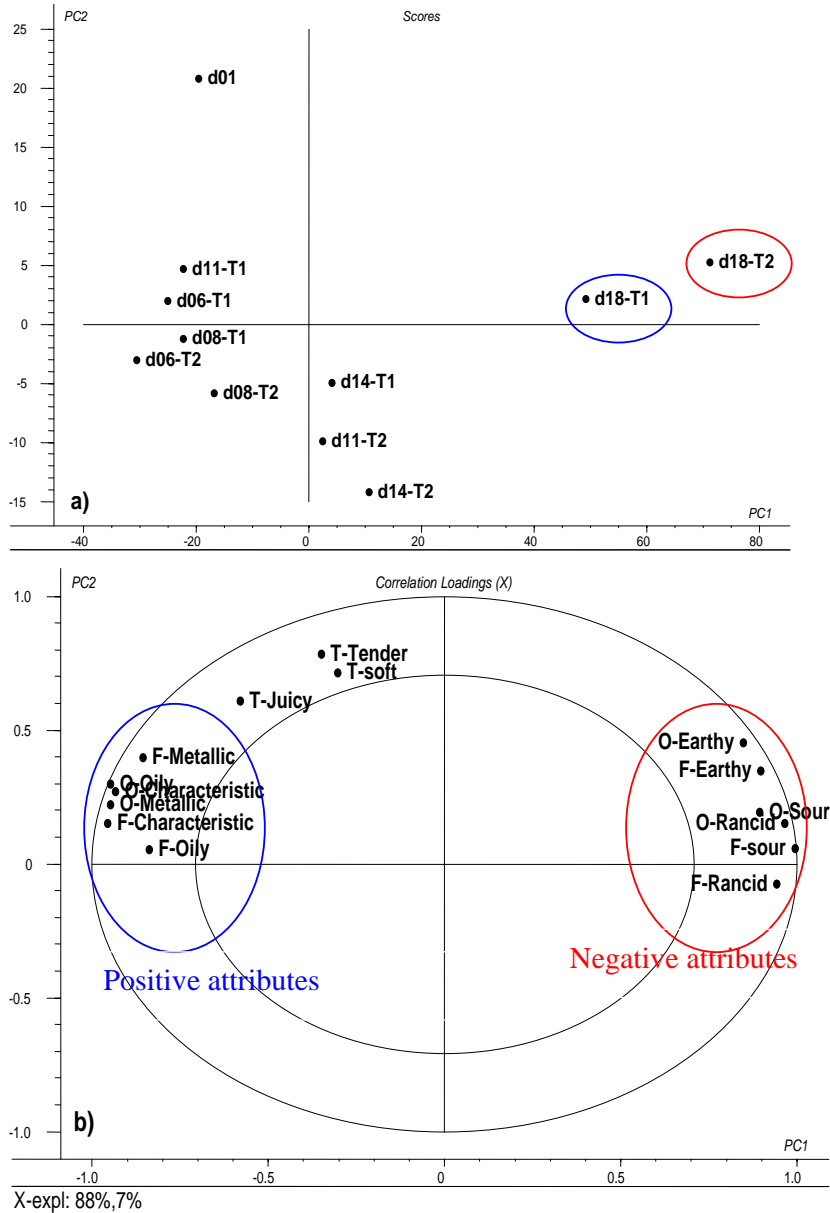Figure 6: PCA describing sensory quality of the Arctic charr as evaluated by a trained sensory panel. Scores (a) and correlation loadings (b). PC 1 (88%) vs. PC 2 (7%). D"xx" = days of storage; T1 = trial 1 (iced the whole storage time); T2 = trial 2 (stored at higher temperature for 24 hours on day 2 of storage, then iced during the remaining storage time); O = odour; F = flavour; T = texture.

4.3.1 Odour

The changes in odour are shown in Figures 7 and 8 and Table 4. The positive odour attributes (characteristic, metallic and oily) were prominent at the beginning of storage time and remained apparently stable up to day 11 of storage for both lots. These attributes became less evident towards the end of the storage time, but differed by groups as the iced lot appeared to keep these characteristics longer (Figures 6 and 7). Characteristic Arctic charr appeared to be stronger compared to metallic and oily odour characteristics throughout the storage time in both lots. Whereas the negative attributes that indicated spoilage of the samples: sour and rancid, became prominent with increasing storage time, especially between days 14-18 of storage in ice (Figure 8). Those changes occurred over different time periods for the lots.

For the negative odour attributes, the two groups differed mainly with regard to sour at the end of the storage time (Figure 8). In the temperature abused lot, sour odour increased sharply to a score of 49 while it only increased to 25 in the well handled lot. Sour odour for the abused fish on day 18 was significantly different to all earlier days of storage, including sour odour for well handled fish on that day. While rancid odour on day 18 did not differ between the lots, it differed to all the earlier storage days for both lots (Table 4).

Table 4: Mean sensory scores of odour, flavour and texture attributes of cooked Arctic charr fillets. T1 = trial 1 (iced throughout storage time); T2 = trial 2 (temperature abused for 24 hours prior to re-icing); d = storage time (days in ice).

Groups:		d01	d06-T1	d06-T2	d08-T1	d08-T2	d11-T1	d11-T2	d14-T1	d14-T2	d18-T1	d18-T2
Attributes	Significance											
Odour												
Characteristic	***	58 ^{ac}	53 ^{bcde}	54 ^{bcde}	57 ^{cf}	57 ^{cf}	61 ^{cf}	53 ^{dfg}	38 ^{efg}	36 ^{fh}	39 ^{gh}	32 ^{h}
Metallic	***	27 ^{a}	37 ^{a}	34 ^{ab}	48 ^{ab}	43 ^{ab}	47 ^{a}	38 ^{ab}	26 ^{ab}	22 ^{ab}	27 ^{bc}	19 ^{cd}
Oily	***	25 ^{a}	29 ^{a}	30 ^{a}	39 ^{a}	39 ^{a}	40 ^{a}	30	26 ^{ab}	16 ^{bc}	21 ^{bc}	17 ^{cd}
Earthy	***	22 ^{b}	14 ^{b}	14 ^{b}	24 ^{b}	22 ^{b}	25 ^{b}	24 ^{b}	22 ^{b}	20 ^{b}	45 ^{a}	47 ^{a}
Sour	***	1 ^{c}	6 ^{c}	4 ^{c}	5 ^{c}	8 ^{c}	8 ^{c}	11 ^{cd}	10 ^{cd}	10 ^{c}	25 ^{bd}	49 ^{a}
Rancid	***	0 ^{b}	8 ^{b}	5 ^{b}	8 ^{b}	8 ^{b}	6 ^{b}	10 ^{b}	12 ^{b}	9 ^{b}	29 ^{a}	32 ^{a}
Flavour												
Characteristic	***	61 ^{a}	57 ^{a}	58 ^{a}	59 ^{ab}	62 ^{ab}	64 ^{ab}	54 ^{bc}	37 ^{d}	36 ^{d}	37 ^{d}	24 ^{e}
Metallic	***	45 ^{a}	38 ^{ab}	41 ^{ab}	54 ^{ab}	48	51 ^{ab}	46	24 ^{bc}	23 ^{bc}	28 ^{cd}	21 ^{cd}
Oily	***	37	36	34	50 ^{a}	43	53 ^{a}	41	39 ^{a}	34	31 ^{b}	34 ^{b}
Earthy	***	24 ^{b}	17 ^{bc}	11 ^{cd}	26 ^{bc}	27 ^{bc}	28 ^{bc}	28 ^{bc}	22 ^{bc}	27 ^{b}	47 ^{a}	53 ^{a}
Sour	***	1 ^{c}	4 ^{c}	0 ^{c}	5 ^{c}	7 ^{c}	5 ^{c}	10 ^{c}	12 ^{c}	13 ^{c}	28 ^{b}	42 ^{a}
Rancid	***	0 ^{b}	5 ^{b}	1 ^{b}	8 ^{b}	10 ^{b}	6 ^{b}	19 ^{b}	16 ^{b}	17 ^{b}	42 ^{a}	39 ^{a}
Texture												
Soft		69	64	61	56	53	60	55	58	53	58	59
Juicy		67	61	61	63	60	64	61	58	50	63	59
Tender	*	72 ^{a}	72	65	67	64	72	64	62	58 ^{b}	69	68

* p<0,05; ** p<0,01; *** p<0,001 (significant difference in a sensory attribute between sample groups, different letters indicate significantly different values between samples within a line).
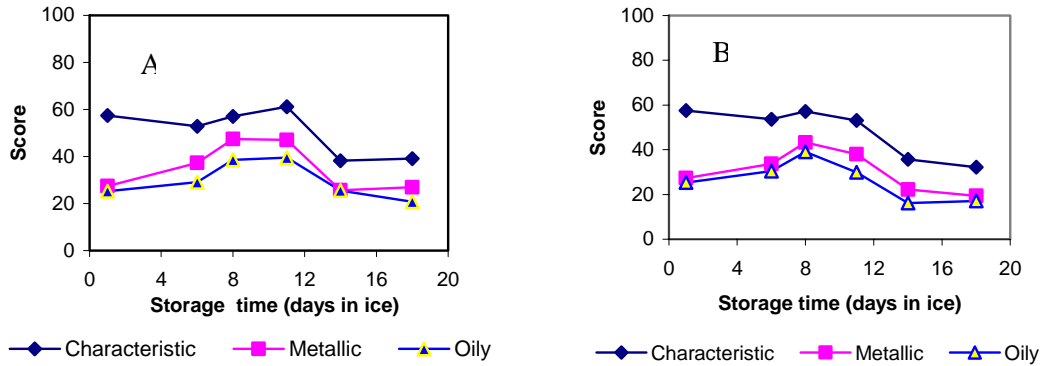
Figure 7: Changes in the mean odour positive attributes score for cooked iced (A) and temperature abused (B) Arctic charr with storage time as observed by a trained sensory panel.
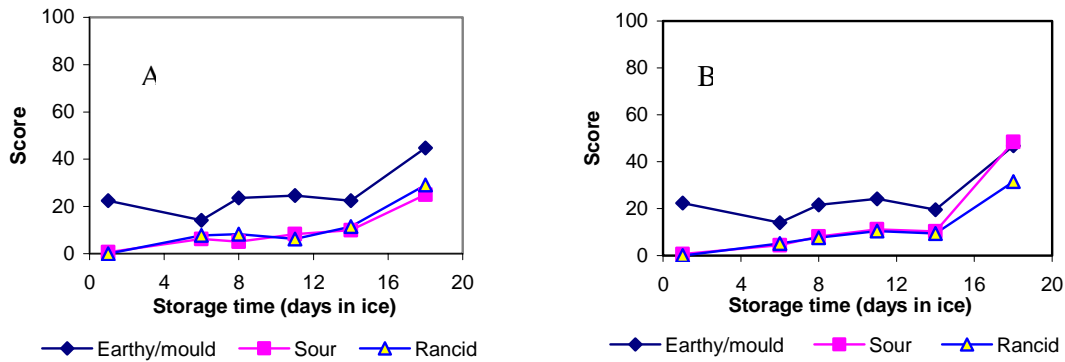

Figure 8: Changes in the mean odour negative attributes score for cooked iced (A) and temperature abused (B) Arctic charr with storage time as observed by a trained sensory panel.

4.3.2   *Flavour*

As for the odour attributes, flavour attributes did not show much change during preliminary storage time in both lots. The positive attributes prominent at the beginning of shelf life, decreased with storage time in both lots (Figure 9 and Table 4). Flavour negative attributes had the same trends as for odour in both lots. There was a sharp increase in the attributes after day 14 of storage with the sour flavour score increasing above rancidity for the temperature abused lot unlike the well handled lot. The negative attribute scores were higher in the abused lot than the well handled (iced) during the last day of storage except flavour rancidity (Figure 10). Sour flavour on day 18 was different for the abused fish compared to the well handled fish, like all earlier storage days that had no difference between the lots (Table 4). The same applied to rancid odour, even though on day 18 there was no significant difference between the lots.
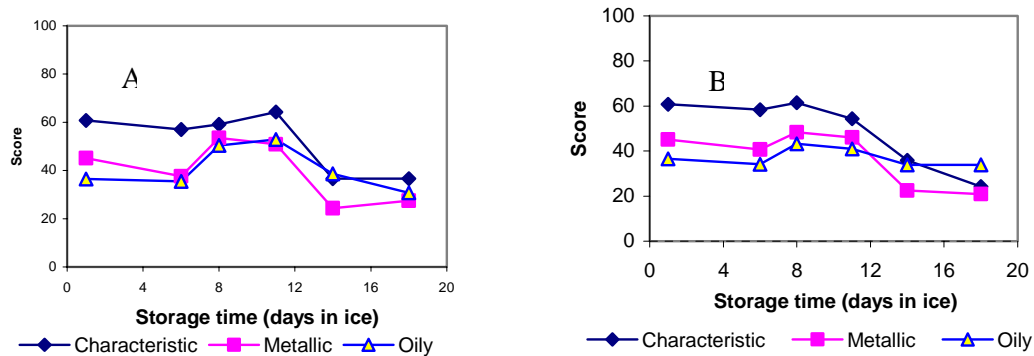
Figure 9: Changes in the mean flavour positive attributes score for cooked iced (A) and temperature abused (B) Arctic charr with storage time as observed by a trained sensory panel.
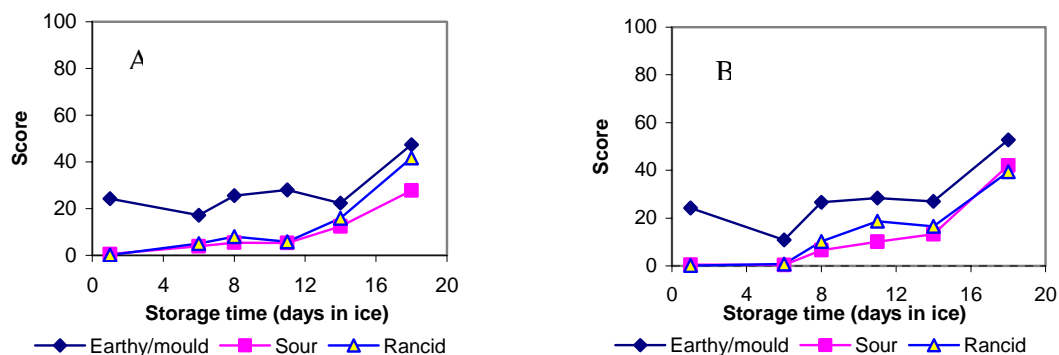
Figure 10: Changes in the mean flavour negative attributes score for cooked Arctic charr iced (A) and temperature abused (B) with storage time as observed by a trained sensory panel.

4.3.3 Texture

Little variation in the texture attribute parameters was recorded during fish storage in ice for both lots (Figures 6, 11 and Table 4). Texture attributes (soft, juicy and tender) recorded scores between 60 and 75 for well handled fish, and between 50 and 70 for abused fish. Tender appeared prominent throughout the storage period in both lots as shown in Figure 10. Texture attributes were not significantly different between the lots or storage days, except tender which was higher on day 1 compared to day 14 for the abused lot.

Figure 11: Changes in the mean texture attributes score for cooked iced (A) and temperature abused (B) Arctic charr with storage time as observed by a trained sensory panel.
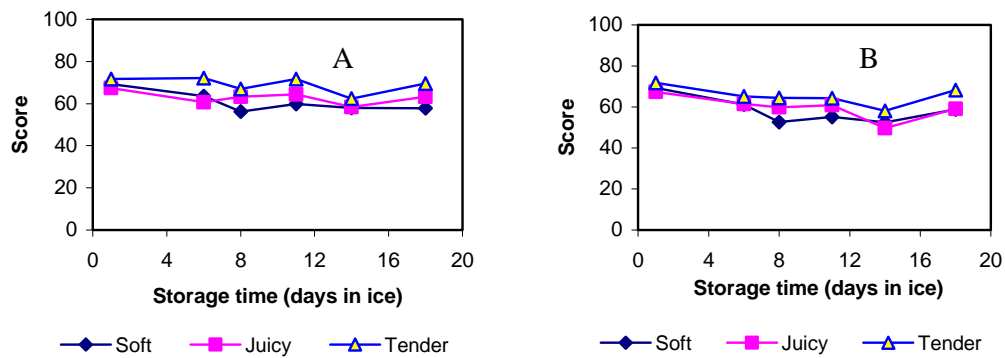
4.4 Microbial counts and pH

4.4.1 Microbial counts

Changes in TVC and counts of H_2S producing bacteria are presented in Figure 12. The results show a low initial bacterial load on day 1 of storage (iced), and an increasing trend for the following days of storage in both lots. In well handled fish the increase in TVC and H_2S producing bacteria was moderate throughout the study, while the abused fish had an exponential increase between days 14 and 18 of storage. This was in accordance with the results of negative taste attributes (sour flavour and sour odour) in evaluation of cooked samples (Figures 8 and 10).

During the first 14 days of storage in ice, H_2S producing bacteria in Arctic charr were present in low numbers and not exceeding 5% of the total flora. At the end of the storage time of the well handled lot, TVC was 10^5-10^6 CFU/g and H_2S-producing bacteria comprised about 6.5% (about 10^4 CFU/g), whereas in the abused lot TVC was 10^6-10^7 CFU/g and H_2S-producing bacteria comprised about 25% (10^5-10^6 CFU/g). The TVC were significantly lower in the well handled compared to the abused fish on days 6, 8, 11 and 18 of storage.
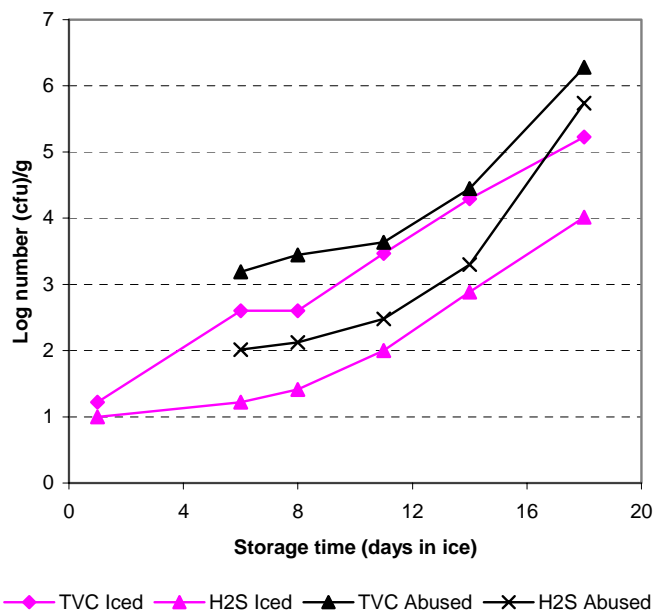
Figure 12: Changes in the total count (TVC) and H$_2$S producing bacteria on iron agar during iced storage of Arctic charr (iced and temperature abused).

4.4.2 pH

pH changes were uniform for both lots, increasing from 6.3 on day 1 to 6.45 on day 6 and thereafter gradually reduced to 6.4 on day 11 of storage, subsequently rising towards the end of storage life to a score of 6.54 and 6.52 for abused and well handled fish respectively on day 18 (Figure 13). The pH was not significantly different between lots throughout the experiment.
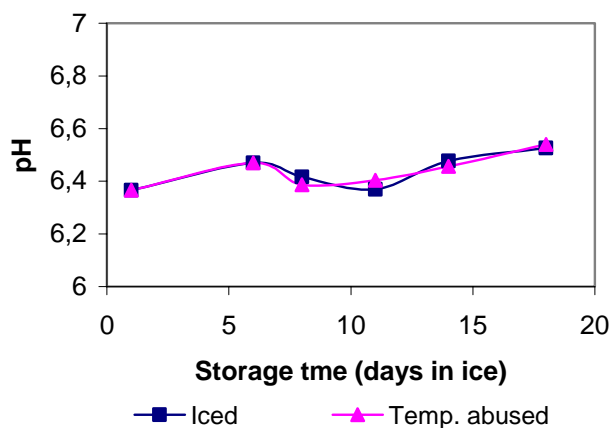
Figure 13: Changes in the pH levels of Arctic charr (iced and temperature abused) with storage time.

4.5   Comparison of evaluation methods

A high correlation was found between QI and TVC, QI and selective counts of H_2S-producing bacteria for both lots. While QI correlated strongly with TVC in the iced lot compared to the abused, it correlated almost equally with TVC and H_2S producing bacteria in the abused lot (Figure 14). At the beginning of the storage period, when bacterial counts were low, the QI scores were likewise low and both increased with storage time.
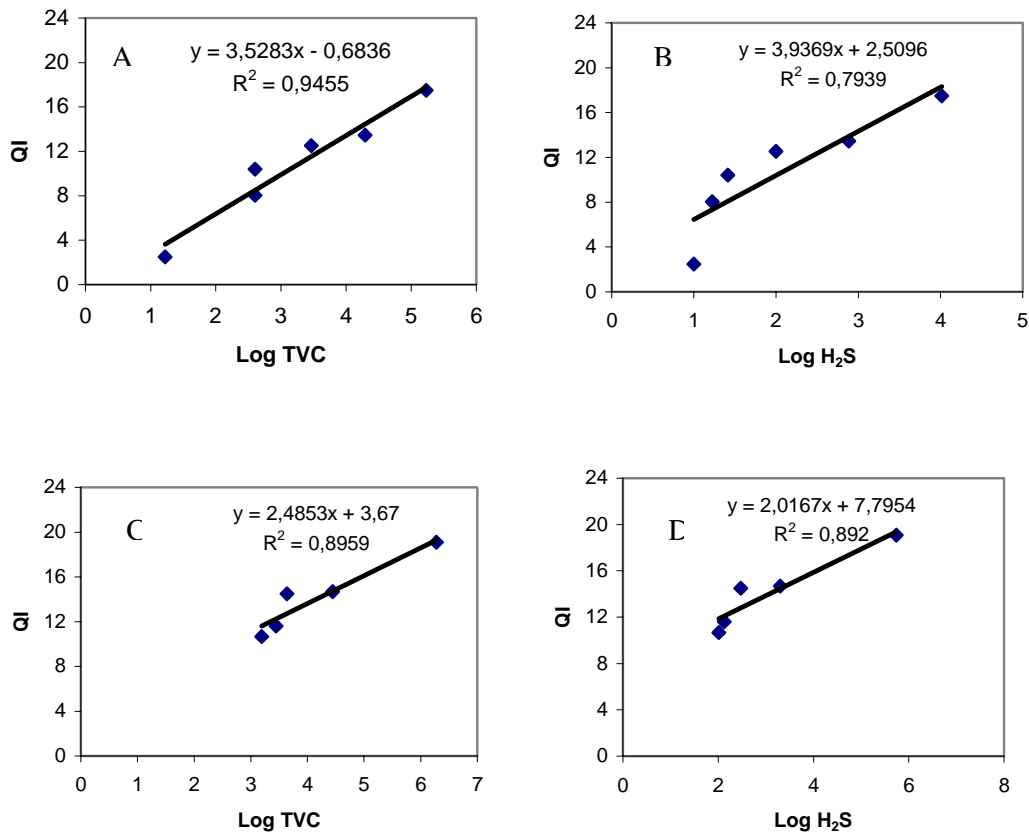
Figure 14: Correlation between bacterial counts (Log TVC and H_2S) and Quality Index of iced (A and B) and temperature abused (C and D) Arctic charr.

5   DISCUSSION

5.1   Shelf life

Comparing QDA, QI and microbiology results, Arctic charr had a shelf life of 17 and 15 days of storage in ice for the iced and temperature abused lots respectively. According to sensory evaluation, the iced group reached the QDA limits after approximately 16-17 days of storage, whereas the temperature abused lot attained the limits on day 15 but was past the consumption limit on day 16 of storage in ice. In the iced lot, H_2S-producing bacteria reached maximum consumption limits of 10^4 CFU/g on day 18 of storage, which was past the acceptable limits according to sensory evaluation and thus the maximum shelf life was 17 days of storage in ice. While the temperature abused lot reached the 10^4 CFU/g (H_2S-producing bacteria) limits on day 15 of storage, which is in accordance to sensory evaluation (QDA limits).

Although QI values showed a constant linear increase with storage time for both lots, there was on average a score difference of 1.7 between well handled and abused fish on each evaluation day. It was noted that at the end of shelf life determined with QDA, both groups received the QI score 17, which is almost the same as the QI reported earlier for Arctic charr by (Milanes 2004) at the end shelf life on day 17 of storage in ice. Therefore it can be assumed that any QI score above this indicates that the fish is no longer fit for human consumption.

5.2   Temperature changes

The short time abuse increased the temperature in the boxes holding arctic charr (temperature abused) to the normal water temperature under optimal growth conditions (10-17°C) reported in various Arctic charr growth studies (Jensen 1985, Larsson and Berglund 1998, Gines *et al.* 2004). This narrow increase in temperature negatively affected the shelf life of the abused lot compared to the iced fish (T1). This indicates that any slight increase in temperature during storage affects positively microbial growth that is responsible for spoilage in iced fish and consequently decreases the shelf life.

5.3   Sensory evaluation of whole fish using QIM

Most of the descriptors/attributes were well used in both lots (Figure 3), at the end of shelf life when Arctic charr received higher QI all descriptors scored above average for well handled fish and were almost fully used for abused fish. However, as expected, higher descriptor scores were given as the storage time increased although in many cases the highest points were not used for the well handled fish. This is in accordance with how the QIM scheme is constructed, where iced fish evaluated shortly after catch should be given the lowest points that subsequently increase with storage time reaching maximum scores at the end of shelf life (Martinsdottir *et al.* 2001).

It is assumed in the QIM that the scores for all quality descriptors increase with storage time in ice. This was observed in the study, but to some extent a few descriptors showed a decrease in between the storage days, especially for the abused fish (Figure 3). The average scores given for gill mucus and odour on storage day 11 were higher than the scores given for storage day 14 for the abused fish. Descriptor scores for temperature abused fish were always above the well handled fish except for eye pupils on day 11, gill colour day 14 and gill mucus day 18.

This may have been due to individual variations present in Arctic charr of the same storage time as found out by Gines *et al.* (2004).

Quality Index showed a high correlation of $R^2 = 0.9727$ with storage time for the well handled (iced) lot compared to $R^2 = 0.9517$ reported earlier by Milanes (2004), implying that the modified draft QIM scheme is applicable in sensory evaluation of Arctic charr. On average a score difference of 1.7 in QI was recorded between the lots on each evaluation day after the abuse. This shows that the QIM scheme developed for Arctic charr stored in ice functions well for temperature abused Arctic charr prior to storage in ice. According to QDA results, the end of storage time for iced lot (T1) was day 17, the assumed QI should be according to the equation $Y = 0.8404*17$(storage days) $+2.6095 = 16.9$ (Figure 4). The end of shelf life of the temperature abused lot (T2) was 15 days. The average difference in QI between the lots (T1 and T2) was 1.7. Therefore, on day 15 QI should have been according to the equation: $Y = 0.8404*15$(Storage days) $+2.6095 = 15.2$ for T1, adding 1.7 to the QI results gives the QI as16.9 for T2.

Panellist 10 was removed from the analysis as he/she gave uniform scores for different storage days in both lots. This was because he/she was not experienced in using the QIM scheme and lacked adequate training prior to evaluation. The results from the other panellists participating in the QIM evaluation of Arctic charr showed that they were able to use the scheme correctly throughout the experiment, even though there were some differences between panellists. It is clear that the panellists' prior experience using QIM schemes worked well. The panellists were used to evaluating fish freshness using QIM schemes, though different species. However, even though the schemes are somewhat different between fish species, the evaluated attributes and descriptors are often similar and this experiment seems to have resulted in rather low variation in scores between panellists. The variation was even lower than was described in Sveinsdottir *et al.* (2003) comparing individual panellist scores.

5.4   Sensory evaluation of cooked samples using QDA

Negative attributes (earthy, sour and rancid) did not change significantly during the preliminary days of storage in ice for both lots up to day 14, but afterwards progressed very rapidly. This indicates that changes in eating quality of Arctic charr are very slow until day 14, when the fish showed signs of spoilage in accordance with Milanes (2004). Based on the judgement of the taste panellists and using the QDA score of 20-30 for negative attributes as the value which indicates the fish to be unacceptable for human consumption, on day 18 of storage in ice both lots were past their shelf life. However, the iced lot was within the unacceptable limits for consumption, whereas for the temperature abused lot it was clear that on day 18 of storage in ice it was far past its shelf life, since negative attributes were different from the iced lot and the scores recorded were beyond unacceptability limits (Figures 8 and 10).

In this study, the end of shelf life was determined when spoilage related attributes such as sour and rancid became evident (between 20 and 30), which is in agreement with earlier studies on shelf life of Atlantic salmon and cod fillets by Sveinsdottir *et al.* (2002) and Bonilla *et al.* (2007) respectively, who found out that the end of shelf life is determined when the average negative sensory score is above 20.

Spoilage of Arctic charr during storage in ice might be due to the combined effects of chemical and bacterial activity as sour and rancid (odour and flavour) were both evident during

evaluation of cooked samples. In temperature abused fish, sour (flavour and odour) was more pronounced during spoilage than in well handled fish, where both sour and rancid were felt almost equally. This can be explained by the initial change in temperature of the abused fish creating an environment conducive to bacterial growth, as indicated in the bacterial load (total counts) difference between the two lots (Appendix 7 and Figure 12). These findings are similar to previous studies (Olafsdottir and Fleurence 1997, Shewan 1977), which reported sour and rotten odour, to originate from short chain fatty acids, alcohols, sulphur compounds and amines generated by microbial activity.

Rancid (flavour and odour) appeared to be higher in the abused fish than the well handled fish but the difference was not significance. The reason behind this could be that the abused fish was not wet as it tended to dehydrate, which sped up the oxidation rate, because oxygen was put in contact directly with unsaturated lipids. Likewise in well handled fish, ice on the surface could not prevent air from being in contact with the fish and thus stop rancidity. It is well known that the autoxidation process leads to the formation of hydro-peroxides whose degradation gives rise to aldehydes, ketones and alcohols (secondary products of lipid oxidation) that have a strong rancid flavour even at very low concentrations (Gram and Huss 1996, Olafsdottir 2005).

5.5   Microbial counts and pH

Growth curves for TVC and counts of H_2S producing bacteria had a very similar shape, though the proportion of H_2S producing bacteria to the TVC increased with storage time. On all occasions, lower numbers were obtained from the lot that was well handled (iced) compared to temperature abused. The total viable counts increased exponentially for the abused fish after day 14 of storage. A similar pattern was noted for negative attributes (QDA) in eating quality of fish in the same lot.

The initial total bacterial load in the fish muscle on day 1 of storage (iced) was 17 colony forming units per g of flesh (CFU/g), with H_2S producing bacteria only sometimes detected at less that 10 CFU/g. The low total counts in early storage days are because the flesh of newly caught fish is sterile since the immune system of the fish prevents the bacteria from growing in the flesh, but when the fish dies, the immune system collapses and consequently during storage bacteria invade the flesh (Sveinsdottir *et al*. 2002). At the end of the storage time of iced fish, H_2S-producing bacteria comprised 6.5% (about 10^4 CFU/g), while in the abused fish it comprised 25% (10^5-10^6 CFU/g) of the total flora This shows that the abused fish was more spoiled than the well handled fish, since H_2S-producing bacteria associated with spoilage often constitute a major proportion of the microbial flora of spoiling fish.

The TVC in Arctic charr flesh at the end of the storage time observed in this study was considerably higher than what was found earlier in similar studies on day 17 of storage (Milanes 2004). This could be due to the low proportion of H_2S-producing bacteria (associated with spoilage) observed in the well handled fish (iced). However, comparing these results to a previous study on salmon, which is in the same family salmonidae as Arctic charr and is closely related in many characteristics, similar values were obtained when salmon was considered unfit for consumption on day 21 of storage in ice (Sveinsdottir *et al*. 2002). The same TVC values were recoded when cod (temperate marine species) was considered unfit for consumption by Magnusson and Martinsdottir (1995). The values were likewise in range with Gram and Huss' (1996) findings that when the number of TVC exceeds 10^6 CFU/g a

significant amount of volatile sulphur-containing compounds are produced and spoilage becomes evident by sensory evaluation.

There were small variations in the pH of muscle tissue (6.3-6.6) with storage time in both lots, even though it appeared to increase towards the end of shelf life. The variations were likewise observed in the texture of cooked flesh (Figure 11) because the post-mortem pH according to (Huss 1988), is the most significant factor influencing the texture of the meat and the degree of "gaping", i.e. the rupture of the connective tissue. One of the reasons for this is that even minor changes in pH drastically affect the properties of the connective tissue.

5.6   Comparison of evaluation methods

A good agreement between the results from different methods was found. In comparing QI to TVC/ H_2S-producing bacteria, there was a strong correlation indicating that both methods can be used in quality evaluation of Arctic charr. It is therefore possible to use one as an alternative to the other, although since the consumer is the ultimate judge of quality, it's important to compare microbiological results to sensory evaluation. High correlation established between QI and TVC, compared to QI and H_2S-producing bacteria may be explained with the proportional increase in TVC with QI, unlike in H_2S-produsing bacteria whose proportion to TVC at a later stage of storage increased un-proportionally.

Comparing QIM and QDA results, QIM increased linearly with storage time in ice whereas QDA attributes showed different trends. Positive QDA attributes remained stable during the initial storage days and decreased after day 11 of storage while negative attributes used to predict storage time increased when spoilage became evident. These methods can be related with regard to shelf life as QDA provides information about maximum shelf life which makes the use of the QIM scheme for management realistic in estimating the past and remaining shelf life.

6   CONCLUSIONS

Based on QDA, Arctic charr showed little changes in quality during the preliminary storage time. This indicates that changes in the eating quality of Arctic charr are very slow until day 14 of storage in ice when the fish began to show signs of spoilage. The maximum storage time was 15 days for the temperature abused fish and 17 days for the well handled fish (iced). The little increase in temperature for a short time at the beginning of storage led to the storage life of Arctic charr being 2 days shorter. Spoilage in the well handled fish was predominantly chemical while in the abused fish it was as a result of both bacterial and chemical activities.

In evaluation using the draft QIM scheme (Milanes 2004), many descriptors were fully used in both lots and thus the developed scheme is applicable in sensory evaluation of Arctic charr during its storage in ice. Due to inadequate training prior to sensory evaluation, one panellist, who was not experienced in using the QIM scheme, was removed from analysis. It is therefore important to recommend proper training of inexperienced panellists prior to use of the scheme and use of the same panellists during evaluation to get good reliable and consistent results.

The results of QDA did not give significant information on a particular day which the abused fish was unfit for consumption, although the scores for negative attributes were beyond acceptable limits on day 18 of storage in ice. This is because there was no evaluation on days 15, 16 and 17 of storage which is thought to have been the limit of acceptability for both lots.

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7   APPENDIX

Appendix 1: Temperature changes in the boxes containing temperature abused Arctic charr during storage in ice
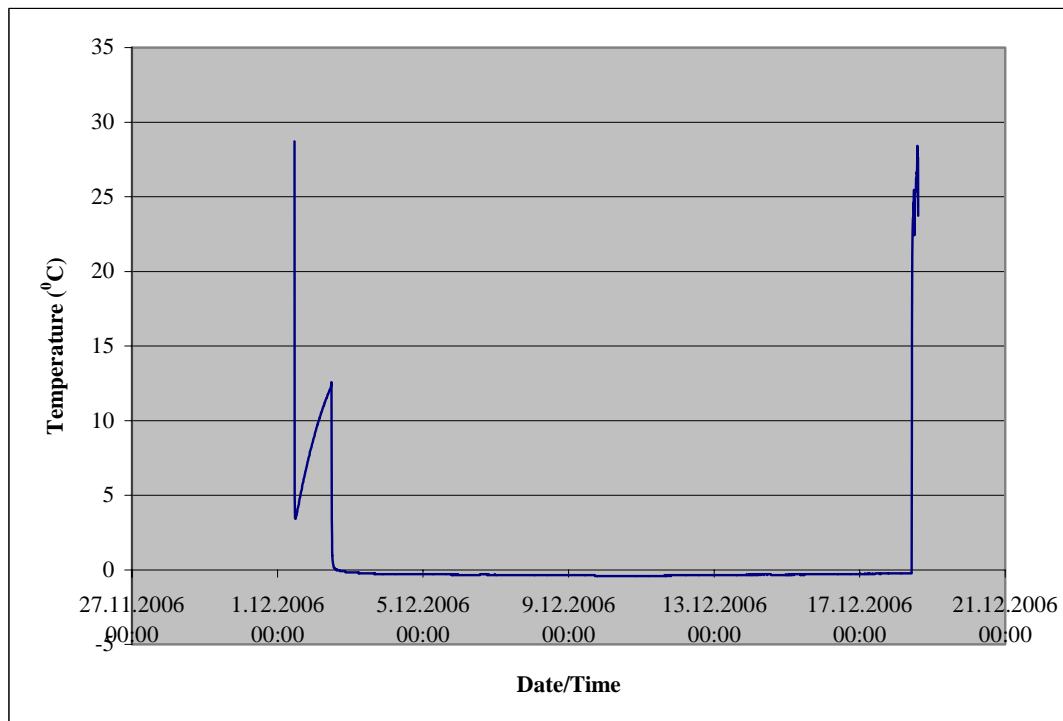
Appendix 2: Temperature changes in the boxes containing Arctic charr iced throughout storage time (well handled)
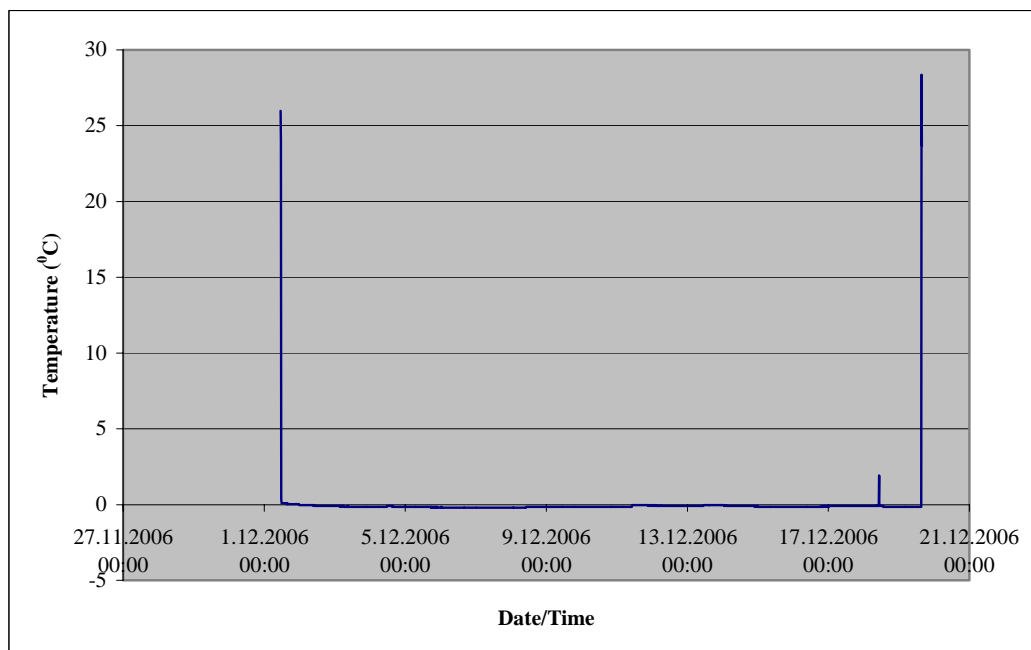
Appendix 3: Changes in ambient/cold room temperature with styropore boxes containing temperature abused Arctic charr
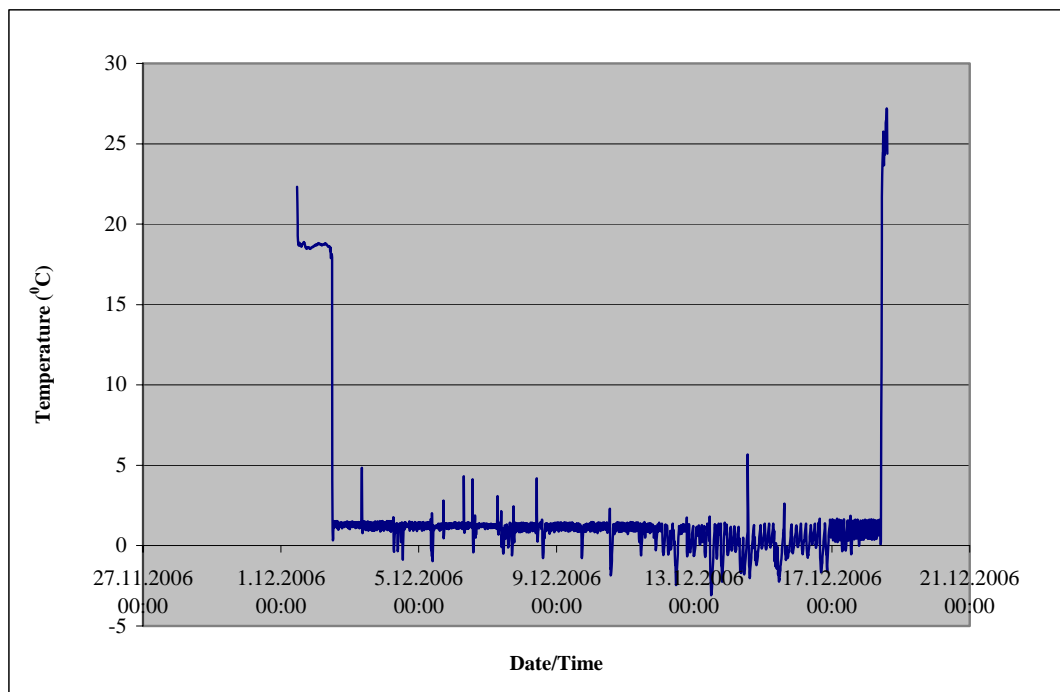
Appendix 4: Changes in cold room temperature with styropore boxes containing iced Arctic charr
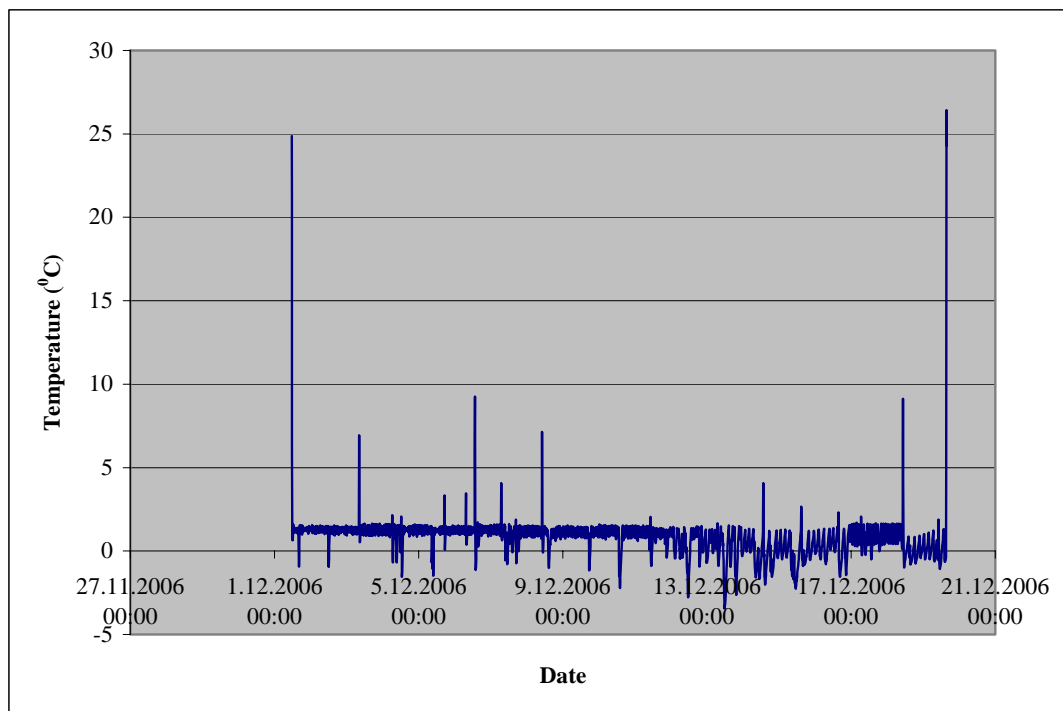
Appendix 5: QI and SD by panelist during QIM evaluation

Well handled fish

Days of storage	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	QI	SD
1	2,8	4	2	2,6	1							2,48	0,98
6	6	10		7	7,8	8,6	8,2	11,4	5,4			8,05	1,86
8	10,6	12,4			9,6	11,4			9		9,4	10,6	1,2
11	11,8	12	14,4		11,6	16,4		12	11,2		10,8	12,5	1,77
14	14,4		14,4	10,4	13,2			15,4	13,6		12,8	13,4	1,48
18	17,2	19			15,8	20,4		15,4	16,6		18	16,9	1,67

Temperature abused fish

Days of storage	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	QI	SD
1	2,8	4	2	2,6	1							2,48	0,98
6	10	12		9,4	9,2	10,6	12,2	14	8			10,7	1,82
8	11	13			10,2	15			10		10,4	12,1	1,83
11	13,6	14,6	16,6	12,2	12	19,8		13	14,4		12	14,2	2,42
14	15,6		14,6		14,2			17,2	14,8		14,2	15,1	1,05
18	19	18,6			17	22,6		17,6	19,2		19,6	18,4	1,68

Odoli

Appendix 6: QDA sensory attributes for cooked Arctic charr (Milanes, 2004).

Name_____ Date_____
Code_____

Odour

Characteristic

| Week | Strong |

Metallic

| Week | Strong |

Oily

| Week | Strong |

Earthy/mould

| Week | Strong |

Sour

| Week | Strong |

Rancid

| Week | Strong |

Flavour

Characteristic

| Week | Strong |

Metallic

| Week | Strong |

Oily

| Week | Strong |

Earthy/mould

| Week | Strong |

Sour

| Week | Strong |

Rancid

	Week	Strong

Texture

Softness

	Firm	Soft

Juiciness

	Dry	Juicy

Tenderness

	Tough	Tender