



CHARACTERIZATION OF SENSORY INDEXES OF ARTIC CHARR (*SALVELINUS ALPINUS*) AND DETERMINATION OF ITS SHELF LIFE

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ABSTRACT

Sensory indexes of Artic Charr (*Salvelinus alpinus*) in ice were characterized to analyse and define the parameters to be used in the development of a Quality Index Method (QIM) scheme for the species. The shelf life of artic charr in ice was determined using sensory, chemical and microbial analysis.

Artic charr was stored in ice for 21 days, and changes during storage were observed with sensory evaluation using the Quality Index Method (QIM) and Quantitative Descriptive Analysis (QDA) as well as total viable counts (TVC), hydrogen sulfide (H₂S) producing bacteria, total volatile nitrogen (TVN) and pH measurements.

The QIM developed for whole raw artic charr consists of 10 parameters, grouped in four main categories, resulting in a total of 21 demerit points. A high correlation between QI and storage time in ice was found. Storage time could be predicted to approximately 14-17 days in ice. TVC and H₂S- producing bacteria increased exponentially with storage after eight days in ice, but at the end of storage their values were not high. TVN and pH measurements were useless as a spoilage indicator for artic charr.

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1 INTRODUCTION

The quality of fresh fish is an important concern of industry and consumers. Marine and fresh water fish are perishable food commodities. Deterioration of fish mainly occurs as a result of bacteriological activity. Freshness is the single most important attribute when assessing fish quality.

Sensory evaluation methods are useful to evaluate quality and shelf life of fresh fish. Fish research institutes in Europe have made considerable efforts to develop rapid and objective sensory evaluation methods for fish freshness. To determine freshness of fish, the sensory methods are not used alone; these methods are used together with chemical and microbiological methods to establish levels of tolerance through chemical indicators and microbiological deterioration. The Icelandic Fisheries Laboratories (IFL) has extensive experience in the use of these methods.

Aquaculture in Cuba is important in economic terms and as a consequence aquaculture has become a source of quality food. A reduction of marine fisheries because of environmental problems and other causes, has led to increased efforts to develop aquaculture.

There are 38 farms (covering an area of 535 hectares) in Cuba, 168 dams and 295 micro dams (covering an area of 114,000 hectares) which are used for the farming of different species. The main farmed species in Cuba are: Chinese carp and tilapia, which represent 95% of the total production (Figure 1).

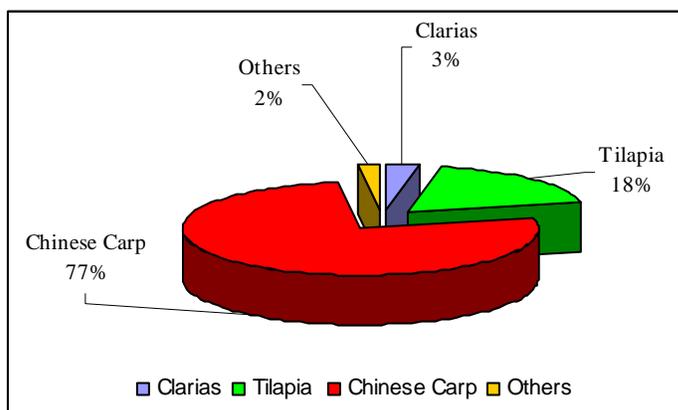


Figure 1: Main farmed species in Cuba. (INDIPES 2004).

During the last four years, aquaculture has been increasing, reaching 20,000 tons last year (Figure 2).

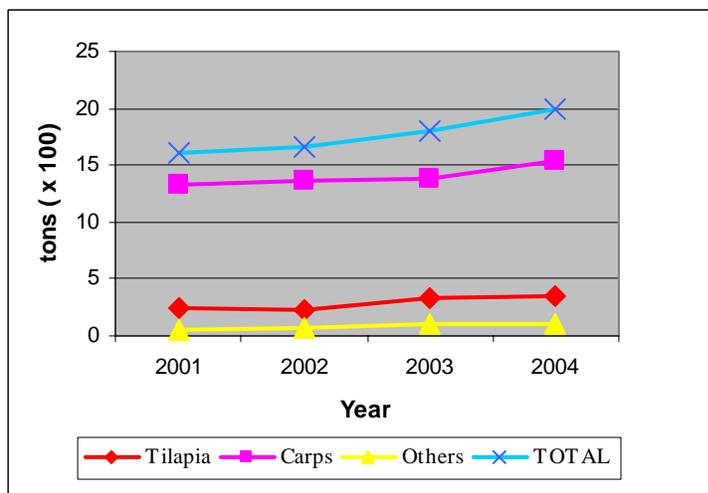


Figure 2: Aquaculture production in Cuba (INDIPES 2004).

The development of aquaculture and fish production has been identified as a priority area by the Cuban authorities.

The main objectives of the present project are:

- To analyse and define the parameters to be used for the development of a Quality Index Method (QIM) scheme for arctic charr (*Salvelinus alpinus*) stored in ice.
- To study the shelf life of arctic charr stored in ice.
- To compare the results from sensory analysis with the chemical and microbiological test results, such as:
 - o TVB- N (total volatile Basic Amines) and pH
 - o Total Viable Counts and H₂S producing bacteria

The main research questions to be answered are :

- What changes occur in gutted arctic charr during its storage in ice?
- Are there correlations among the results of the sensory, chemical and microbiological analysis carried out on gutted arctic charr?
- What is the shelf life of arctic charr?

The methodology to develop a QIM scheme will be adapted to important species in Cuba, such as tilapia and carp.

2 LITERATURE REVIEW

2.1 Fish freshness

Most often “quality” refers to the aesthetic appearance and freshness or degree of spoilage which the fish has undergone. It may also involve safety aspects such as being free from harmful bacteria, parasites or chemicals (Huss 1995). One of the most important aspects of fish and fish product is freshness. Due to consumer preferences there is a strong tendency to select very fresh fish (Luten and Martinsdóttir 1997).

Loss of freshness and spoilage of fish is a complex process and depends on various factors such as species and different storage conditions. Consequently, it has been suggested to use a combination of selected indicators that represent the different changes occurring during spoilage (Olafsdóttir *et al.* 1997b).

2.1.1 Changes in raw fresh fish

The first sensory changes observed in fish during storage are related to appearance and texture. During the first couple of storage days in ice the characteristic taste of the species is usually developed (Huss 1995).

The most dramatic change is the onset of rigor mortis. Immediately after death, 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 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).

2.1.2 Spoilage

Spoilage of fish is not clearly defined. Obvious signs of spoilage are formations of off-odours and off-flavours, slime formation, gas production and changes in texture.

The development of these spoilage conditions in fish and fish products is due to a combination of microbiological, chemical and autolytic phenomena (Huss 1994).

The physical, chemical, and bacteriological characteristics of fish vary with species, seasons, methods of capture, fishing grounds etc. However, it is possible to describe the changes that take place after death, until the fish is totally spoiled and unfit for human consumption (Reay and Shewan 1949).

The changes in fish have three main groups of causes: bacteria, digestive enzymes and others (e.g. oxidation leading to rancidity) (Borgstrom 1965). The total number of bacteria on fish rarely indicates sensorial quality or expected characteristics (Huss *et al.* 1974). However, it is well recognized that certain Gram-negative bacteria are the main cause of spoilage (Huss *et al.* 1974).

2.1.2.1 Microbial spoilage

Microbial spoilage of foods may take diverse forms, but all of them are the consequence of microbial growth, which are manifested as changes in the sensorial characteristics as shown in Table 1.

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

Microbiological activity	Sensory manifestation
Breakdown of food components	Production of off-odours and flavour
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

Important factors contribute to the microbiological complexity of fish and fish products. They are:

- Contamination of the environment where the animal lives and of the products during processing
- Growth conditions for microorganisms due to specific intrinsic and extrinsic factors (temperature, A_w , pH, oxygen, light and microbial interactions).

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).

Microbial activity is also the cause of spoilage of many preserved fish products stored at temperature > 0 °C. However, in most cases the specific spoilage bacteria are not known (Huss 1994).

The spoilage of marine temperate- water fish is characterized sensorically by the development of offensive fishy, rotten, H₂S off-odours and off-flavours. This behaviour is different from some tropical and fresh water fish, where fruity, sulphhydryl off-odours and off-flavours are more typical (Gram and Huss 1996).

2.1.2.2 Chemical spoilage

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 discoloration of the fish tissue. The degradation of hydro-peroxides gives rise to 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).

2.1.2.3 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 denaturation of fish tissue, changes in texture and loss of water retention capacity (Huss 1994).

2.1.3 Control of spoilage

All foods spoil sooner or later, but diverse measures can be taken to reduce spoilage rate. Of most importance is to control storage temperature.

Chemical spoilage or development of rancidity can be prevented by rapid handling of the catch on board and storage of the fish products under anoxic conditions (vacuum packed or modified atmosphere packed). Antioxidants may be used too (Huss 1994). Spoilage rate decreases considerably at temperatures below – 20 °C.

2.2 Methods to evaluate freshness

Numerous methods may be used to evaluate freshness of fish. In general the main practical methods of determining seafood freshness quality involve chemical analysis, physical instruments and sensory evaluation (Botta 1995). Microbiological and biochemical methods may also be used, as well as some instrumental methods.

2.2.1 Sensory methods

Characteristic sensory changes occur in appearance, odour, taste and texture during storage of fish (Olafsdóttir *et al.*1997a). Sensory evaluation is one of the most important methods for freshness and quality evaluation in the fish sector and the fish inspection services (Luten and Martinsdóttir 1997).

2.2.1.1 EU scheme

The EU scheme is the most common method for quality assessment in the inspection service of the fishing industry used in Europe. It was introduced in Council decision No. 103/76 of January 1976. This method has been questioned because the scheme does not take into account differences between species; it only uses general parameters (Luten and Martinsdóttir 1997).

There are three levels in the EU scheme, E (Extra), A and B, where E is the highest quality and below B is the level where fish is discharged for human consumption (Hyldig and Nielsen 1997).

2.2.1.2 Quality Index Method

The Quality Index Method (QIM) is a relatively recent method that has been developed for various species (Luten and Martinsdóttir 1997). The scheme was originally developed by the Tasmanian Food Research and has been tested in many European countries. It has been developed for fresh herring, saithe and cod and frozen cod, for red fish, sardines and flounder and for Atlantic mackerel, horse mackerel and European sardine (Nielsen 1997).

The QIM scheme is a rapid and objective method. It is based on significant and well defined characteristic changes of outer appearance attributes (eyes, skin, gills, smell) for raw fish through a score system from 0 to 3 demerit (index) points. QIM gives scores of zero for very fresh fish and increasingly larger total results as the fish deteriorate (Hyldig and Nielsen 1997). The total number of index points can also be used to predict the remaining shelf life (Luten and Martinsdóttir 1997).

The first step in developing a QIM scheme is to perform a parallel sensory analysis of raw and cooked fish. The purpose of the sensory evaluation of the raw fish is to describe (literally) all detectable aspects of changes on/in the fresh fish during cold storage in ice. This involves a detailed description of all possible changes/deviations of sensory parameters such as appearance, texture and odour. Every deviation for a specific parameter has to be described in common words for each evaluation during the storage trials (Hyldig and Nielsen 1997).

2.2.1.3 Torry scale

For sensory evaluation of fish fillets, it is common to cook the fillets and evaluate their odour and flavour. The Torry scale is the most used scale for evaluating the freshness of cooked fish, but sensory profiling is also used in research laboratories in Europe (Hyldig and Nielsen 1997).

The Torry scale is a descriptive 10 point scale developed at the Torry Research Station. This scale has been developed for lean, medium fat and fat fish species (Martinsdóttir *et al.* 2001).

The panellist evaluates the odour and flavour of the cooked fillets using the Torry-scale, the scores are given from 10 (very fresh) to 3 (spoiled), and the average score of 5.5 may be used

as the limit for consumption. It is considered unnecessary to have descriptions below 3, as the fish is then not fit for human consumption (Martinsdóttir *et al.* 2001).

2.2.1.4 Quantitative Descriptive Analysis (QDA)

Quantitative descriptive analysis (QDA) is a method used at the Icelandic Fisheries Laboratories by a sensory panel to evaluate cooked fish (Sveinsdóttir *et al.* 2002). This method uses a linear scale, which has anchored in their ends words or terms that describe intensity or character of attributes evaluated. The intensity increases from left to right, for example, firm to soft, dry to juicy, 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 analyzed statistically (Meilgaard *et al.* 1999).

2.2.2 Chemical methods

Chemical methods of measuring freshness quality are considered to be objective and consequently superior to methods involving sensory evaluation (Botta 1995).

Odour is one of the most important parameters to evaluate fish freshness. During storage of fish the odour undergoes changes, from fresh odour, sweet and stale odours until the final phase of spoiled or putrid odours. Volatile compounds contributing to odour changes can be measured to evaluate the freshness and spoilage of fish (Olafsdóttir and Fleurence 1997).

During the deterioration of fish amines are formed. The measurements of total volatile bases (TVB) have been used in the fish industry as an indicator of quality for fish and fish products (Olafsdottir and Fleurence 1997). TMA is one of the substances and has been used widely as an indicator of spoilage in marine fish.

2.2.3 Physical methods

Several physical measurements have been used to evaluate fish freshness (Heia *at el.* 1997). These include measurements of time- temperature indicators (TTI), mechanical, microstructure, electrical properties, colour measurements, spectroscopy and pH.

Time –temperature indicators are devices or materials that can be attached to, or incorporated into foods in order to give an indication of the time-temperature history of the food. The

mechanism of recording the time –temperature history is through some biological or chemical process, which depends on time and temperature.

Mechanical measurements can be used to evaluate structural changes. The instruments used are texturometers fitted with a wide variety of accessories for different types of analysis.

Another way to assess structure of fresh fish is by microstructural characterization of the fish muscle.

Changes in freshness can also be measured by changes in the electrical properties of the fish muscle.

Colour changes may be related to changes in fresh fish. Instrumental measurements of colour are becoming increasingly important in quality control in the food industry.

Spectroscopy methods have gained importance in evaluation of food quality parameters because of how rapid they are.

Most microorganisms grow best at pH values around 7.0 (6.6 - 7.5), whereas few grow below 4.0. Adverse pH affects at least two aspects of a respiring microbial cell: the functioning of its enzymes and the transport of nutrients into cells (Jay 1996). The post mortem reduction in the pH of fish muscle has an effect on the physical properties of the muscle. When the pH decreases, the net charge of protein on the muscle is reduced, causing them to partially denature and lose some of their water- holding capacity. In the state of rigor mortis the muscle tissue loses its moisture when the fish is cooked (Huss 1995).

2.2.4 Microbiological methods

The aim of microbiological examination of fish is to evaluate the possible presence of bacteria of public health significance. The number of specific spoilage bacteria will give information on the remaining shelf life which can be predicted from such numbers. Microbial measurements can be used to evaluate the degree of fish freshness. When such microbiological measurements are needed it is recommended to use the numbers of specific spoilage organisms (SSO) or classical total viable counts (TVC) measurements (Olafsdottir *et al.* 1997).

Volatile sulphur compounds are typical components of spoiling fish and most bacteria identified as specific spoilage bacteria produce one or several volatile sulphides. The volatile sulphur compounds are foul smelling and can be detected even at ppb levels, so even minimal quantities have a considerable effect on quality. Fruity, rotten, sulphhydryl odours and flavours are typical of the *Pseudomonas* spoilage of iced fish (Huss 1995).

The number of microorganisms as determined by the aerobic plate count (APC) is variable and depends on the original contamination, increase or decrease of microorganisms during processing, recontamination of processed product and growth or death during storage, retailing and handling. The microbial flora is constantly changing (Banwart 1981).

The medium most widely used in North Europe for determining total counts is Iron Agar. Furthermore, Iron Agar gives the number of hydrogen sulphide producing bacteria, which in some fish products, are the specific spoilage bacteria. It is relevant when examining products where psychrotrophs are the most important organisms to use pour plating and three to four days incubation at 25 °C, while the products where the psychrophilic *Photobacterium phosphreum* appears should be examined by surface plating and incubation at a maximum of 15 °C (Huss 1995).

All agar-based methods have a common disadvantage, due to the extensive period of incubation required.

Strachan and Nicholson developed a method using Gastec detector tubes for the selective analysis of amines, hydrogen sulphide (H₂S) and ammonia in the headspace of gills and found that H₂S analysis gave the best results to predict storage period (Olafsdottir and Fleurence 1997).

3 MATERIALS AND METHODS

A new QIM scheme of arctic charr was developed and evaluated in a shelf life study including both the sensory evaluation with the QIM scheme and sensory evaluation of cooked fish to determine the end of shelf life. The sensory results were compared to other traditional methods, such as microbial counts and chemical indicators of spoilage measured during the shelf life study.

The new QIM scheme was developed in three steps:

- A pre- observation stage;
- Training of sensory panel and development of the QIM scheme; and a
- Shelf life study

The preliminary observation stage is a necessary step to know all the changes related with the appearance of gills, eyes and skin, which occur in fish during their time in storage, because not all species spoil in the same way. During the preliminary observation stage two persons observed fish at different storage times and listed the changes in a preliminary scheme.

During the training panel step, the panellists observed fish at different storage times (in this step the panellists were informed of the storage time of the fish) and evaluated the fish using the preliminary QIM scheme. On finishing the training, the QIM scheme that was used in the following evaluations, was drafted.

The shelf life study was performed using, sensory, chemical and microbiological methods.

3.1 Artic charr

A total of 60 fresh iced artic charr (*Salvelinus alpinus*) of approximate 1 kg farmed in the Silungur Farm arrived at the laboratory on 18/ 11 /04 (10 fish) and 29/ 11 /04 (50 fish) iced in a container, one day after slaughter. The first 10 fish were used to draft a QIM scheme for the species.

The container of 50 fish was divided into five groups, four containers with eight fish each for the sensorial analysis (QIM scheme and QDA), and one container with 10 fish for the microbiological and chemical analysis. The remaining eight fish were used in the first training session of the panel. The fish were iced in a container in alternating layers of ice and fish. Afterwards, the five containers were placed in cold storage at 0 - 2 °C. The containers were opened three times for sampling and adding of ice.

3.2 Sampling plan for all measurements

The following table shows the sampling plan developed.

Table 2: Sampling plan developed for all measurements.

Pre – observation						
# fish used	Day zero 18-11-04	First day 19-11-04	Fifth day 22-11-04	Seventh day 24-11-04	Ninth day 26-11-04	Twelfth day 29-11-04
	2	1	2	1	2	2
Main experiments						
Training of panel and development QIM scheme						
Date	# fish used					
30-11-04	QIM scheme			QDA		
One session	4 fish 1 st day 2 fish 12 th day			2 fish 1 st day 2 fish 12 th day		
Sensory analysis – Shelf life study						
# fish used	First day 30-11-04	Eighth day 7-12-04	Fourteenth day 13-12-04	Seventeenth day 16-12-04	Twenty-first day 20-12-04	
	4 QIM 2 QDA	4 QIM 2 QDA	4 QIM 2 QDA	4 QIM	Sensorial analysis was not made	
Chemical and microbiological analysis – shelf life study						
# fish used	First day 30-11-04	Eighth day 7-12-04	Fourteenth day 13-12-04	Seventeenth day 16-12-04	Twentieth one day 20-12-04	
	2	2	2	2	2	

3.3 Preliminary observation

The fish were observed during 12 days to study the changes that take place in them during storage time in ice at 0 - 2 °C. A QIM scheme of salmon was used (Sveinsdóttir *et al.* 2002) as a model for the arctic charr scheme because salmon and arctic charr are similar. Both belong to the same family *Salmonidae* (British Encyclopedia).

After concluding the observations, the changes observed in appearance, odour and texture during storage were listed in a preliminary scheme.

3.4 Training of panel, development of a QIM scheme and shelf life study

3.4.1 Quality Index Method (QIM)

A total of 20 fish were analyzed with a QIM scheme during the training and evaluation period.

3.4.1.1 Training of judges

Training of 10 QIM judges was carried out in one session. The judges were all employees at the Icelandic Fisheries Laboratories and had experience in assessing fish with a QIM scheme. The judges were introduced to the scheme developed during the pre- observation of arctic charr stored in ice.

The procedure of evaluation was introduced to the judges and each parameter evaluated was discussed. The judges were informed of the plan to develop a QIM scheme for arctic charr stored in ice and were asked to comment on the QIM scheme. The judges observed the fish (the storage time in ice was given) and the scheme was explained to them at the same time.

3.4.1.2 Sample preparation and QIM scheme

The fish were placed on a table, 30 minutes before the evaluation. Each fish was coded with a number consisting of three digits that did not indicate the storage time or condition of the fish.

For the QIM evaluation (four sessions) 16 fish at different storage times were evaluated,(four on each sampling day). All observations of the fish were carried out under standardised conditions at room temperature using electric light and with as little distraction as possible.

The QIM scheme for arctic charr was applied for the sensory analysis of the raw fish. Six to eight QIM judges evaluated the fish individually and registered their evaluation for each quality parameter in the scheme. The quality index was calculated as a sum of the score for evaluated parameters. The average score of each panellist for each fish was calculated. After each sampling day the average QI of four fish was calculated. The judges had no information about the storage time in ice. The evaluation took 25-30 minutes each time.

3.4.2 *Sensory evaluation of cooked fish*

3.4.2.1 Training of judges

Prior to the experiment, the panel was trained during one session in the use of the QDA for sensory evaluation. The members of the panel were familiar with the QDA method and experienced in sensory analysis of fish. Sensory evaluation of the cooked artic charr was performed parallel to the QIM assessment.

A preliminary objective of the training was to develop a scorecard to use to score the products. The panellists as a group met with the panel leader and developed a common language that described their perception of the product. The panel leader facilitated the discussion.

A scorecard lists the attributes in order of occurrence, starting with the first perception and ending with the last. The scorecard of artic charr is shown in Appendix 1.

The average of the panellists score for each attribute and the average of duplicate was calculated.

3.4.2.2 Sample preparation and development of QDA

Each panellist evaluated samples from different storage times. Samples collected from each fish without skin came from the loin part of the fish. The samples were placed in aluminium boxes. Each sample was coded with three random digit numbers and cooked at 95-100 °C for seven minutes in a pre-warmed oven. The boxes were closed with plastic covers and then served to the panel. Each sample was evaluated in duplicate.

3.4.3 *Microbial counts and pH measurements*

On each sampling day, two samples from each trial were taken to the microbiological laboratory of IFL for microbial counts, performed by staff. Initially the skin was disinfected with ethanol (70%) and removed. The underlying muscle was minced and blended in a Butterfield's buffer to make up a 1/10 solution. The following microbial counts were carried out: total plate count and H₂S producing bacteria on Iron Agar at 15 °C for three to four days (surface plating). These measurements were carried out at the microbiology laboratory of IFL by the staff. The pH of the mince was then determined (5 g mince+ 5 ml buffer) in the Radiometer PHM 80.

3.4.4 *Chemical analysis*

The mince from two fish was combined for TVB measurements which were done by steam distillation (Billon et al. 1979). The measurements of TVN were carried out at the chemistry laboratory of IFL by the staff.

3.5 Data analysis

The equation of best fit and correlation coefficients (R^2) of QI, total viable count and H_2S producing bacteria of raw arctic charr against storage time in ice were calculated using Microsoft Excel.

The results of QDA were treated with analysis of variance (ANOVA) to analyze if some difference existed between samples. Multivariate comparisons were calculated using Duncan's Multiple-Comparison Test. In the ANOVA when $p < 0.05$ there are significant differences between samples.

4 RESULTS

4.1 Pre-observation results

Changes were not observed in the appearance of the fish during the first and second day of storage time. On the fifth day of the preliminary observation changes became apparent. The first changes recognised were: the skin was less shiny, the pupils of the eyes became dark grey and flat, the gill odour changed from metal to metal/cucumber and the abdomen began to become yellowish.

On the seventh day, the skin colour near the abdomen was more yellowish, and the odour changed to strong metal. The odour of the gills became strong metal, and a little sour.

By the twelfth day the fish was beginning to show decomposition, as the odour in the abdomen became sour, indicating spoilage.

No changes were observed in the appearance of the pupils from the fifth to the twelfth day. They stayed flat and dark/mat grey and the colour of the gills remained dark brown. Texture was the other quality attribute that did not change.

Parameters that describe changes in skin, gills, eyes and abdomen were listed in a preliminary scheme after completing the preliminary observation of the raw arctic charr. The original appearance of the fish is shown in Figure 3. Figure 4 shows the appearance of the fish after 12 days of storage.



Figure 3: Appearance of arctic charr on day zero. Pearl –shiny skin, red gills, no blood present in the abdomen and black coloured pupils were observed in the fish on day zero.



Figure 4: Appearance of artic charr after 12 days of storage. Yellowish skin mainly near the abdomen, paled red/ light brown gills, blood became brown /yellowish around the abdomen and the colour of the pupils turned to dark grey.

4.2 Training panel

During the first session, the panellists worked with the QIM scheme for farmed salmon. The panellists compared the QIM scheme of salmon with the preliminary QIM scheme of artic charr and the pertinent changes were made to adapt it for artic charr. The preliminary QIM scheme was changed in the descriptions corresponding to skin (mucus, odour and texture), gills (colour, mucus and odour) and in the abdomen odour. A score from 0-1, 0-2, or 0-3 demerit points (index) was given for changes occurring in odour and texture and in the external appearance of eyes, skin, and gills and also the abdomen (Table 3).

Table 3: The QIM scheme for farmed articharr- draft.

Quality parameters	Description	Points	
Skin	Colour/ appearance	Pearl-shiny all over the skin	0
		The skin is less pearl-shiny	1
		The fish is yellowish, mainly near the abdomen	2
	Mucus	Clear, no clotting	0
		Milky, clotted	1
		Yellow and clotted	2
	Odour	Fresh seaweed, neutral	0
		Metal, cucumber, grass	1
		Hey, sour	2
		Rotten, dish cloth	3
	Texture	In rigor	0
		Finger mark disappears rapidly	1
Finger leaves mark over 3 seconds		2	
Eyes	Pupils	Clear and black, 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
Abdomen	Blood in abdomen	Rotten	3
		Blood red/ not present	0
	Odour	Blood more brown, yellowish	1
		Neutral	0
		Cucumber, melon	1
		Sour, reminds of fermentation	2
	Rotten, rotten cabbage	3	
Maximum sum (Quality Index)		24	

4.3 Verification of Quality Index scheme

The sum of the scores evaluated according to the QI scheme (Table 3) was presented as the Quality Index (QI). The QI was calculated for four different storage days (one, eight, 14 and 17 of the fish and formed a linear relationship with the storage time (Table 4, Figure 5).

Table 4: Average QI for four fish, average day scores and standard deviation of four fish.

Sample / fish no.	Day 1	Day 8	Day 14	Day 17
1	3.2	8.5	15.0	15.6
2	3.8	9.9	8.9	16.0
3	6.0	10.6	13.0	16.9
4	4.3	8.2	10.1	17.7
Average QI	4.3	9.3	11.7	16.5
Standard dev.	1.2	1.2	2.7	0.95

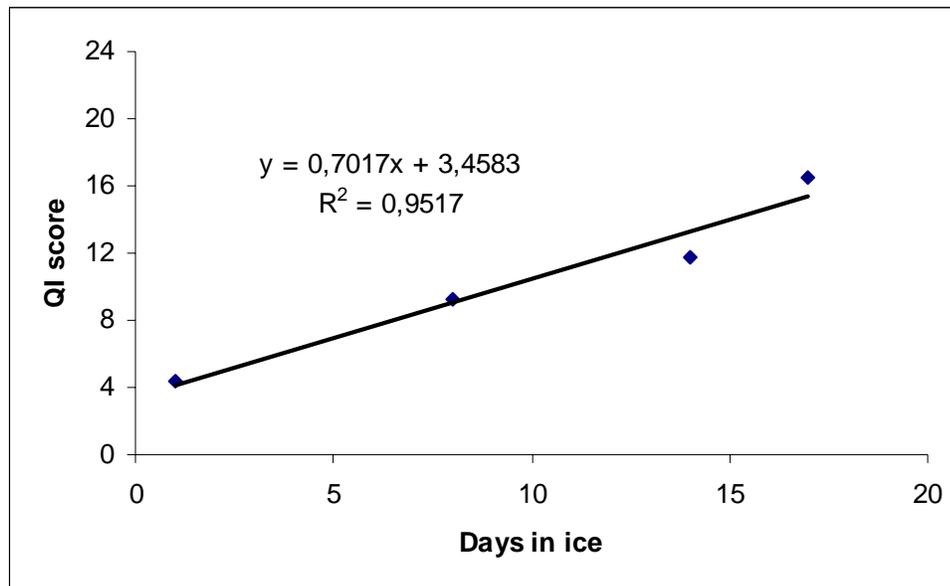


Figure 5: Quality Index of artic charr. Average QI of each storage day versus days in ice.

High correlation between the average QI and days in ice was obtained.

QIM assumes that scores for all quality attributes increase with storage time in ice. This was observed except for the form of the eyes (Figure 6). The rate of increase was different for different attributes, all the scores increased but for some attributes the increase was less pronounced than for others such as skin mucus and pupils of the eyes. For the rest of the attributes the growth was high.

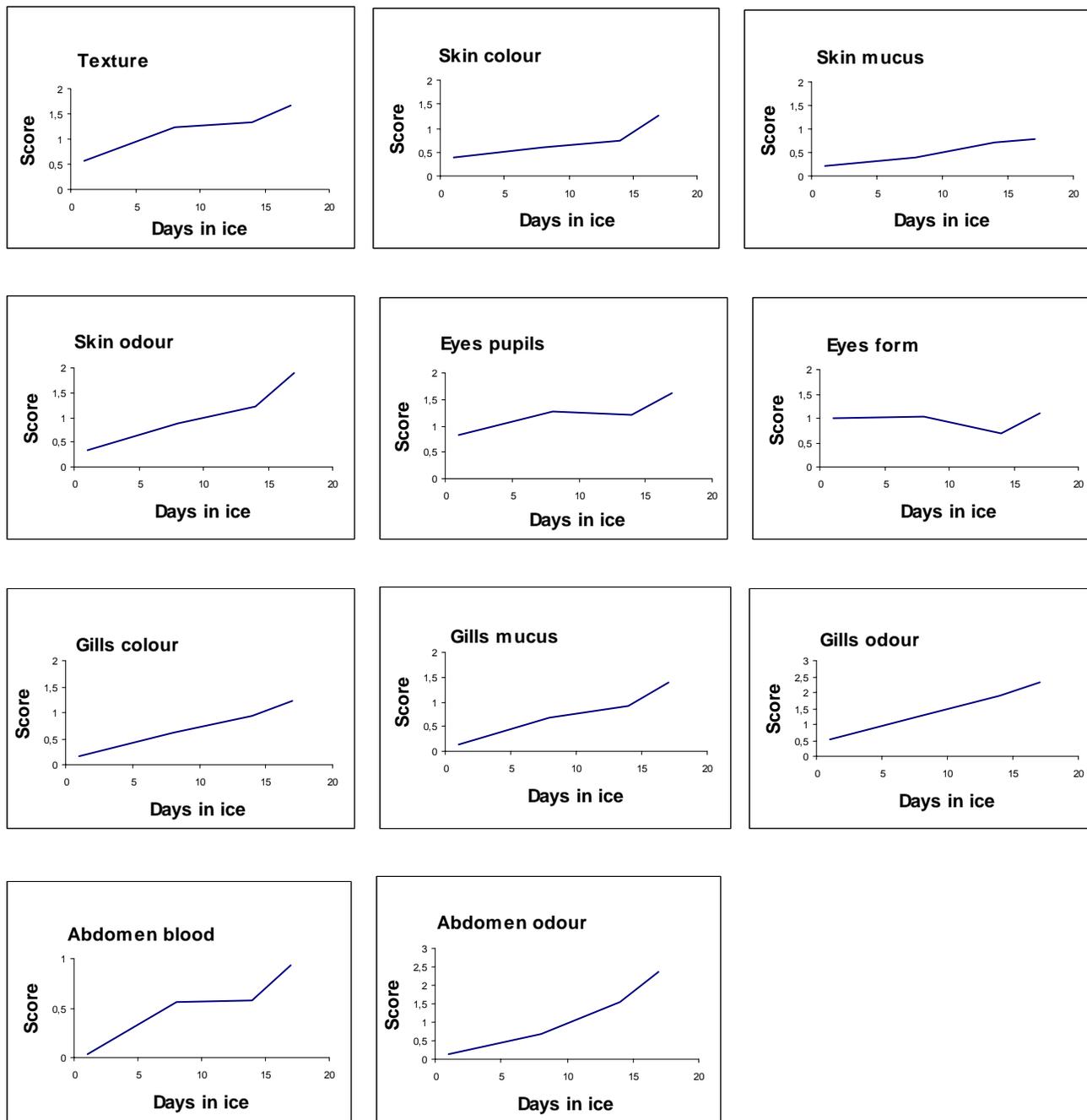


Figure 6: Average scores of each quality attribute assessed with the QIM scheme for artic charr in ice against days in ice.

The skin texture increased from day zero to day eight, between day eight and 15, the score suffered little change. After day 15, the scores increased until the end of the storage time. The skin texture score increased from 0.6 to 1.7. On day 17 most of the panellists gave the maximum point demerit to this attribute.

The average skin colour score increased from 0.4 to 1.3. It was scored as 0.4 on storage day one (,very fresh and pearl- shiny all over the skin). On day eight it was evaluated as less pearl

shiny, with this evaluation remaining until day 17. On day 17 some panellists gave the maximum demerit points (2).

The skin odour, gills odour, gills mucus, gills colour and abdomen odour increased considerably through the days of storage.

At the beginning of the storage when the fish were very fresh, the skin odour was described as fresh seaweed or neutral and then, a metal or cucumber odour dominated the artic charr skin odour. At storage day 17 the average score reached a value of 1.9, corresponding to hey or sour odour. The score 3 (rotten) was rarely used by panellists.

The skin mucus scores were from 0.2 to 0.8, increasing throughout the storage time, but the variation among samples was little.

Skin mucus and eye pupils were the attributes that showed the least variation during the assessment. The pupils of the eyes scored from 0.9 to 1.6.

The assessment of eye form was the quality attribute that presented most difficulties in evaluation, but during the storage time it constantly recorded 1 demerit point.

The score for gills colour increased throughout the storage time,, reaching the maximum score at the end of the storage period.

The gills odour was described at the beginning as fresh metal/ metal cucumber, because the score average was 0.5 (11 samples were evaluated as fresh and 13 were evaluated as metal/cucumber). At the end of the study the average score was 2.3, described as sour, mouldy.

The average scores of gills mucus were very low until after day eight of storage (0.1- 0.7). At the end of the storage time,, the scores reached values close to the maximum score.

The average scores for quality attributes of abdomen were very low until after day eight of storage when they began to increase with storage time.

The average scores of abdomen blood increased from 0.04 to 0.9. On day 17 most of the panellists gave the maximum demerit point score to this attribute.

The abdomen odour was described at first as neutral with an average score of 0.13, finishing, at the end of the evaluation, with a score of 2.4, described as sour, reminds of fermentation. This was the quality attribute with the most variation in the score, coming close to a rotten odour at the end of the evaluation.

4.4 Sensory evaluation of cooked arctic charr fillets

Training sessions produced a list of words that described the quality parameters of odour, flavour and texture of cooked arctic charr (Table 5).

Table 5: Attributes that describe arctic charr fillets assessed by the QDA method.

Odour	Flavour	Texture
Characteristic for species	Characteristic for species	Softness
Metallic	Metallic	Juiciness
Musty/mould	Musty/mould	Tenderness
Sour	Sour	
Rancid	Rancid	

4.5 Quantitative Descriptive Analysis

The positive attributes for flavour and odour of cooked arctic charr were described as characteristic arctic charr, metallic and oil on a scale ranging from 0 to 100 %. The negative attributes were described as musty/mould, sour and rancid flavour and odour. Mean scores for the arctic charr samples (over a time period of one, eight and 14 days of storage time in ice at 0 – 2 °C) were evaluated by the trained panel with p values shown in Table 6.

4.5.1 Positive attributes

Average scores for positive attributes did not change significantly during the storage time (Figure 7). The average scores of characteristic arctic charr were between 57 and 63 throughout the storage time, while the average scores of metallic and oil flavour were between 30 and 38 throughout the storage time. The lowest value was observed on day eight for all three attributes.

No significant differences were found in the positive attributes odour scores, except in the oil odour, where the scores changed significantly with the storage time, decreasing from 40 to 18. The average scores characteristic for arctic charr were between 57 and 62 during the storage time, while the average scores of metallic odour were between 23 and 27. These three attributes decreased with the storage time (Figure 8).

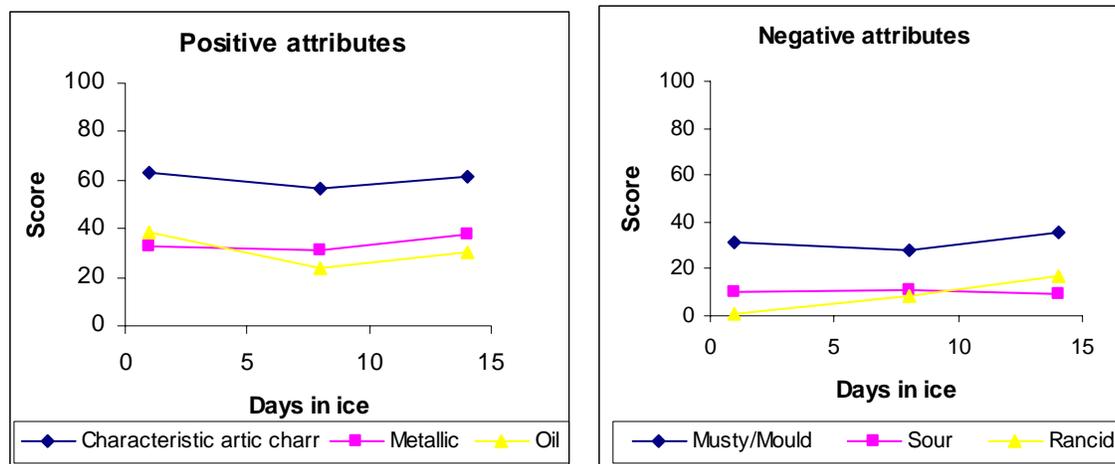


Figure 7: Changes in flavour attributes of cooked arctic charr (average score) against storage of the raw arctic charr in ice observed by a trained QDA panel.

4.5.2 Negative attributes

The negative attributes behaved similarly; no significant difference was found in the scores. The scores for sour and rancid attributes were low, approximately 1 to 17. The scores of musty/mould attribute were a little higher, approximately 28 to 36. The musty/mould and rancid attributes reached their maximum value score at day 14, whereas the sour attribute reached its maximum value on day eight (Figure 7).

The negative odour attributes also behaved similarly, however, the rancid attribute increased markedly as the storage time increased.(from 0 score to 17). The average scores of musty/mould were between 25 and 31 during the storage time, while the average scores of sour were between 5 and 11 during the storage time. All the negative attributes increased with the storage time (Figure 8).

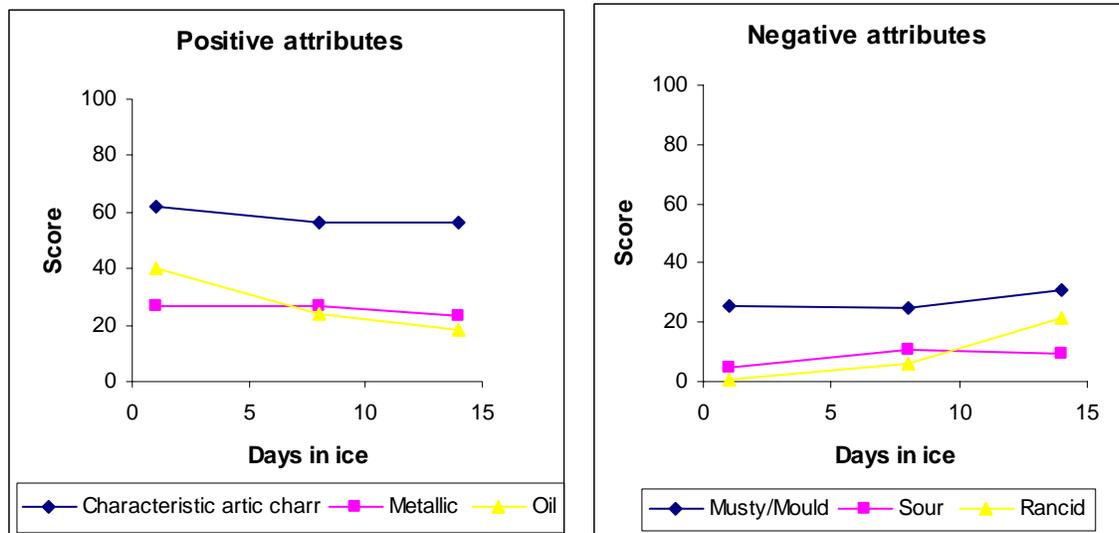


Figure 8: Changes in odour attributes of cooked articharr (average scores) against storage time of articharr in ice observed by QDA panel.

The oil odour and oil flavour were the positive attributes that showed more variation throughout the storage time.

The rancid odour and rancid flavour were the negative attributes that showed more variation throughout the storage time.

The other positive and negative attributes of odour and flavour did not show significant changes during the storage time.

Little variation in the texture attribute parameters was recorded during the storage (Figure 9).

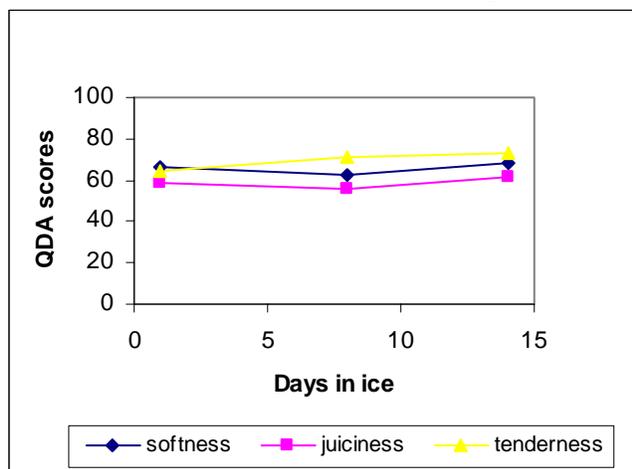


Figure 9: Changes in the texture attributes of cooked articharr (average scores) against storage time of articharr in ice observed by QDA panel.

The softness and juiciness parameters recorded the same behaviour with the score decreasing on day eight compared to day one, but increasing on day 14.

The tenderness parameter increased with storage time.

There was not statistical difference between average QDA scores during storage time of ice, except on oil odour attribute and rancid odour attribute (Table 6).

The oil odour attribute was markedly different on day one with respect to days eight and 14. The panellists identified that the oil odour was more intense on day one than days eight and 14. The intensity of this attribute decreased during the storage time (Figure 8). In this case the probability was < 0.05 .

Reverse behaviour was observed for the rancid odour negative attribute. This attribute was different on day 14 with respect to days one and eight. On day 14 the rancid odour was at its most intense in comparison to days one and eight. The rancid odour increased during storage time, the p-value was < 0.01 .

Rancid odour also increased during storage time, but the p-value was 0.09 ($p > 0.05$).

The rests of the attributes (negative and positive) did not record significant differences between sampling days, the panellists' perceived little differences.

It is necessary to evaluate more storage days of the fish to find big differences between sampling days.

Table 6: Mean scores for the artic charr samples (one, eight and 14 days of storage time in ice at 0 – 1 °C) evaluated by trained sensory panel with p-values.

Attribute		Days of storage			Prob.level
		1	8	14	
Odour	Characteristic of artic charr	62.0	56.6	56.6	0.495
	Metallic	27.0	27.0	23.2	0.82
	Oil	40.2 ^a	23.8 ^b	18.4 ^b	< 0.05
	Musty/mould	25.6	24.6	30.6	0.574
	Sour	4.8	11.0	9.6	0.389
	Rancid	0.67 ^a	6.36 ^a	21.7 ^b	< 0.01
	Characteristic of artic charr	63.3	56.5	61.8	0.555
Flavour	Metallic	33.0	31.0	37.4	0.763
	Oil	38.4	23.8	30.0	0.112
	Musty/mould	31.2	28.0	36.0	0.688
	Sour	10.33	11.07	9.33	0.961
	Rancid	1.25	8.64	17.33	0.09
	Softness	66.08	62.29	67.92	0.614
Texture	Juiciness	58.6	56.2	61.7	0.765
	Tenderness	64.5	71.14	73.33	0.311

(a,b: different letters indicate significant differences between the samples).

4.6 Shelf- life

According to the results of QDA, on day 14 the fish started to show signs of spoilage (characterised by the rancid odour at that stage), although the scores for this attribute were not high. On the same day using the QIM scheme, the odour attributes gave signs of spoilage (the average odour scores of gills was nearly 2, characterised by the sour odour and score of abdomen odour of 1.5, with the odour changing from cucumber/melon to sour and reminds fermentation). On day 17, the odour was stronger still, and the fish presented signs of spoilage (the gills average odour scores reached 2.3(sour/mouldy) and the average abdomen odour scored 2.4 (sour/reminds fermentation).

4.7 Microbiological and chemical results

During the first eight days of chilled storage, no microorganisms were detected (Figure 10).

After day eight, increasing growth was found for the remaining storage period, reaching the highest number at the end of storage (day 21). When the sensory panel judged the fish unfit for consumption (QDA) after 14-17 days of storage, the total count was ca. $10^3 - 10^4$ / g. At the same time the numbers of H₂S producing bacteria were less than 10^2 /g.

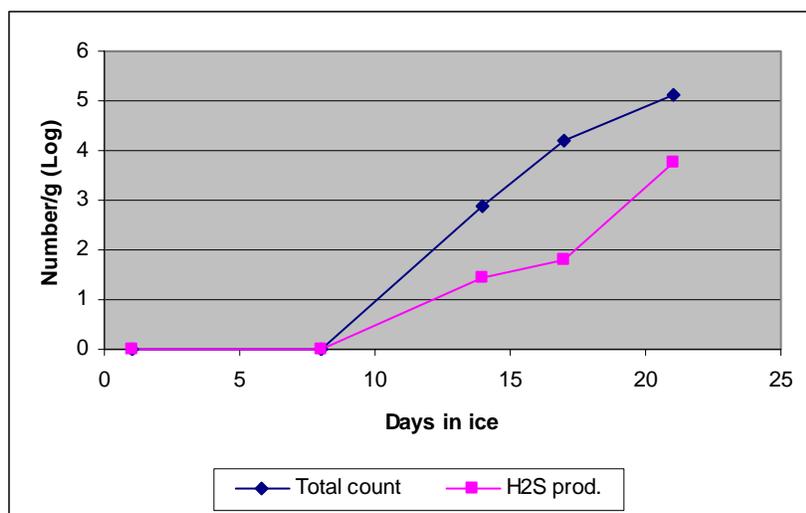


Figure 10: Microbiological counts on Iron agar at 15 °C in the muscle of artic charr against the storage time.

Measurements of TVN fluctuated throughout the storage period (Figure 11). TVN values ranged from 15.6 mg N/100g to 19.8 mgN/100g. During the first 14 days the TVN value decreased.

No increase was observed in TVN values during the storage period, indicating that formation of basic volatile substances like TMA and NH₃ was minor.

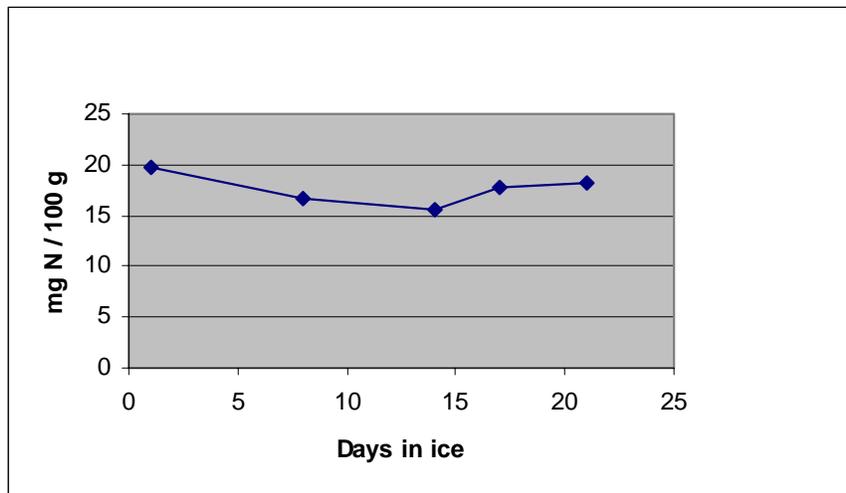


Figure 11: Changes in total volatile nitrogen compounds (TVN) during storage of gutted arctic charr stored in ice.

There was no significant difference in the pH values with increasing storage time (Figure 12).

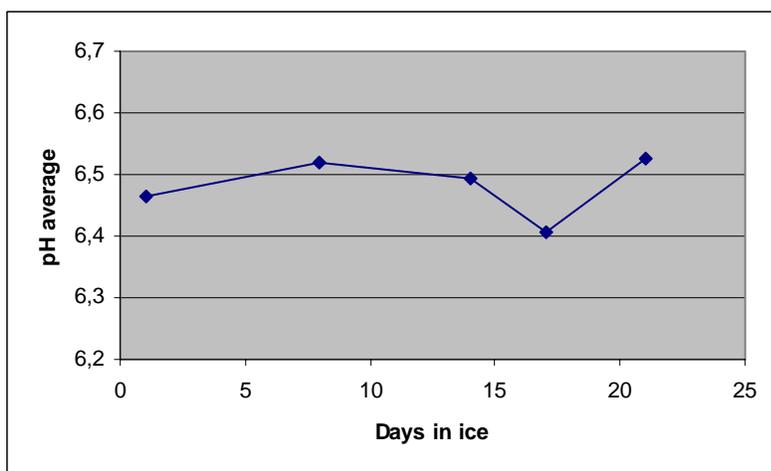


Figure 12: Changes in pH of arctic charr against storage of raw arctic charr in ice.

At the beginning of the storage period, when microorganisms were not detected, the QIM scores were low. A high correlation was found between QI and total count on flesh, and some correlation was seen for H₂S producing bacteria., These values increased proportionally during the later stages of storage (Figure 13).

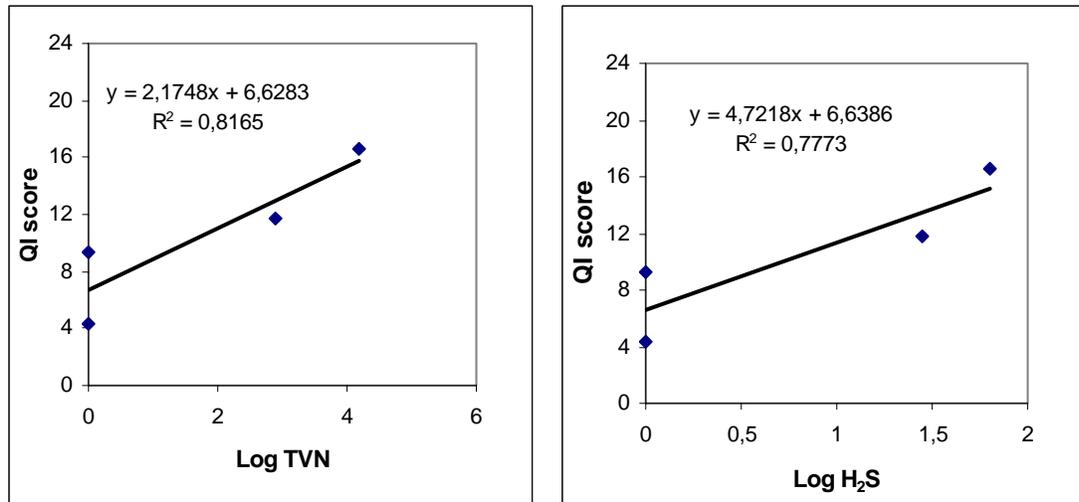


Figure 13: Correlation between bacteria counts and Quality Index of raw arctic charr stored in ice at 0 – 1°C.

5 DISCUSSION

5.1 Sensory analysis using the Quality Index Method

During the evaluation of sensory attributes of arctic charr it was found that some attributes did not have significant variation with storage time, such as skin mucus and eye form, while other showed strong correlation with storage time like gill and abdomen odour. At day 17 of storage time the sour odour was very strong and the panel decided not to evaluate the cooked fish. Eye form did not change during storage time and was therefore excluded from the QIM scheme. The final QIM scheme of arctic charr consists of 10 parameters (Table 6), resulting in a total of 21 demerit points.

Table 7: Quality Index Method (QIM) scheme for arctic charr (*Salvelinus alpinus*).

Quality parameters	Description	Points	
Skin	Colour/ appearance	Pearl-shiny all over the skin	0
		The skin is less pearl-shiny	1
		The fish is yellowish, mainly near the abdomen	2
	Mucus	Clear, no clotting	0
		Milky, clotted	1
		Yellow and clotted	2
	Odour	Fresh seaweed, neutral	0
		Metal, cucumber, grass	1
		Hey, sour	2
		Rotten, dish cloth	3
Texture	In Rigor	0	
	Finger mark disappears rapidly	1	
	Finger leaves mark over 3 seconds	2	
Eyes	Pupils	Clear and black, metal shiny	0
		Dark grey	1
		Mat, grey	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 more brown, yellowish	1
	Odour	Neutral	0
		Cucumber, melon	1
		Sour, reminds of fermentation	2
	Rotten, rotten cabbage	3	
Maximum sum (Quality Index)		21	

5.2 Quantitative Descriptive Analysis.

No changes in the positive and negative attributes of flavour during the assessment of cooked fish were found, probably because the spoilage was very slow and did not begin in earnest until day 14, when the fish showed signs of beginning to spoil. Although some significant differences in the attributes corresponding to odour were found, the scores were low.

For the odour and flavour, the attribute that correlated best to spoilage of arctic charr was rancid.

5.3 Shelf-life

Changes in sensory attributes indicated that arctic charr was approaching the end of shelf-life when arctic charr was increasingly characterised by sour odour in the gills and abdomen on day 17, corresponding to the QI results. These results correlated with the QDA methods, which recorded that on day 14 the fish started to present spoilage signs. Rejection of arctic charr was considered to occur approximately after 14- 17 days in ice.

To determine with accuracy the shelf life of arctic charr it is necessary to evaluate the raw fish on day 21 using the QIM scheme method and the cooked fish on day 17 and 21 using the QDA method.

5.4 Total viable counts (TVC) and H₂S –producing bacteria

The flesh of newly caught fish is sterile because 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 (Sveinsdóttir *et al.* 2002).

Comparing these results with microbial results obtained from marine cod (muscle), it is evident that higher values were obtained in cod muscle than in the arctic charr muscle. Thus, when the cod was considered unfit for consumption on day 16 (TMA >10mg N/ 100g), total counts on plate count agar were 10⁴-10⁵ /g (Magnússon and Martinsdóttir 1995).

Higher values were also obtained in salmon muscle than in the arctic charr (Sveinsdóttir *et al.* 2002). The total count at the beginning of storage was around 10 cfu / g for salmon while for arctic charr there was no count. However, at day 21 of storage the value of total count was equal for both species 10⁵ / g. Similar behaviour was witnessed with the counts of H₂S producing bacteria. At the beginning of storage these were not found in either of the two species, and at day 21 the value was 10⁴ for salmon and 10³-10⁴ for arctic charr. This behaviour is normal since both are fresh water species and belong to the *Salmonidae* family.

Comparing all the results obtained in this work with the results on rainbow trout (Chytiri *et al.* 2004) it can be seen that numbers of H₂S producing bacteria were much higher in the rainbow trout than was found for arctic charr. H₂S producing bacteria values ranged from ca. 10 – 10⁶ /g on rainbow trout, while on arctic charr they were less than 10² /g.

5.5 Total volatile nitrogen compounds (TVN)

TVN value decreased during the first 14 days due volatile substances evaporating and/or leaking out through skin and belly.

In a study on rainbow trout (Chytiri *et al.* 2004) found that there was no significant increase in TVN and pH which is in harmony with the results obtained for arctic charr in this study.

No limit of TVN for acceptability of rainbow trout was established by Decision 95/149 (EU 1995). Gimenez *et al.* (2002) proposed a value of 25 mg N/ 100 g flesh as the highest acceptable level (Chytiri *et al.* 2004). Comparing TVN values of arctic charr with this value, they remained below this limit of acceptability throughout the entire storage period in ice.

In the aforementioned decision, the TVN limit values are fixed for salmon at 35 mg N/ 100 g of flesh., The values obtained for arctic charr throughout storage time were lower than that limit.

It can therefore be concluded that TVN measurements are useless as a spoilage indicator for arctic charr.

5.6 pH

The results in the pH indicate that formation of basic substances was negligible during the storage period.

5.7 Comparison of methods

A good agreement between results from different methods was found. During the first eight days of storage the QI scores were low, while the QDA scores for the positive attributes were relatively high. In the same period the values of pH and total volatile nitrogen compounds did not show significant changes.

At the end of the storage time QI scores reached high values while QDA scores for negative attributes also increased. The values of total viable counts and H₂S producing bacteria increased too.

The QDA method was used until day 14, when panellists detected that cooked fish began to present rancid odours, however, rancid flavours were not strong. According to Bremmer (2000) the end of storage time is defined when a trained panel detects spoilage flavour in cooked samples of the fish, therefore, the fish were not spoiled at this storage time..

Comparing the QIM and QDA methods shows that in the former (QIM) most of the parameters changed significantly during storage time, while in the latter (QDA) most of the parameters did not show significant change.

The QIM scheme in this case gave more information for determining the end of shelf life of arctic charr.

On day 17, the values reached a total count of 10^4 cfu/g which is not high compared with values found on spoiling fish (10^7 - 10^8 / g) (Gram *et al.* 1987, Ólafsdóttir *et al.* 1997a). Nevertheless, standards, guidelines and specification often use much lower TVC as indices of acceptability. According to a consumer study carried out in Europe, fish was assumed “not to be in good enough condition to be stored for long” when total count reached 10^6 cfu/g (Anon 1995). Microbial criteria based on low TVC as 10^6 cfu/g can be difficult to use because a correlation between TVC and the remaining shelf life is assumed but generally not known (Ólafsdóttir *et al.* 1997b).

On the other hand, total volatile nitrogen (TVN) values on day 17 did not exceed the legal limit set for these indexes in Directive 95/149/EEC (Ruiz-Capillas and Moral 2000). The TVN value for arctic charr on day 17 did not indicate that fish was spoiled.

The results of microbial and chemical methods did not indicate spoiled fish either.

6 CONCLUSIONS AND RECOMMENDATIONS

The QIM developed for whole raw arctic charr consists of 10 parameters grouped in four main categories, resulting in a total of 21 demerit points. The scores for quality attributes in the QIM scheme increased differently with storage time in ice, but a linear relationship with high correlation was found between QI and storage time in ice.

Based upon the sensory evaluation of raw and cooked fish, the storage time of the arctic charr may be predicted as approximately 14-17 days in ice. Comparing this result with studies developed for other species of the same family it was seen that the shelf life of arctic charr is similar to rainbow trout (15-16 days) (Chytiri 2004) and less than that of salmon (20 days) (Sveindóttir *et al.* 2002).

The results of QDA do not give significant information for the determination of arctic charr shelf life, although the scores for negative attributes increased. This may be because the panellists were not very familiar with the use of QDA methods for arctic charr and needed more training and also that it was necessary to evaluate the cooked fish on days 17 and 21.

Odour was the parameter that defined spoilage of fish.

Total volatile nitrogen and pH measurements are useless as a spoilage indicator for arctic charr.

On the other hand the total viable counts and H₂S – producing bacteria values at the end of storage time did not reach very high values.

Recommendations:

- The panel needs more time for training in the use of the QDA method for arctic charr (perhaps 10-15 hours) in order to understand the meaning of the attributes (Murray *et al.* 2001) and also needs more training in the use of line scales.
- To repeat the study using the QIM scheme, QDA and microbiological methods (total viable counts and H₂S producing bacteria) from day zero (when the fish are slaughtered); to analyze more storage days (e.g. 0, 3, 5, 8, 10, 15, 17, 21); to use 10 panellists in each sensorial analysis session for the whole raw fish and cooked filleted fish; to use larger quantities of the samples than used in this experiment; and, in the case of microbiological analysis to use four samples instead of two.

To develop the aforementioned methods for Cuban species of fish, it would be necessary to keep in mind the above recommendations.

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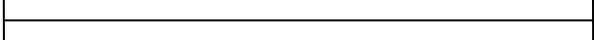
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APPENDICES

Appendix 1: Scorecard for artic charr.

Name: _____ Date: _____ Code: _____

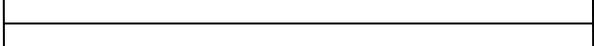
Odour

Characteristic for species 

Week Strong

Metallic 

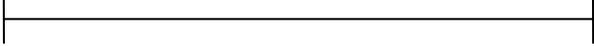
Week Strong

Oil 

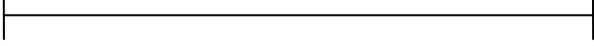
Week Strong

Musty/mould 

Week Strong

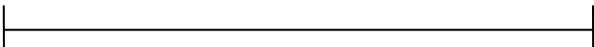
Sour 

Week Strong

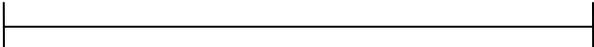
Rancid 

Week Strong

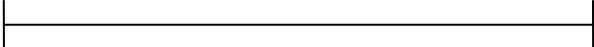
Flavour

Characteristic for species 

Week Strong

Metallic 

Week Strong

Oil 

Week Strong

Musty/mould 