COMBINED APPLICATION OF MODIFIED ATMOSPHERE PACKAGING (MAP) AND SUPERCHILLED STORAGE TO EXTEND THE SHELF-LIFE OF FRESH COD (*GADUS MORHUA*) LOINS

Wang Tao
Dalian Fisheries University
Heishijiao 52, DaLian, 116023
CHINA
wtao1023@dlfu.edu.cn

Supervisors
Emilía Martinsdóttir, emilia@rf.is, Hannes Magnússon, hannes@rf.is
Kolbrún Sveinsdóttir, kolbrun@rf.is, Icelandic Fisheries Laboratories

ABSTRACT

To meet the increasing demand for value-added fresh cod loins in the EU retail market, it is important to develop new technologies and preservation methods to effectively extend the shelf-life and improve the eating quality of fresh cod loins. The aim of this study was to investigate the effects of combined application of MAP and superchilled storage on the shelf-life extension of fresh cod loins. The influence of MA packaging (50.0% CO_2-45.0% N_2 -5% O_2) and storage temperature (1.5°C or -1°C) to prolong the shelf-life of cod loins was evaluated by sensory analysis (Quality Index Method (QIM) and Quantitative Descriptive Analysis (QDA)), physical, chemical and microbial analysis. Compared with traditional chilled storage in polystyrene boxes (1.5°C), MA packaging and superchilled storage alone increased the shelf-life of cod loins from nine to 14 and 16-17 days. When combined, a synergistic effect was observed and the shelf-life might be further extended to at least 24 days. It is noteworthy that the characteristic fresh and sweet taste can be maintained up to 18 days. This could contribute to improved eating quality of fresh cod loins for consumers in distant markets. The results of multi-indicator evaluation by PCA indicated that QIM seems more feasible to evaluate the marketability of the fresh cod loins than other quality indicators, although some modifications are needed. High correlation was found between QIM and TVC, H2S-producer, *P. phosphoreum*, indicating that these microbial variables gave similar information as QI scores. TVB-N, TMA and pH gave more information about the onset of putridity and did not reflect the earlier stages of spoilage. MAP combined with superchilled storage might have an impact on the textural properties. There is a significant difference of meaty texture among superchilled MA packed cod loins and other groups after seven days of storage. Accordingly, drip losses reached levels of 4.7-5.3% after 13 days of storage.

Keywords: modified atmosphere packaging, superchilled storage, fresh cod loins, shelf-life, sensory evaluation, microbial analysis.

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1 INTRODUCTION

Cod is the most valuable seafood resource in Iceland, accounting for 40% of all Icelandic seafood export value in 2004. Export of fresh fillets from Iceland to markets in Europe and in the USA has become increasingly important in recent years, while the export of whole fish in ice has declined (Figure 1).
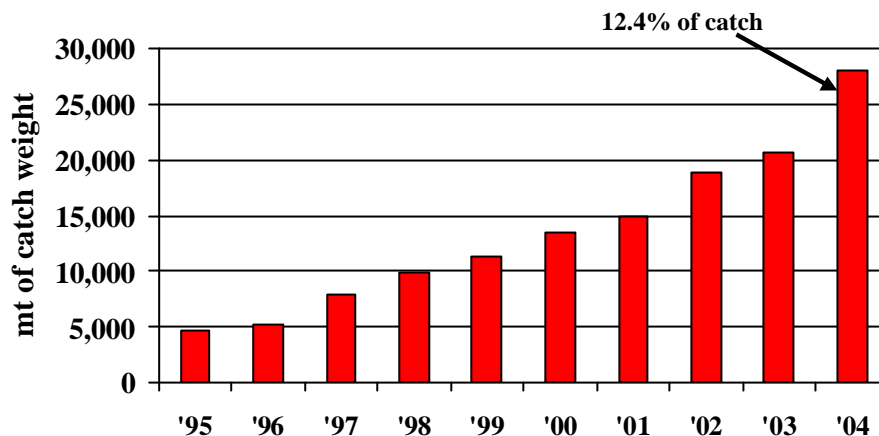
Figure 1: Disposition of the cod catch for fresh fillet processing 1994-2004 (Fisheries Directorate in Iceland 2005.

In the fish sector, as well as in other food sectors, there is a demand for fresh and conveniently packaged boneless fish products. This explains the increasing demand for packaged fresh fish fillets in many European countries. At the same time this drives value-added fresh fillet production in Iceland such as pre-packaging fish fillets.

Fresh cod fillets have a short shelf-life which presents logistical problems during distribution and retailing. The rate of fish spoilage depends primarily on the initial quality (intrinsic factors and treatment received during fishing and handling) as well as the storage conditions under which it is kept. The most important of these are temperature, processing and atmospheric conditions during storage.

The shelf-life of fresh cod fillets can be extended by hygienic handling and processing, proper icing and control of the environmental temperature etc. Recent studies have revealed much interest in the use of superchilling to delay bacterial growth and prolong the shelf-life of chilled fish (Huss 1995, Chang *et al.* 1998).

Modification of the atmosphere within the package has been shown to significantly prolong the shelf-life of perishable food products at chill temperatures (Parry 1993). Sale of fresh fish products in modified atmosphere packaging (MAP) is increasing on the European retail market. The popularity of these products is related to the fact that conveniently packed fresh fish can be sold from chill cabinets e.g. in supermarkets in the same way as other food. However, the shelf-life of fresh MAP seafood is very short in comparison with that of fresh MAP meat products (Farber 1991, Dalgaard 1995a) and a European consumer study reported problems with off-flavors in fresh MAP seafood in

retail (Anonymous 1995). These off-flavors most likely resulted from spoilage bacteria having reached high numbers due to inappropriate time-temperature storage conditions, the use of insufficiently fresh raw material before packaging, or insufficient hygienic practice prior to MA packaging (Bøknæs *et al.* 2001).

The combined effect of superchilling under modified atmospheres as a potential method to extend the shelf-life of Atlantic mackerel (*Scomber scombrus L.*) fillets, smoked blue cod, and Atlantic salmon (*Salmo slar*) fillets have been reported (Hong *et al.* 1996, Penney *et al.* 1994, Sivertsvik *et al.* 2003).

At present there is limited data available in the literature documenting the effects of combined application of modified atmosphere packaging (MAP) and superchilled storage (-1°C) on the shelf-life of fresh cod loins. Also little information is available about whether the QIM scheme developed for fresh cod fillets is suitable for cod loins stored in MA.

A comprehensive study is needed to determine the characteristic spoilage pattern and identify freshness and quality indicators of fresh cod loins stored in MAP at superchilled temperatures. In this project, quality change and shelf-life of fresh cod loins stored under four different conditions was investigated by sensory, chemical, microbiological and physical methods.

In China, it is a common practice to assess the freshness of fish by sensory methods. But well defined methods such as QIM or QDA have not been introduced in the Chinese fisheries sector to date. General limits of TVB-N concentration and total viable counts (TVC) are often used as an index to assess the keeping quality and shelf-life of different kinds of seafood products. Little research has been carried out regarding different spoilage patterns of fish products under different storage conditions. More knowledge on specific spoilage organisms (SSO) and the development of objective and reliable multi-quality indicators for a number of Chinese fish species are needed to monitor and study the quality of seafood in China.

2   LITERATURE REVIEW

2.1   Shelf-life of fish

The shelf-life of food is defined as the maximum length of time that a given product is fit for human consumption. For fish, it is the time from when the fish is caught until it is no longer fit to eat (Huss 1995).

Most fish are caught in nets or with lines with baited hooks. Hence it is difficult to control the initial quality of the raw material with any degree of repeatability. The stress and mechanical damage caused during capture, the structure and composition of the fish, pH and storage temperature prior to landing all influence the spoilage rate of the fish (Church 1998). The spoilage of fresh fish is a rather complex process and is caused by microorganisms and a number of physico-chemical mechanisms, some of which are inter-related and may affect one another. Among the most important degradation mechanisms, microbial spoilage and the biochemical degradation of non-protein nitrogen (NPN) compounds and proteins, with the subsequent formation of a variety of products such as hypoxanthine (Hx), trimethylamine (TMA), should be highlighted (Church 1998, Piñeiro *et al.* 2004).

It is well documented that packed fillets spoil more rapidly and have different spoilage patterns than whole fish (Lindsay *et al.* 1986, Huss 1995, Lauzon *et al.* 2002). Consequently, many studies focus on maintaining the freshness of fish fillets by optimal handling, storage and transport conditions as well as packaging methods to ensure the high quality of the products on the market.

2.2   Specific spoilage organisms (SSO)

Spoilage of fish products can be caused by both chemical reactions and physical damage. However, the major cause of fish spoilage is microbial growth and metabolism resulting in the formation of amines, sulfides, alcohols, aldehydes, ketones, and organic acids with unpleasant and unacceptable off-flavours (Gram and Dalgaard 2002a).

Each food product has its own unique flora, however, some studies showed that seafood products can be categorised into groups with similar microbial ecology (Gram and Huss 2000, Gram and Dalgaard 2002a). During storage, the microflora changes owing to different abilities of the microorganisms to tolerate the preservation conditions.

Previous studies have shown that no direct correlation has been found between the total number of microorganisms and the degree of spoilage. A large part of the bacteria present in spoiled fish plays no role whatsoever in the spoilage. Only a fraction of the total microflora-specific spoilage organisms (SSO), participates in the spoilage process which is responsible for producing the off-odours and off-flavours in the spoiled fish. Each fish product will have its own specific spoilage bacteria and the number of these, as opposed to the total number, will be related to the shelf-life and can be used as objective indices of

spoilage in shelf-life de terminations (Huss 1995, Church 1998). The specific spoilage bacteria for cod stored at different temperatures is given in Table 1.

Table 1: Specific spoilage bacteria for cod and bacterial spoilage compounds (Church 1998).

Storage temp.	Pack atmosphere	Specific spoilage bacteria	Spoilage compounds
0°C	Aerobic	*S. putrefaciens* *P.seudomonas*	TMA, H_2S, HX, CH_3SH, ketones, esters Aldehydes
0°C	Vacuum	*S. putrefaciens* *P. phosphoreum*	TMA, H_2S, HX, CH_3SH, ketones, esters TMA, HX
0°C	MAP	*P. phosphoreum*	TMA, HX
5°C	Aerobic	*S. putrefaciens*	TMA, H_2S, HX, CH_3SH, ketones, esters
5°C	Vacuum	*S. putrefaciens* *Aeromonas spp*	TMA, H_2S, HX, CH_3SH, ketones, esters -
5°C	MAP	*Aeromonas spp*	-

The most relevant SSOs in aerobically stored and vacuum-packed marine fish species are sulphide-producing bacteria (SPB), mainly *Shewanella putrefaciens* in cold water species like cod, and Vibrio sp. in species from tropical waters (Jørgensen and Huss 1989, Gram *et al.* 1987). Some sulphide-producing *Aeromonas* and *Enterobacteriaceae* spp. may occasionally be present (Gram *et al.* 1987).

The specific spoilage organism for MA-packaged cod and salmon has been found to be *Photobacterium phosphoreum* (Dalgaard *et al*. 1993, Emborg *et al*. 2002). A good correlation was found between this SSO and the remaining shelf-life of MAP cod fillets. *P. phosphoreum* is more CO_2-tolerant than the specific spoilage bacteria observed for air stored temperate fish, *Shewanella putrefaciens* (Dalgaard *et al*. 1997a, Devlieghere and Debevere 2000).

2.3 Extension of shelf-life by superchilling

The extension of shelf-life of chilled fish fillets is of importance to allow the transport of products to distant markets at a lower cost (Ólafsdóttir *et al.* 2005). Superchilling has proven to effectively delay bacterial growth and prolong the shelf-life of chilled fish and fish products (Huss 1995, Chang *et al.* 1998).Various types of cooling systems have been used for superchilling (-4 to 0°C) of seafood products including chilled and refrigerated seawater (Smith *et al.* 1980, Ólafsdóttir *et al.* 2000), liquid-ice and brine solutions (Huidobro *et al.* 2000), flake ice or slurry ice (Losada *et al.* 2005, Zeng *et al.* 2005), subzero temperature during storage (-2 and -3°C) (Riza Fatima *et al*. 1988, Sivertsvik *et al.* 2003) and the use of cooling agents like CO_2 snow (LeBlanc and LeBlanc 1992, Jeyasekaran *et al.* 2004).

According to a pioneer report by Ólafsdóttir *et al.* (2005), by using a new CBC (Combined Blast and Contact) technique followed by superchilled storage (-1.5°C), the sensory shelf-life of the cod fillets could by extended for at least three days compared to the traditional process, resulting in a shelf-life of 15 days. Such a gain in shelf-life is of high economical value since it would allow the distant transportation of fresh fillets by ship or truck, which is less costly compared to air freight.

2.4 Modified atmosphere packaging (MAP)

Modified atmosphere packaging (MAP) is an increasingly popular food preservation technique. Consumer demands for fresh and convenient foods free of chemical preservation has led to growth in the use of MAP and this technique may also reduce wastage and significantly prolong the shelf-life of a range of seafoods at chill temperatures (Farber 1991, Haard 1992, Parry 1993, Church 1998).

On the other side, some studies show that MAP can affect the quality of the product, mainly owing to CO_2 dissolved in the muscle tissue, which is associated with an increase of carbonic acid (Sivertsvik *et al.* 2004). A greater loss of water-holding capacity of muscle protein occurs at lower pH values. Randell *et al.* (1997) reported that the shelf-life of rainbow trout and Baltic herring fillet packed in modified atmosphere package (35% CO_2) was limited by the excessive drip formed during storage, that on the other hand was lower when the fish was not packed in a modified atmosphere package. MAP used in retail packs is still an expensive technique and it can not replace good chilling or good hygienic production conditions (Huss 1995). In addition, outgrowth and toxin production of *Clostridium botulinum* is increased under anaerobic conditions which may cause problems for the safety of packed fish (Church 1998, Huss 1995, Sivertsvik *et al.* 2002).

The effects of modified atmosphere packaging on the shelf-life extension of fish products and the most suitable gas composition for a specific application depends extensively on the fish species, fat content, initial microbiological contamination, background of the fish, the treatment the fish undergoes after slaughtering, such us handling and storage conditions, the ratio of gas volume to product volume (G/P), and most importantly the packaging method and storage temperature (Stammen *et al.* 1990, Bøknæs *et al.* 2000, Sivertsvik *et al.* 2002).

The MAP gas mixtures usually consist of normal air gases: carbon dioxide (CO_2), nitrogen (N_2) and oxygen (O_2). CO_2 is the most important gas in the field of MAP technology. Microorganisms, in particular aerobic bacteria, are strongly affected by CO_2. CO_2 inhibits microbial activity by effectively dissolving into the food's liquid phase, thereby reducing its pH and causing changes in permeability and function. Nitrogen is an inert gas; it is primarily used to replace oxygen in packaging and thereby prevents oxidation. Owing to its low solubility in water, N_2 also helps to prevent package collapse by maintaining internal volume. For most foodstuffs, the package should contain as little oxygen as possible to retard the growth of aerobic microorganisms and reduce the degree of oxidation. Several studies have shown that the limiting factor for the shelf-life of fresh MAP cod products is *Photobacterium phosphoreum* growth and trimethylamine (TMA)

production (Dalgaard *et al.* 1993, Dalgaard 1995a). A mixture of $30\%O_2$–$40\%CO_2$–$30\%N_2$ or 40–60% of CO_2 and 60% N_2 has been proposed, respectively, for low fat fish and for fatty fish (Pastoriza *et al.* 1998, Woyewoda *et al.* 1984). Debevere and Boskou (1996) proposed a mixture of $40\%O_2$–$60\%CO_2$ as the most effective for the inhibition of the normal TMA- and H_2S-producing flora *(Shewanella putrefaciens)* but less effective on the inhibition of the growth and activity of the TMAO-reducing large cells of *Photobacterium phosphoreum,* which are not H_2S-producing, and show high resistance against 60% CO_2. In order to decrease the TMAO-reduction by *P. phosphoreum,* O_2 can be added in the packaging atmosphere. The reduction of the rate growth of *P. phosphoreum* by oxygen has been reported (Dalgaard 1995b, Guldager *et al.* 1998). But the organism still dominated the spoilage microflora of cod fillets stored in oxygen containing modified atmospheres (Dalgaard *et al.* 1997b).

2.5   Novel technologies of MAP combined with superchilling

MAP can be combined with superchilling to further extend the shelf-life and safety of fresh fish. The increased shelf-life seems to be increased dissolvement of CO_2 at the superchilled temperature and the suppression of SSOs in the product (Sivertsvik *et al.* 2003).

A typical shelf-life extension of about seven days is obtained for superchilled MAP fish as compared with traditional ice stored fish of the same type (Sivertsvik *et al.* 2002). An acceptable sensory shelf-life of 21 days was observed for mackerel at -2°C in 100% CO_2 (Hong *et al.* 1996). For smoked blue cod a doubling of shelf-life was gained when lowering the storage temperature from 3 to -1.5°C (Penney *et al.* 1994). Recent studies showed that the importance of this technique is the potential to extend the period of prime quality in fish. Fresh Atlantic salmon (*Salmo salar*) fillets packaged under modified atmosphere (MA) were stored in superchilled (-2°C) and chilled (4°C) conditions, and the results show that superchilled salmon stored at -2°C had a 21 day sensory shelf-life. The synergistic effect was observed giving an additional effect on the microbial and sensory quality of salmon fillets. The shelf-life extension was at least 2.5 times that of traditional chilled MA salmon and at least 3.5 times that of chilled storage exposed to air (Sivertsvik *et al.* 2003). Lauzon and Martinsdóttir (2005) reported that the spoilage microflora was better controlled and the characteristic "fresh fish taste" can be maintained longer by superchilling the fillets during processing (a new technique called combined blast and contact freezing, CBC) in combination with MAP and superchilled storage.

2.6   Methods to evaluate fish freshness

Freshness is the most important attribute when assessing the quality of fish or fishery products. Most of the methods that have been used to estimate the quality of fresh fish measure or evaluate parameters that change, disappear or are formed during deterioration of fish. These methods may be divided into several groups such as sensory, physical, chemical and microbiological methods.

2.6.1   Sensory evaluation

Sensory evaluation is an important method for the assessment of freshness and quality, and is commonly used in the fish sector and fish inspection services (Luten and Martinsdóttir 1997). There has been a trend to standardise sensory evaluation to make it an objective measurement to assess freshness (Ólafsdóttir *et al.* 1997). The Quality Index Method (QIM) is a promising method to measure the freshness of whole fish stored in ice, and is both rapid and reliable (Martinsdóttir *et al.* 2001). To evaluate sensory attributes of cooked fish, it is common to evaluate cooked fillets by Torry schemes (Martinsdóttir *et al.* 2001, Huss 1995). In research, Quantitative Descriptive Analysis (QDA) is used for cooked fillets to establish a detailed description and quantify product sensory aspects (Stone and Sidel 1985).

2.6.1.1   Quality Index Method (QIM)

The Quality Index Method (QIM) is a grading system for estimating the freshness and quality of seafood, which has been demonstrated to be relatively fast, objective and non-destructive for many fish species (Martinsdóttir 2002). In addition, the QIM is usable in the first part of the storage period where other instrumental methods are inaccurate (Nielsen *et al.* 1992).

The method is based upon a scheme originally developed by the Tasmanian Food Research Unit (CSIRO). The technique is based on selecting a number of significant sensory parameters (skin, eyes, gills, etc) characteristic for a particular species and allocating scores to each attribute depending on the state of freshness or quality of the selected fishery products (Martinsdóttir 2002, Sveinsdóttir *et al.* 2003). The score zero is given for very fresh fish and an increasingly higher score as the fish deteriorate. The scores for all the characteristics are then added to give an overall sensory score, the so-called Quality Index. As no excessive emphasis is laid on a single attribute a sample cannot be rejected on the basis of a single criterion and minor differences in results for any of the criteria do not unduly influence the total QIM score (Luten and Martinsdóttir 1997). The aim is to achieve a linear correlation between the sensory quality expressed as the sum of demerit scores (QI) and storage life in ice, which makes it possible to predict the remaining storage life (Nielsen and Jessen 1997, Hydilg and Nielsen 1998, Martinsdóttir *et al.* 2001).

QIM was primarily used for the evaluation of whole and gutted fish. Up to now, QIM schemes have been developed for a number of fish species including fresh herring and cod (Jonsdottir 1992, Larsen *et al.* 1991), red fish (Martinsdottir and Arnason 1992), Atlantic mackerel, horse mackerel and European sardine (Andrade *et al.* 1997), brill, dab, haddock, pollock, sole, turbot and shrimp (Luten 2000), gilthead seabream (Huidobro *et al.* 2000), frozen cod fillets (Warm *et al.* 1998), farmed Atlantic salmon (Sveinsdottir *et al.* 2003) and fresh cod fillets (Bonilla *et al.* 2006).

Further research is needed to evaluate the applicability of the QIM for fish stored under different conditions such as frozen-thawed fish, storage in ice slurry, temperature abuse during storage, etc. Furthermore, new preservation techniques (superchilling, VP, MAP) extend the shelf-life of fish, alter the spoilage pattern and this has to be taken into account when using the QIM evaluation (Martinsdóttir 2002).

2.6.1.2   Quantitative Descriptive Analysis (QDA)

Quantitative Descriptive Analysis (QDA) can be used on cooked fish samples to determine the maximum storage time in addition to giving a detailed description of the sensory profile of the fish. The method involves detection and description of the qualitative and quantitative sensory aspects of a product by a trained panel of 10–12 people (Stone and Sidel 1985). With the QDA, all detectable aspects of a product are described and listed by a trained panel under the guidance of a panel leader. The list is then used to evaluate the product and the panelists quantify the sensory aspects of the

product using an unstructured scale. The panelists are trained in using an unstructured scale for each of the attributes, before participating in the sensory analysis of the product. The words used to describe odour and flavour of the fish can be grouped into 'positive sensory parameters' and 'negative sensory parameters', depending on whether they described fresh fish or fish at the end of the storage period (Martinsdóttir 2002, Sveinsdóttir *et al.* 2002).

Applications of QDA are very wide. The method has been applied to the mapping of consumer preferences (Helgesen *et al.* 1997) and for relating sensory textural attributes to instrumental measurements (Reyes-Vega *et al.* 1998). Other applications include its use in measuring shelf-life of products without dependence on standards or control products (Sveinsdóttir *et al.* 2003, Bonilla *et al.* 2006, Magnússon *et al.* 2006).

2.6.2 *Physical and chemical analysis*

Besides sensory methods, chemical (biochemical), physical and microbiological analyses are also used to assess the freshness quality of fish.

Compared with microbiological methods, which are slow, physical and chemical analyses may be significantly faster. Chemical (biochemical) and physical methods measure the concentrations of breakdown products from bacterial or enzymatic activity. However, for some compounds measurable concentrations are not present until close to spoilage (Gram *et al.* 2002a). Classical single-compound quality index (SCQI) for seafood includes measurements of total volatile basic nitrogen (TVB-N), trimethylamine (TMA) and the formation of biogenic amines (Botta *et al.* 1984, Hebard *et al.* 1982, Mietz and Karmas 1978), whereas nucleotide degradation product ratios (such as hypoxanthine, ratios between ATP degradation products (K values), Ki values) have been used as freshness indicators (Saito *et al.* 1959, Karube *et al.* 1984, Luong and Male 1992, Dalgaard 2000b). Multiple-compound quality indices (MCQI), in which combinations of several metabolites are identified by statistical methods, have recently been introduced and correlate better with sensory properties and/or shelf-life in some products (Jørgensen *et al.* 2000, Jørgensen *et al.* 2001, Leroi *et al.* 2001).

2.6.2.1 <u>Total volatile basic nitrogen (TVB-N)</u>

Total volatile basic nitrogen (TVB-N) is one of the most widely used measurements of seafood quality. It is a general term which includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia and other volatile basic nitrogenous compounds associated with seafood spoilage. Although TVB-N analyses are relatively simple to perform, they generally reflect only later stages of advanced spoilage and are generally considered unreliable for the measurement of spoilage during the first 10 days of chilled storage of cod as well as several other species (Huss 1995). The concentration of TVB-N in freshly caught fish is typically between 5 and 20 mg N/100 g muscle, whereas levels of 30–35 mgN/100 g muscle are generally regarded as the limit of acceptability for ice-stored cold water fish (Huss 1988a, Connell 1995).

2.6.2.2 <u>Trimethylamine (TMA)</u>

Among chemical indexes used to evaluate the freshness of fish, the most commonly used is TMA production; it is produced during chilled fish storage. This compound is found in very low levels in fresh fish, and its formation is associated with bacterial spoilage (Fernandez-Salguero and Mackie 1987). TMA is formed from enzymatic decomposition and bacterial use of TMAO, naturally occurring osmo-regulating substance found in most marine fish species (Koutsoumanis and Nychas 1999). The quantity and presence of this compound depends on the species, size, sex, station of year, etc. (Tsigarida *et al.* 2003). Some studies have shown excellent correlation between TMA level and eating quality (including iced cod) (Wong and Gill 1987).

2.6.2.3 <u>Hypoxanthine</u>

The development of TMA is in many fish species parallel to the production of hypoxanthine. Hypoxanthine can be formed by the autolytic decomposition of nucleotides, but it can also be formed by bacteria and the rate of bacterial formation is higher than the autolytic. Both Jorgensen *et al.* (1988) and Dalgaard *et al.* (1993) showed a linear correlation between the contents of TMA and hypoxanthine during iced storage of packed cod.

2.6.2.4 <u>K-value</u>

For fish, shellfish, crustaceans and cephalopods, shortly after death, ATP is rapidly degraded to inosine monophosphate (IMP) by endogenous enzymes (autolysis). The further degradation of IMP to inosine and hypoxanthine is much slower and is catalysed mainly by endogenous IMP phosphohydrolase and inosine ribohydrolase with a contribution from bacterial enzymes as storage time increases. The degradation of ATP was found to parallel the perceived loss of freshness of fish as determined by trained analysts (Gill 1995). Therefore, the K-value gives a relative freshness rating based primarily on the autolytic changes which take place during *post mortem* storage of the muscle.

A shortcoming of the K-value as a freshness index is that it varies between species owing to differences in rates of ATP degradation. It also varies with postmortem time and temperature, storage conditions, handling conditions and method of kill (Ólafsdóttir *et al.* 1997). The K-value has been reported as not being a suitable predictor of shelf-life for CO_2 packed refrigerated striped bass strips (Handumrongkul and Silva 1994).

Several studies suggested that none of these chemical indicators that include total base nitrogen (TVB-N), biogenic amines, trimethylamine (TMA), dimethylamine (DMA), K value, etc., are universally applicable (Gill 1990, Botta 1995).

2.6.2.5   Physical parameters

Physical changes in fish that result in the decline of freshness are mainly related to structure and colour. Texture is a very important property of fish product whether it is raw or cooked. Texture measurements can be used to determine structural changes. Comparisons of texture measurements of fish with sensory analysis have shown good correlation in some cases (Ólafsdóttir *et al.* 1997). Changes in fish freshness can also be determined by measuring the microstructural characterisation of the fish muscle, change in electrical properties, changes in colour and pH etc. During the past few years, spectroscopic methods and electronic noses have been introduced as alternative rapid techniques to supplement or replace traditional quality control techniques in the food industry.

2.6.3   Microbiological methods

The activity of microorganisms is the main factor limiting the shelf-life of fresh fish. Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms varies enormously and Liston (1980) states a normal range of 10^2-10^7 cfu/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g. When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. On the skin surface, the bacteria, to a large extent, colonise the scale pockets. During storage, they invade the flesh by moving between the muscle fibers. Murray and Shewan (1979) found that only a very limited number of bacteria invaded the flesh during iced storage.

2.6.3.1   Total viable counts (TVC)

An estimation of the total viable counts (TVC) is usually used as an acceptability index in standards, guidelines and specifications (Ólafsdóttir *et al.* 1997). But this figure is seldom a good indicator of the sensorial quality or expected shelf-life of the product. In ice-stored Nile perch, the total count was 10^9 cfu/g for days before the fish was rejected (Gram *et al.* 1989) and in lightly preserved fish products high counts prevail for a long time before rejection. At the point of sensory rejection, the TVC of fish products are typically 10^7-10^8 cfu/g. Nevertheless, standards, guidelines and specifications often use much lower TVC as indices of acceptability (Ólafsdóttir *et al.* 1997).

2.6.3.2   Specific spoilage organisms (SSO)

Considering the contribution of specific spoilage organisms (SSO) to developing microbial metabolites causing spoilage of fish, the detection of SSO like *Shewanella putrefaciens, Photobacterium phosphoreum* and *Pseudomonas* ssp. is regarded more reliable than total viable counts (TVC) to accurately evaluate the freshness or spoilage level of fish and fish products (Dalgaard 2000a, Gram *et al.* 2002b).

When stored aerobically, levels of 10^8-10^9 cfu/g of specific spoilage bacteria (*Shewanella putrefaciens*) in the flesh are normally required to cause spoilage of ice-stored fish. A

much lower level (10^7CFU/g) of P. *phosphoreum* is needed to spoil chilled fish packed in modified atmosphere (Gram and Huss 1996).

Shewanella putrefaciens is a well-known fish spoilage bacterium that produces intensive off-odours, TMA and H_2S and this organism was found responsible for spoilage of both packed and unpacked cod stored under aerobic conditions (Gram *et al.* 1987). This microorganism can be enumerated in iron containing agar and correlation coefficients as high as -0.97 were achieved when comparing log numbers of *S. putrefaciens* with the remaining shelf-life of aerobically stored fish, as determined by sensory evaluation. Owing to the selection of microorganisms in chilled fish, the correlation between SSO and freshness is usually higher than between TVC and freshness (Gram *et al.* 1987, Ólafsdóttir *et al.* 1997).

In CO_2-packed fish, the growth of *S. putrefaciens* and of many other micro-organisms found in live fish is strongly inhibited. In contrast, *Photobacterium phosphoreum* was shown to be highly resistant to CO_2 (Dalgaard 1995a). It was also shown that the growth of this bacterium corresponds very well with the shelf-life of modified atmosphere packed (MAP) cod fillets (Sivertsvik *et al.* 2002).

3   MATERIALS AND METHODS

3.1   Sample preparation

Cod (*Gadus morhua L.*) was caught with long line and bought directly from the boat which landed at Sandgerði, Iceland on the 28 November 2005 and stored in isothermic boxes containing flake ice. The whole cod arrived at Nýfiskur Company at 14:00 in the afternoon of 29 November. Six random samples were selected during weighing of the fish and evaluated with the QIM scheme for whole cod. All six cod had a QIM score between 0 and 2 (score 1 was given for appearance-skin, texture, gills-odour for three cod fish). After assessment, 25 cod of medium size were selected, gutted, washed and placed in polystyrene boxes with ice and transported to the Icelandic Fisheries Laboratories (IFL) for a storage study. The average weight and length of the whole cod samples were 2.90±0.48 kg and 69.48±3.96 cm, respectively.

The rest of the lot was gutted and headed and stored for further processing the following day. The lot was processed at 14:30 on 30 November (two days from catch). Fresh cod loin samples were taken directly from the processing line and placed in foam polystyrene boxes with false bottoms to allow for drip and transported to IFL. The boxes were stored in a chilled storage chamber at 1 to 2°C before packaging.

As to the cod chain study (part of the SEAFOODplus project), the fresh cod loins from the same batch were transported by air-cargo at 15:00 the same day to Belgium. In Belgium the cod loins were transported to Fjord Seafood Pieters in Brugge and collected by RIVO on 1 December and transported by car to the laboratory. The samples were stored at 0°C in original polystyrene packaging with ice until sensory assessment. The

QIM scheme for fresh cod loins and QDA for cooked loins used in RIVO was the same or slightly modified as in IFL.

3.2   Experimental design

A total of 260 fresh cod bins were used in this experiment. The samples were randomly divided into four sample groups and kept under different storage conditions on 1 December (three days from catch) (Table 2).

For sample groups M1 and M2 (stored in modified atmosphere), two to three cod bins (around 500 g) were placed on trays (expended polystyrene, E39-34 (LINSTAR 3536)) with built-in absorbent drip pads, each tray was put into a vacuum bag (55PA/60LDPE, 25×32. 5 cm×115 cm, Plastprent: 082020135). A gas mixture of 50.0% CO_2-45.0% N_2 - 5% O_2 was injected to modify the atmosphere and the bag was heat-sealed by Henkovac, Heavy Duty 2000.

At the beginning of the experiment (day 0), eight fresh cod loins were randomly selected for analysis. Three loins were used for sensory evaluation of fresh loins (QIM). Four to six loins were used for sensory evaluation of cooked loins (QDA). Three bins were used for microbiological analyses including numbers of total viable psychrotrophic count (TVC), hydrogen sulfide-producing bacteria, *P. phosphoreum* and Lactic acid bacteria; physical and chemical analysis including pH, TVB-N, TMA. All the microbiological, physical and chemical analyses were carried out in triplicate.

Table 2: Experimental groups and storage conditions.

Group name	Storage temperature	Storage method
A1	at 1.5°C	In foam polystyrene boxes aerobically (with false bottom)
A2	at -1°C	In foam polystyrene boxes aerobically (with false bottom)
M1	at 1.5°C	On trays with built-in absorbent drip pads, in vacuum bags, MA packed.
M2	at -1°C	On trays with built-in absorbent drip pads, in vacuum bags, MA packed.

During the storage trial, samples were taken from the four different groups of cod bins stored under different conditions at predetermined time intervals namely: 1, 4, 7, 11, 13, 15, 18 and 21 days after packaging (experimental design in Appendix I). The samples were submitted to sensory, microbiological, physical and chemical analysis.

For MAP group, on each sampling day, three separate packs of cod loins from each batch were analysed for QIM, pH and all the chemical and microbiological parameters, two separate packs from each batch were used for evaluation of drip loss and QDA. Gas composition of the modified atmosphere was determined for all five packs.

3.3 Temperature profile

Temperature data loggers were used during all periods of chill and superchilling storage (Stow Away®, Onset Computer Corporation, USA) to monitor the temperature of the ambient storage environment. The loggers were located in two cold chambers to follow the environment variations of each storage condition. Temperature recordings were at 3 minutes intervals.

3.4 Gas composition

For all packs from MA storage in this study, before the packs were opened for further analyses the gas composition in the headspace was analysed by a gas analyser (PBI, Dansensor, Checkmate 9900, Denmark) by penetrating a needle through a gas-tight septum.

3.5 Sensory evaluation

3.5.1 Sensory panels

QIM: Nine to 12 trained panelists of the Icelandic Fisheries Laboratories' sensory panel participated in the evaluation of whole cod and cod loins with the QIM scheme. The members of the panel were all trained according to International Standards (ISO 1993) before and familiar with the QIM scheme and experienced in sensory evaluation of cod.

QDA: Nine to 12 panelists of the Icelandic Fisheries Laboratories' sensory panel participated in the QDA of the cooked samples. The members of the panel were previously trained in QDA for different cod products. The descriptors developed by Bonilla *et al.* (2006) for fresh cod fillets and Magnússon *et al.* (2006) for desalted cod fillets were used as a basis for this experiment.

Prior to the shelf-life study one session was held for training by using cod loin samples with different storage times in ice. The list of descriptors was adapted to describe fresh and MA packed loins during the storage trial and the intensity of each attribute for a given sample was discussed under the guidance of the panel leader.

3.5.2 Quality index method (QIM) for whole gutted cod

The evaluation was carried out under standardised conditions at room temperature using fluorescent light and a table provided with white covering. Samples were transferred from the cold chambers and placed on the table for 30 minutes before the evaluation

The quality index method (QIM) for whole cod (Bremner 1985, Martinsdóttir *et al.* 2001) was used in the evaluation of whole gutted cod. Sensory attributes relating to the gills, eyes, skin and intestinal cavity were evaluated and a quality index score was given for all the attributes assessed. The quality index score was at 0 for very fresh fish and 23 for very spoiled fish with an assumed rejection level at 17-18 (Martinsdóttir *et al.* 2001).

3.5.3 Quality index method (QIM) for fresh cod loins

The methodology and QIM scheme used for assessing the raw cod loins was based on the method described by Martinsdóttir *et al.* (2001), Martinsdóttir (2002), Sveinsdottir *et al.* (2003) and Bonilla *et al.* (2006).

Nine sessions were carried out lasting from 10 to 40 minutes each. The panelists assessed three cod loins from four different storage groups (A1, A2, M1 and M2) in each session during the shelf-life study. Each sample was coded with a random three-digit number unrelated to storage time and group. The cod loins were served in a random order and evaluated individually.

3.5.4 Quantitative descriptive analysis (QDA) of fresh cod loins

The QDA method, introduced by Stone and Sidel (1985), was used to evaluate the cooked cod loins in parallel to the QIM sessions. Samples weighing about 40–50 g were taken from the cod loins and placed in aluminum boxes coded with three-digit random numbers. The samples were cooked at 95 to 100°C for seven minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) with air circulation and steam, and then served to the panelists.

All sample observations were conducted according to International Standards (ISO 1988). The panelists evaluated the samples without information about the storage times and groups. Each sample was evaluated randomly in duplicate. A computerised sensory registration system (FIZZ Network, Version 2.0, 1994-2000, Biosystèmes, Couternon, France) was used for data recording and for further processing. Average scores of the judges were calculated for each sample from different storage groups and the reported values were the average of the duplicate samples.

3.6 Physical and chemical measurements

3.6.1 pH measurement

The pH was measured by mixing 5 g of mince with 5 mL of deionised water. The pH meter was previously calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.00 ± 0.01 (20°C) (Radiometer Analytical A/s, Bagsvaerd, Danmark).

3.6.2 Drip loss

The drip loss of the samples was measured by gravimetric method. Each raw cod loin was weighted before packaging and at each storage time, after it was removed from the package. The difference in weight (? g) was divided by the initial weight of the product (g) and expresses as ? g /g%.

3.6.3   TVB-N and TMA measurement

Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) were determined using steam distillation in the minced cod tissue, followed by the titration method (AOAC 1990). The TVB-N was performed through direct distillation into boric acid using a Kjeldahl-type distillator (Struer TVN distillator) (Malle and Poumeyrol 1989), the acid was titrated with diluted H_2SO_4 solution. To determine TMA the same method was used as for TVB-N but adding 20 ml of 35% formaldehyde to the distillation flask to block the primary and secondary amines, an alkaline binding mono-and di-amine, TMA being the only volatile and measurable amine (Malle and Poumeyrol 1989). The TVB-N and TMA content was expressed in mgN/100 g cod tissue.

3.7   Microbiological analysis

Pieces of flesh were aseptically taken from cod loins and comminuted in a mixer, 25 g of minced flesh were homogenized in 225 mL of cooled Maximum Recovery Diluent (MRD, Oxoid) for 1 minute in a stomacher (Lab. Blender, London, UK) to make 1/10 dilution. Further decimal dilutions were made and then 0.1 ml of each dilution was transferred with pipettes onto the surface of Petri plates.

Total viable psychrotrophic count (TVC) was evaluated by spread-plating aliquots on pre-chilled plates of Modified Long and Hammer's medium (LH) containing 1% NaCl (Van Spreekens 1974), incubated aerobically for five days at 15°C.

TVC and selective counts of H_2S-producing bacteria were enumerated on iron agar (IA) as described by Gram *et al.* (1987) with the exception that 1% NaCl was used instead of 0.5%. Plates were surface-plated and incubated at 15°C for five days.

Analysis of lactic acid bacteria (LAB) counts was done by spread-plating aliquots on pre-chilled plates of NAP (Nitrite-Actidione-Polymyxin) medium. The medium was prepared according to Davidson and Cronin (1973). Plates were incubated at 22°C for five days under microaerophilic conditions.

Numbers of *Photobacterium phosphoreum* were enumerated specifically by a conductance methods described by Dalgaard *et al.* (1996).

3.8   Statistical analysis

In order to identify the effects of various storage treatments on the measured quality attributes (QDA attributes, pH, drip loss, TVB-N, TMA, TVC, H_2S-producing bacteria, *Photobacterium phosphoreum* and LAB counts) at each day of storage, one-way analysis of variance (ANOVA) was performed. Furthermore, Duncan's multiple-comparison test was used to determine the significant difference between different sample groups. Significance of differences was defined at the 5% level (p<0.05). Comparison tests were performed using the statistical programme-Number Cruncher Statistical Software (NCSS, PASS Trial 2000). All quality attributes were presented as the average of the replicates.

All data were analysed by use of multivariate techniques-principal component analysis (PCA) to identify similarities and differences amongst samples on the basis of sensory, microbiological, physical and chemical analysis. Multivariate analysis was conducted in the software Unscrambler version 9.5 (CAMO, Trondheim, Norway). Prior to the statistical analysis, a logarithmic transformation was applied to the microbial counts in order to compare values that range over several orders of magnitude (Martinsdottir et al 2001). In addition, each element in the matrix was weighted with the inverse of the standard deviation of the corresponding variables in order to compensate for the different scales of the variables. A full cross validation method was used. The data matrix consists of 25 samples (column) differing with respect to storage time and storage groups. Composition rows (variables) in the data matrix integrated by 30 quality attributes: pH, drip loss, TVB-N, TMA, TVC, H_2S-producing bacteria, *Photobacterium phosphoreum* and LAB counts, QIM and all QDA attributes. An unweighted PCA was also performed for QDA attributes to study the main tendencies in the data and to illustrate the effects of different storage conditions on the sensory quality of cod loins.

4   RESULTS AND DISCUSSION

This chapter looks at the results from the shelf-life studies of cod loins stored under four different conditions (carried out at IFL). The comparison of the results obtained from the cod-chain study (SEAFOODplus project-SEAFOOD SENSE) is shown in Appendix .

4.1   Surrounding temperature profiles during storage

The average temperature of the cold storage chamber 1, in which group A1 and M1 were stored, was 1.5°C (Figure 2). Another cold storage chamber 2, in which group A2 and M2 were stored, was -2.0°C from first sampling day (day 0) to day 4 and -1.0°C after day 4 throughout the study period. Ice crystal formation was observed in cod loins stored under superchilled temperature (group A2 and M2), because of the lower surrounding temperature during the first four days of storage. Unfortunately the temperature changes of the cod loins during storage were not measured in our study. However, influence on the texture was found in superchilled cod loins presumably due to ice crystal formation.
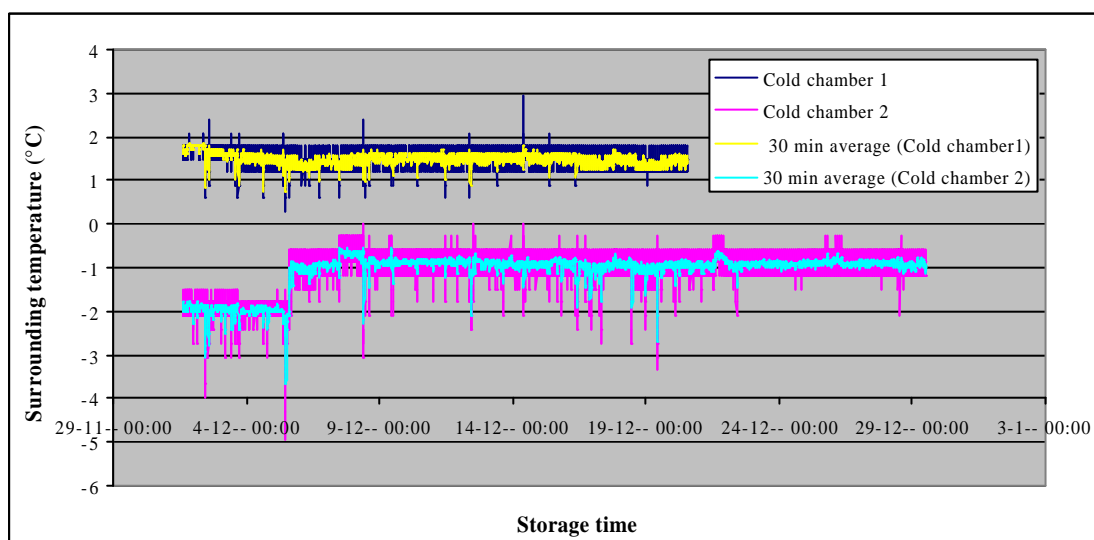
Figure 2: The surrounding temperature during the storage period [1].

4.2   Gas composition analysis

Previous experiments showed that a higher CO_2 concentration than 50% has negative effects on the textural properties in cod muscle (IFL unpublished data). Based on these studies, the composition of gas mixtures used in the present experiment was 50.0% CO_2-45.0% N_2 -5% O_2

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[1] The positive or upward spikes in the graph are mainly when the doors to the storages were opened.
The minus or downward spikes are when the cooling system starts and cold air is blown over the boxes.

Average initial gas composition after packaging was CO_2 /O_2/N_2: 50.7/4.6/44.7. After four days storage, the CO_2 level in the headspace of the chilled MA packages was reduced to 33.1%±1.8% probably as a consequence of CO_2 dissolution into the water phase of the fish muscle (Ruiz-Capillas and Moral 2001a, Sivertsvik *et al.* 2004). From day 11 on, the content of CO_2 increased again due to bacterial and enzymatic activity (Table 3). Contrary to CO_2 content, the proportion of O_2 increased up to day 4. Due to respiration of bacteria the proportion of O_2 decreased later on. Similar results were reported by Randell *et al.* (1997) that oxygen concentration decreased and carbon dioxide concentration increased during storage of MA packed rainbow trout and Baltic herring as a result of microbial metabolism. They also reported that in the MAP package, carbon dioxide concentration decreased at the early stage of storage and then remained almost constant.

Table 3: Change of headspace gas composition of the cod loins in MAP during storage.

Storage time (days)	M1			M2		
	CO_2 (%, v/v)	O_2 (%, v/v)	N_2 (%, v/v)	CO_2 (%, v/v)	O_2 (%, v/v)	N_2 (%, v/v)
0	50.7±0.2	4.6±0.2	44.7±0.2	50.7±0.2	4.6±0.2	44.7±0.2
1	37.8±2.6	6.4±0.4	55.8±2.3	37.1±1.0	6.7±0.4	56.2±0.7
4	33.1±1.8	7.2±0.7	59.6±1.1	33.1±2.1	6.7±0.3	60.2±1.8
7	33.2±1.7	6.3±0.4	60.5±1.6	33.1±0.8	6.6±0.2	60.2±0.8
11	34.5±3.1	4.7±1.8	60.8±1.7	31.6±2.0	6.7±0.3	61.7±2.0
13	36.2±1.8	3.1±1.0	60.7±1.1	29.9±3.5	8.0±1.9	62.0±1.8
15	37.6±1.4	1.7±0.3	60.6±1.3	32.5±1.6	6.3±0.9	61.3±1.1
18				32.5±1.8	4.7±0.8	62.8±2.4
21				34.0±2.3	4.4±1.2	61.6±1.5

As to group M2, CO_2 concentration decreased until day 4 and remained around 32-33% until day 18 (except on day 13). Sivertsvik *et al.* (2003) reported that superchilled MA packed salmon dissolved more CO_2 compared with the chilled salmon which resulted in a higher decrease of CO_2 concentration in the headspace seven days after packaging. This is in agreement with the theory that solubility of CO_2 in water increases with decreasing temperature (Carroll *et al.* 1991). In our experiment, no significant CO_2 concentration decrease was found for superchilled MA packed cod loins compared with the chilled MAP group. The probable reason might be the formation of ice crystals in cod loins caused by slow freezing while CO_2 will not dissolve into ice (Monnin *et al.* 2001). After 18 days of storage, the content of CO_2 increased again. The only exception has been observed on day 13, where the concentration of CO_2 and O_2 was 29.9% and 8.0% respectively. This is probably caused by fluctuations in gas composition or improper sealing of the vacuum bags during packaging.

Headspace gas analysis could be an effective and easy index of microbial growth (Torrieri *et al.* 2005). Similarly in our experiment, the change of headspace gas composition during storage of MA packed cod loins showed, coincide with microbiological results, a little oxygen decrease and CO_2 increase, respectively after 11 and 18 days of storage.

4.3 Sensory evaluation

4.3.1 Sensory evaluation of whole cod (QIM)

During the storage period, cod showed gradual and consistent changes for all the parameters of sensory evaluation(Figure 3). The scores were around 2-3 on storage day 2, reaching a total score of 18 demerit points at day 15, indicating that it had reached the rejection point at day 15 of storage time on ice, which is in accordance with Martinsdóttir *et al* (2001).
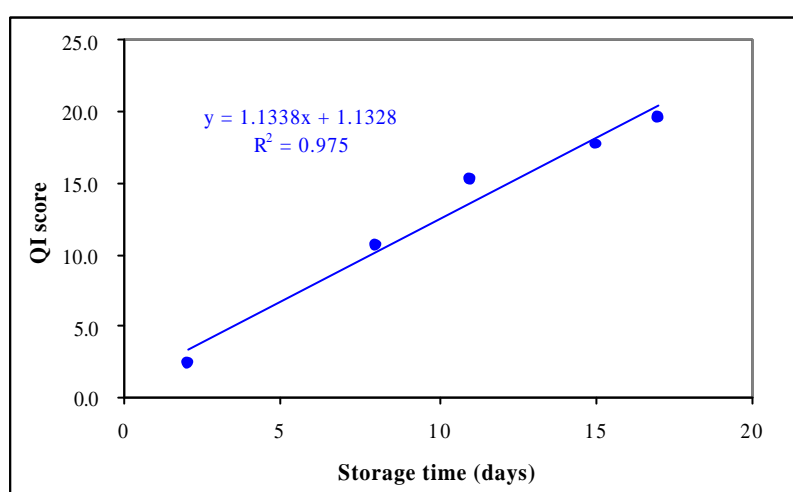
Figure 3: Average QI scores of each storage day analysed for whole gutted cod ($N = 3$) versus storage days in ice.

4.3.2 Sensory evaluation of cod loins stored under different conditions

4.3.2.1 <u>Sensory evaluation of cod loins (QIM)</u>

The results from the sensory evaluation of fresh cod loins stored under different conditions with the QIM scheme are shown in Figure 4.

The total sum of the scores (average scores of nine to twelve panelists) in the QIM was designated as the Quality Index (QI). There was a linear relationship with a high correlation (for group A1, $r^2=0.961$; for group A2, $r^2=0.986$; for group M1, $r^2=0.980$) between the average QI and storage time in polystyrene boxes under chilled and superchilled temperature and in chilled MA packaging (Figure 4 and Figure 5). To the contrary, lower correlation ($r^2=0.883$) was found between the average QI and storage time for superchilled MA packaging. The development of the QI score shows a slow and rather irregular increase with storage time with an abnormal sharp increase of QI scores on day 13. This increase could be linked to relatively lower CO_2 content in the packs for QIM evaluation on day 13, possibly due to the fluctuation of gas composition or improper sealing of the vacuum bags during packaging. The data from the superchilled MA packs

on day 13 were therefore excluded from further analysis of the QIM data. The slope of the regression line for group s A1, A2, M1 and M2 was 0.655, 0.523, 0.532 and 0.246 respectively. The results apparently indicated that fresh cod loins stored under chilled temperature spoil faster than those stored under superchilled temperature. There was no significant difference in average QI scores between the A2 and M1 samples during the whole storage time. The superchilled MAP cod loins had the slowest spoilage rate as seen by the slow increase rate of the QI score vs. days. The microbial growth is effectively delayed under superchilled MAP conditors, some other enzymically derived degradation compounds might contribute to the sensory rejection (Ólafsdóttir *et al*. 2005). Further modification of the QI scheme might be necessary to effectively evaluate the sensory quality of cod loins preserved by this new preserva tion technique.
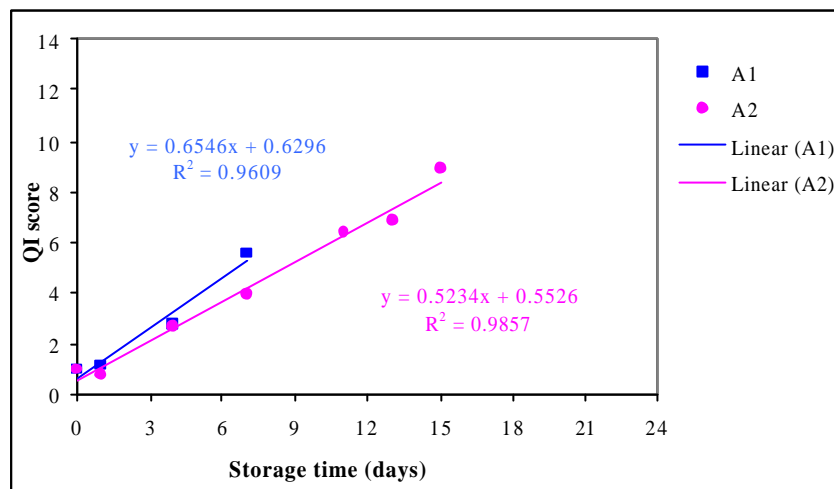
Figure 4: Average QI scores of each storage day analysed for fresh cod loins ($N = 3$) versus storage days. A1- stored in polystyrene boxes at 1.5°C and A2- stored in polystyrene boxes at -1.0°C.
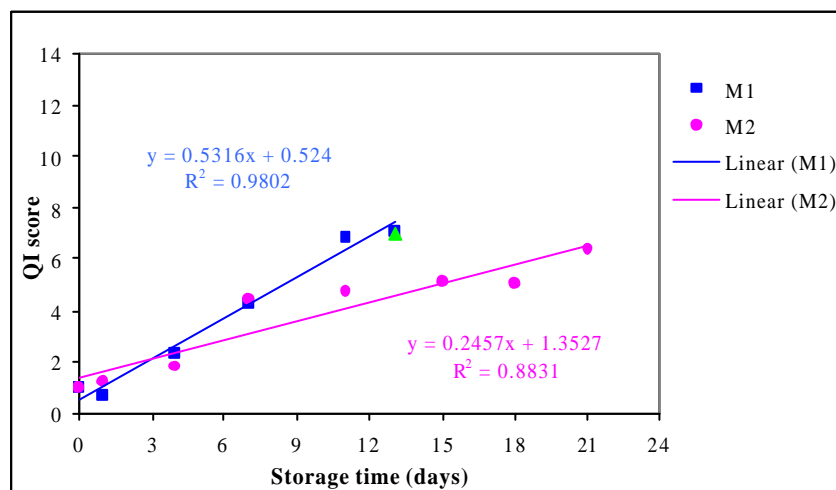
Figure 5: Average QI scores of each storage day analysed for fresh cod loins ($N = 3$) versus storage days. M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

It was assumed in the Quality Index Method that the scores for all quality attributes increased with storage time. But in our storage trial, the rates of change of the different parameters were not the same (Figure 6).

For groups A1, A2, M1, the scores for texture, odour, colour and brightness increased consistently throughout the storage time even though at the end of the storage time, the scores for texture were much lower than other attributes. The assessment of blood and gaping appeared to be difficult since the average scores increase slowly and irregularly throughout the storage time, especially for gaping. In the process of gaping, as described by Botta (1995), the flakes that are originally connected to each other by connective tissue in a fresh fillet, separate and the fillet loses the appearance of a continuous muscle. The increased gaping during ice-storage might be due to degradation of proteoglycans and glycoproteins, important for the spatial organisation of the collagen fibres and in anchoring cells in the ECM to the collagen network (Ofstad *et al.* 2005). The rate of deterioration depends on the biological status, catch or slaughter history and temperature during storage (Robb *et al.* 2000, Sheehan *et al.* 1996). In present studies, the cod loin samples from four different storage groups did not show much change in flesh gaping and scores for gaping did not change much during the whole storage time. Gaping results in fillets with unacceptable appearance might also influence textural properties (Ofstad *et al.* 2005). The number and deepness of gaping present in the flesh affects the total quality of fresh cod loins and it is an important parameter in the evaluation of the freshness of cod loins. However, the results of our study indicated that gaping did not contribute much to the freshness changes through the storage time. For group M2, the average scores fluctuated considerably throughout the storage time. Especially on day 13, most scores for the quality parameters increased sharply. As mentioned before, this could be explained by relatively lower CO_2 content in the packs for QIM evaluation on that day. Furthermore, the results strongly suggest that if the gas composition in the packs is not controlled adequately, the spoilage reactions will accelerate.

According to the guidelines for freshness assessment of whole fish given by Martinsdóttir *et al.* (2001), a minimum of three (large fish) to 10 (small fish) random samples should be taken to cover the biological differences in spoilage rates of fish. Sveinsdottir *et al.* (2002) reported that when more than three whole fish per batch of storage time were assessed, it could increase the precision of the prediction of storage time. Bonilla *et al.* (2006) suggested that due to individual variation present in the samples, a minimum of three samples should be used for freshness assessment of fresh cod fillets. Our study suggested that for MAP cod loins, more than three samples should be used in sensory evaluation to cover the individual variation between samples and different CO_2 content in MA packs. Moreover, the gas composition in the headspace should be measured for all packs from MA storage before the packs are opened for further analyses. Those packs with relatively lower CO_2 content should be excluded. It also needs to be noted that odour and brightness seem to be the most sensitive attributes among the sensory prosperities evaluated under all different storage conditions. On the contrary, gaping did not adequately describe the freshness change during storage. The results showed that the category giving 3 points should be included in the 2 points category, due to the important influence of gaping on the overall quality of cod loins.

This modification will need additional trials.
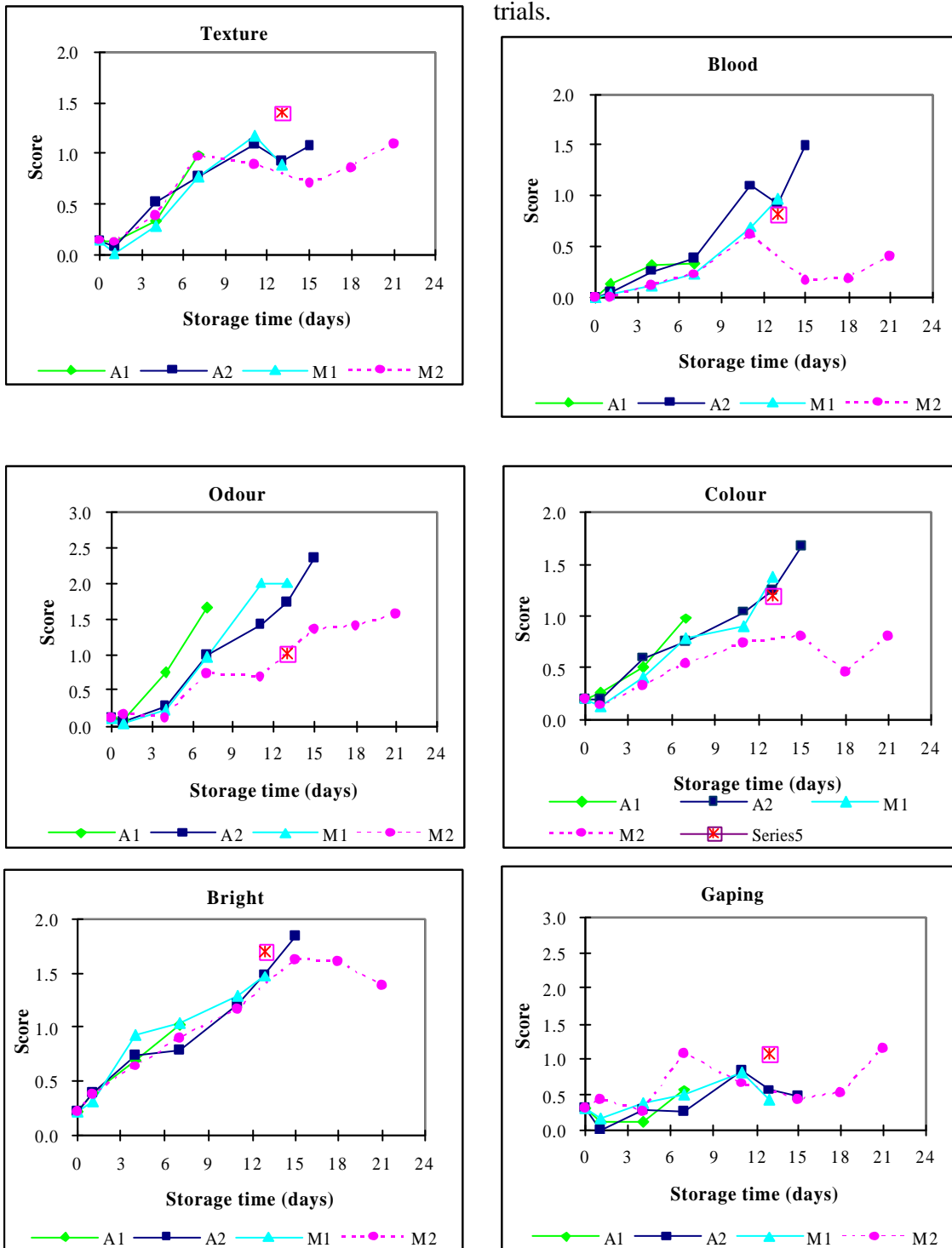
Figure 6: Average scores of each quality attribute evaluated with QIM scheme for cod loins (*N* = 3) versus storage days. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

4.3.2.2   Quantitative descriptive analysis

The attributes which were perceived at the beginning of storage may be considered to be positive. The attributes which were not present at the beginning of the storage time but were detected closer to the end of shelf-life described spoilage and were considered negative. At the beginning of storage, the positive attributes such as sweet, boiled milk and vanilla odour, sweet and metallic flavour, juicy texture were prominent (Figure 7, Figure 9 and Table 4). After that, the samples were less described by positive attributes. However, the decrease rates were different among these 4 groups. The least changes were noted in group M2 (superchilled cod loins), followed by groups A2 and M1 and a rapid decline was observed in group A1. The average scores of characteristic fresh cod attributes such as sweet, boiled milk odour, sweet and metallic flavour were between 20 and 70 through 18 days of storage in superchilled MAP group (M2).

The scores of the negative attributes: TMA odour and flavour, sour odour and flavour, putrid odour and flavour, table-cloth odour and bitter flavour were low at the beginning of storage and then increased with storage time. Similarly, the least changes were observed in group M2, followed by groups A2 and M1 and rapid increase in group A1. These negative odour and flavour attributes increased rapidly between days 4 and 7 of storage in sample group A1, and days 7 to 11 in sample groups A2 and M1. These unpleasant odours and tastes became obvious on day 7 for group A1 (Figure 8, Figure 10 and Table 4). Regarding sample group M2, the evolution of average scores showed an almost horizontal profile up to day 13 and day 15 respectively for negative odour attributes and negative flavour attributes, followed by a slow increasing trend up to the end of the storage period. Group M2 received significantly lower scores for these attributes on days 11, 13 and 15 (Table 4) compared with groups A2 and M1. This strongly indicates that the production of TVB-N was effectively delayed under superchilled MAP conditions. Changes in sensory attributes indicated that the samples were approaching the end of shelf-life when the cod loins were increasingly characterised by TMA, sour and putrid odours and flavours.

It is of interest to compare the storage time of "marketable quality" of cod loins when the samples are more described by important positive QDA odour and flavour attributes such as sweet, boiled milk and vanilla odour, sweet and metallic flavour. Previous studies showed that shelf-life extension of fresh fish can be reached by MAP and other storage methods, but usually the period of moderate to low quality (neutral taste period) is extended rather than earlier stages of prime quality. At the neutral stage the fillets are more described by neutral odour and taste attributes and have lost their characteristic fresh, sweet taste (Sivertsvik *et al.* 2003, Ólafsdóttir *et al.* 2005, Lauzon and Martinsdóttir 2005). It is important to evaluate whether the combination of MAP with superchilled storage can extend the earlier phase, since this would provide an important market value.

The scores of these positive odour and flavour attributes decreased rapidly in conventional chilled cod loins (A1). Samples were hardly described by these attributes after day 6 of storage. Superchilled storage (A2) and chilled MAP (M1) could maintain characteristic fresh fish odours and tastes longer, however, they were difficult to detect after 9-10 days (Figure 7 and Figure 9). The slowest change rate was found with superchilled MA packed cod loins (M2) which received significantly higher scores for sweet and boiled milk odours, sweet and metallic flavours on days 11, 13 and 15 (Table 4) compared to groups A2 and M1. This shows that superchilled MAP cod loins will have an extended marketable shelf-life. The importance of this new technique is the potential to maintain the characteristic fresh sweet taste of the cod loins longer and extend the period of prime quality. This could contribute to improved eating quality of fresh cod loins for consumers in distant markets (Ólafsdóttir *et al.* 2005, Lauzon and Martinsdóttir 2005).

An interesting observation is that superchilled MA packed cod loins always got higher scores for meaty texture during storage except on day 4. A significant difference was found between group M2 and the other three groups after day 7 of storage. This indicated that MAP combined with superchilled storage might have an impact on the textural properties. To the contrary, M2 samples were less described by soft, tenderness, juicy and mushy texture (Figure 11). The most likely explanation might be excessive drip loss and decrease of water holding capacity under superchilled MAP conditions. Unfortunately, water holding capacity was not measured in the present study and further investigations are needed. Sample group A2 showed some tendency to mushy and soft texture, this could be linked to cell destruction caused by partial freezing.
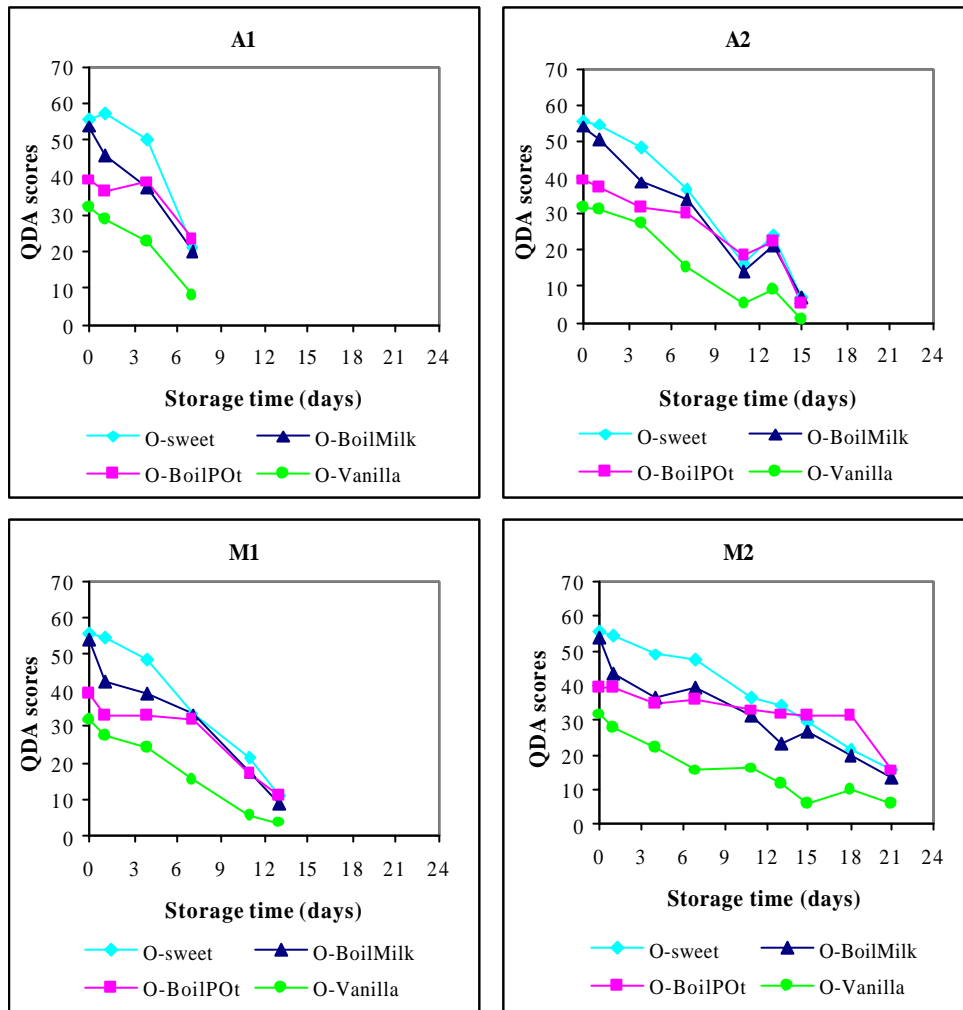
Figure 7: Positive odour attributes (average scores, $N = 2$) of cooked cod loins versus storage days. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.
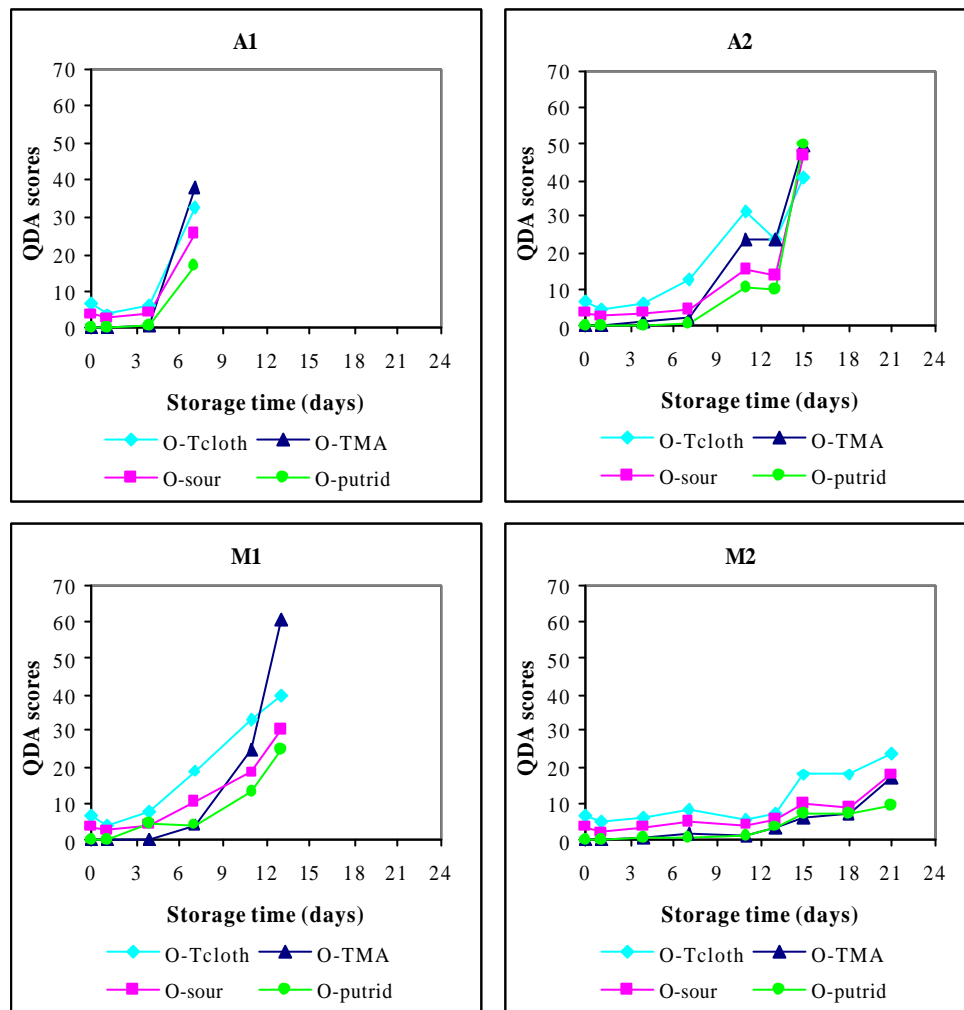
Figure 8: Negative odour attributes (average scores, $N = 2$) of cooked cod loins versus storage days. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.
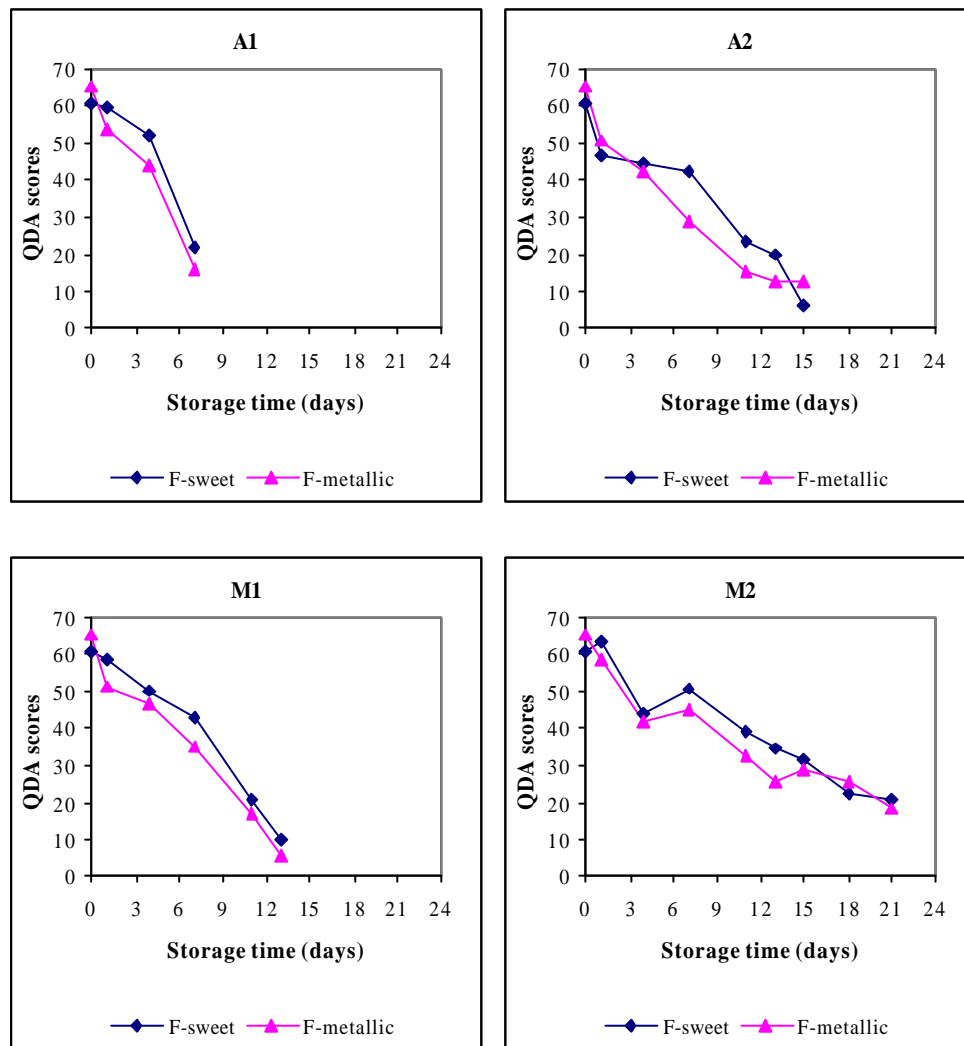
Figure 9: Positive flavour attributes (average scores, $N = 2$) of cooked cod loins versus storage days. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.
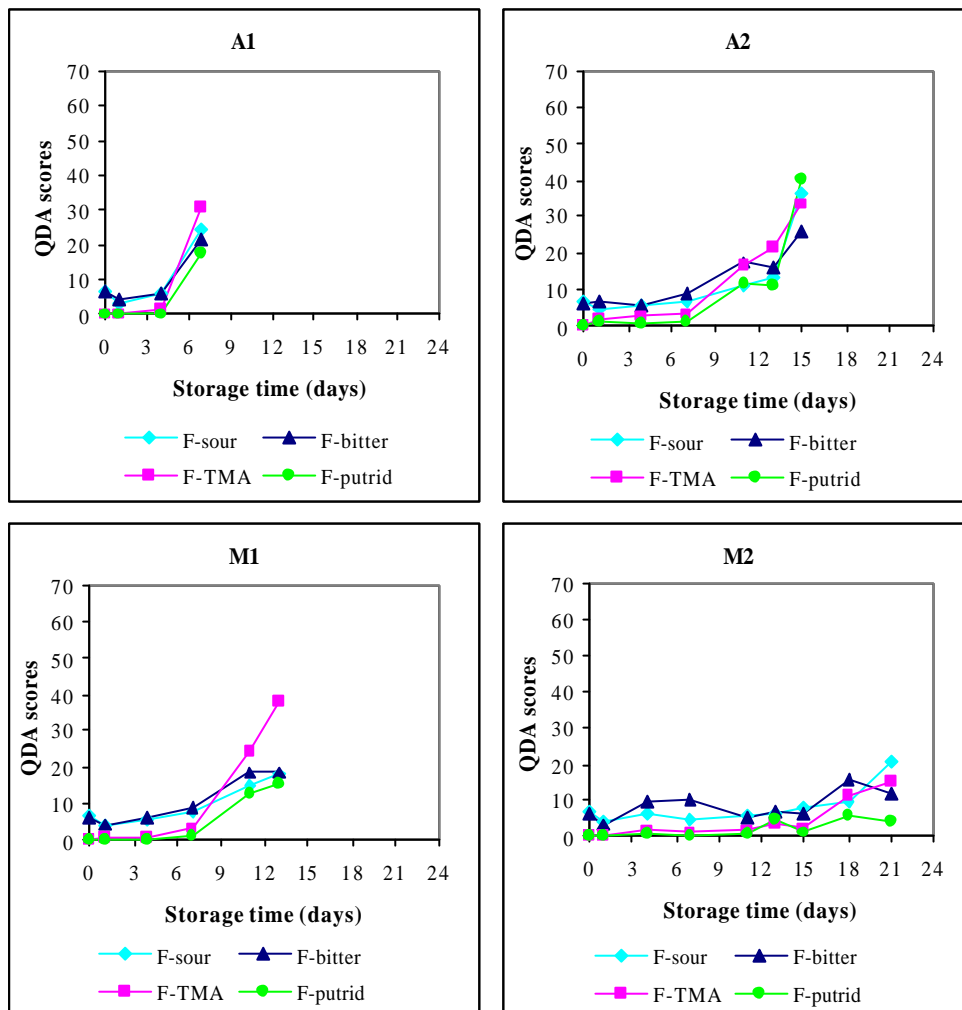
Figure 10: Negative flavour attributes (average scores, $N = 2$) of cooked cod loins versus storage days. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.
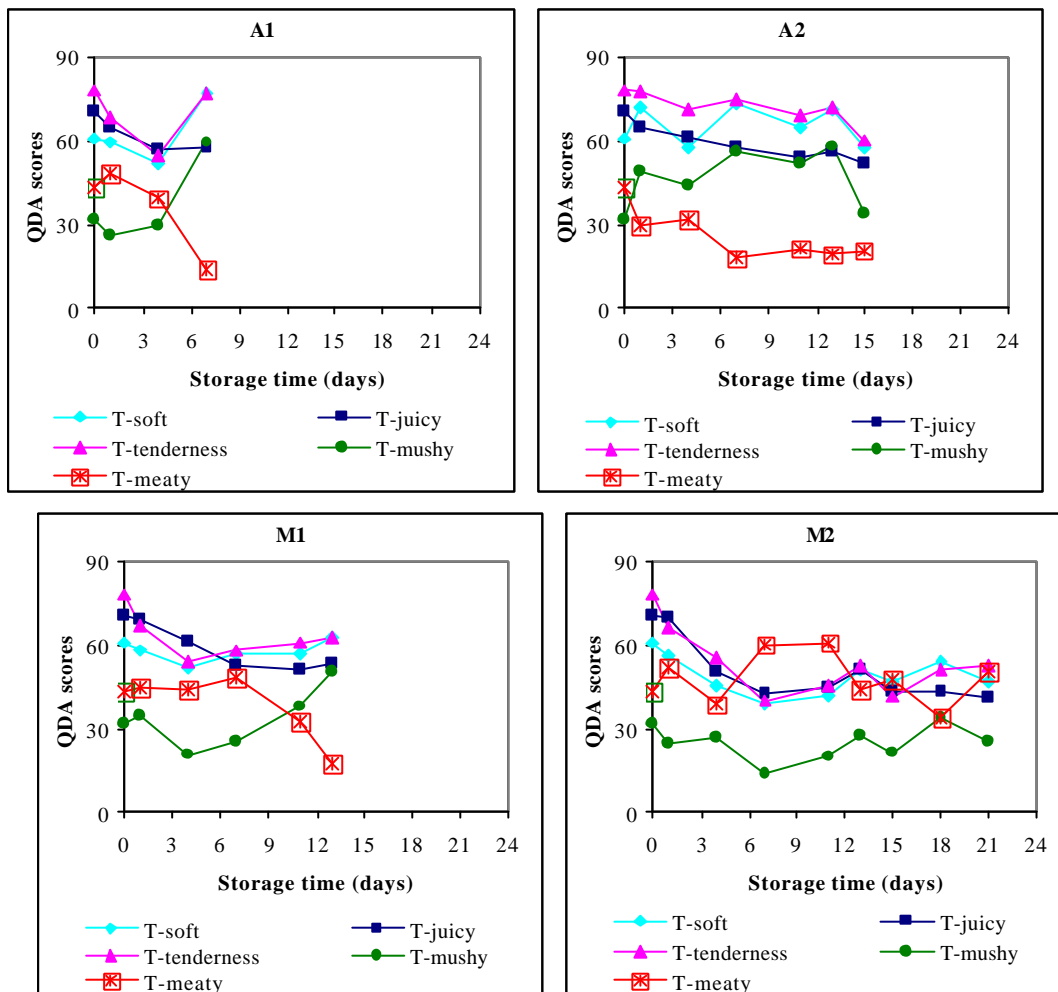
Figure 11: Texture attributes (average scores, $N = 2$) of cooked cod loins versus storage days. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

Table 4: Mean sensory scores of QDA attributes of cooked cod loins under different storage conditions.

Storage time (days)	group	O-sweet	O-BoilMilk	O-BoilPOt	O-Vanilla	O-Tcloth	O-TMA	O-sour	O-putrid	A-colour	A-Discoloration	F-sweet	F-metallic	F-sour	F-bitter	F-TMA	F-putrid	T-soft	T-juicy	T-tenderness	T-mushy	T-maety
0	AM0	56	54	39	32	7	0	4	0	11	12	61	65	7	7	0	0	61	71	79	32	43
1	A1	57	46	36	29	4	0	2	0	13	15	59	54	4	5	0	0 b	60	65	69	26 b	48 a
	A2	54	50	37	31	5	0	3	0	19	23	47	51	5	7	2	1 a	72	65	77	50 a	30 b
	M1	54	42	33	28	4	0	3	0	19	19	58	51	4	4	0	0	58	69	67	34 b	45 a
	M2	54	43	39	28	6	0	2	0	11	16	63	58	4	3	0	0 b	56	70	67	25 b	52 a
	p-value	0.9762	0.7613	0.9082	0.9728	0.9602	0.5649	0.9765	0.5967	0.0672	0.2307	0.1388	0.7312	0.9749	0.7260	0.1822	0.0442	0.0657	0.6139	0.2434	0.0048	0.0149
4	A1	50	37	39	23	6	1	4	0	19	24	52	44	6	6	2	0	52	57	55 b	30	39
	A2	48	39	32	27	6	1	3	0	20	21	44	42	6	6	3	0	57	61	71 a	44	32
	M1	49	39	33	24	8	0	4	5	21	23	50	46	6	6	1	0	52	62	54 b	21	44
	M2	49	37	35	22	6	1	4	0	17	20	44	42	6	10	2	0	46	50	55 b	27	39
	p-value	0.9936	0.9862	0.7910	0.9053	0.9658	0.3030	0.9938	0.3952	0.8305	0.8385	0.5340	0.9178	0.9968	0.7011	0.3021	0.3732	0.2685	0.0858	0.0185	0.0192	0.4619
7	A1	21 b	20	23	8	32 a	38 a	26 a	17 a	34 a	43 ab	22 b	15 b	25 a	22 a	31 b	18 a	77 a	57 a	77 a	59 a	14 c
	A2	37 a	34	30	15	13 b	2 b	5 b	1 b	21 b	28 cd	43 a	29	7 b	9	3 b	1 b	74 a	58 a	75 a	56 a	18 c
	M1	34	33	32	15	19 b	4 b	11 b	4 b	26	37 bc	43 a	35 a	8 b	9	3 b	1 b	57 b	53 a	58 b	25 b	48 b
	M2	47 a	39	36	16	9 b	2 b	5 b	0 b	19 b	25 d	51 a	45 a	5 b	10 b	1 b	0 b	39 c	43 b	40 c	14 b	61 a
	p-value	0.0012	0.0105	0.1872	0.2014	0.0006	0.0000	0.0000	0.0000	0.0051	0.0008	0.0005	0.0027	0.0000	0.0600	0.0000	0.0000	0.0000	0.0017	0.0000	0.0000	0.0000
11	A2	16 b	14 b	18 b	5 b	31 a	24 a	15	10	28	35 a	23 b	15 b	11	17 a	17 b	12 a	65 a	54	70	52 a	21 b
	M1	22 b	17 b	17 b	6 b	33 a	25 a	19 a	13 a	24	28	21 b	17 b	15	18 a	24 a	13 a	57 b	51	61	38 a	32 b
	M2	37 a	31 a	33 a	16 a	6 b	1 b	4 b	1 b	16	22 b	39 a	32 a	6	5 b	2 b	1 b	42 b	45	46	20 b	61 a
	p-value	0.0162	0.0154	0.0199	0.0071	0.0005	0.0005	0.0299	0.0362	0.0815	0.0535	0.0376	0.0211	0.1480	0.0308	0.0002	0.0144	0.0014	0.2341	0.0050	0.0004	0.0000
13	A2	24	21 a	22	9	24 b	24 a	14 a	10 b	26	33	20	12	13	16	22 b	11	71 a	56	72	58 a	19 b
	M1	11 b	9 b	11 b	4	40 a	61 a	30 a	25 a	29	40	10 b	6 b	18 a	19	38 a	16	63 a	53	63	51 a	17 b
	M2	35 a	23 a	32 a	11	7 c	3 b	6 b	4 b	25	31	34 a	26 a	6 b	7	4 c	5	51 b	51	53	28 b	44 a
	p-value	0.0174	0.0290	0.0101	0.1780	0.0000	0.0000	0.0013	0.0046	0.7211	0.2437	0.0333	0.0202	0.0481	0.1362	0.0005	0.1504	0.0013	0.6376	0.0538	0.0026	0.0035
15	A2	7 b	7 b	5 b	1 b	41	50 a	47 a	50 a	28	46	6 b	12	37 a	26 a	33 a	40 a	58	52	61	34	21 b
	M2	29 a	27 a	31 a	6 a	18	6 b	10 b	8 b	21	33	31 a	29	8 b	6 b	2 b	1 b	47	44	42	21	47 a
	p-value	0.0097	0.0026	0.0005	0.0160	0.0516	0.0000	0.0009	0.0004	0.2126	0.1416	0.0225	0.0763	0.0007	0.0091	0.0000	0.0000	0.2977	0.2940	0.0495	0.2762	0.0129

A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C. Values in the same column followed by different superscript letters are significantly different.
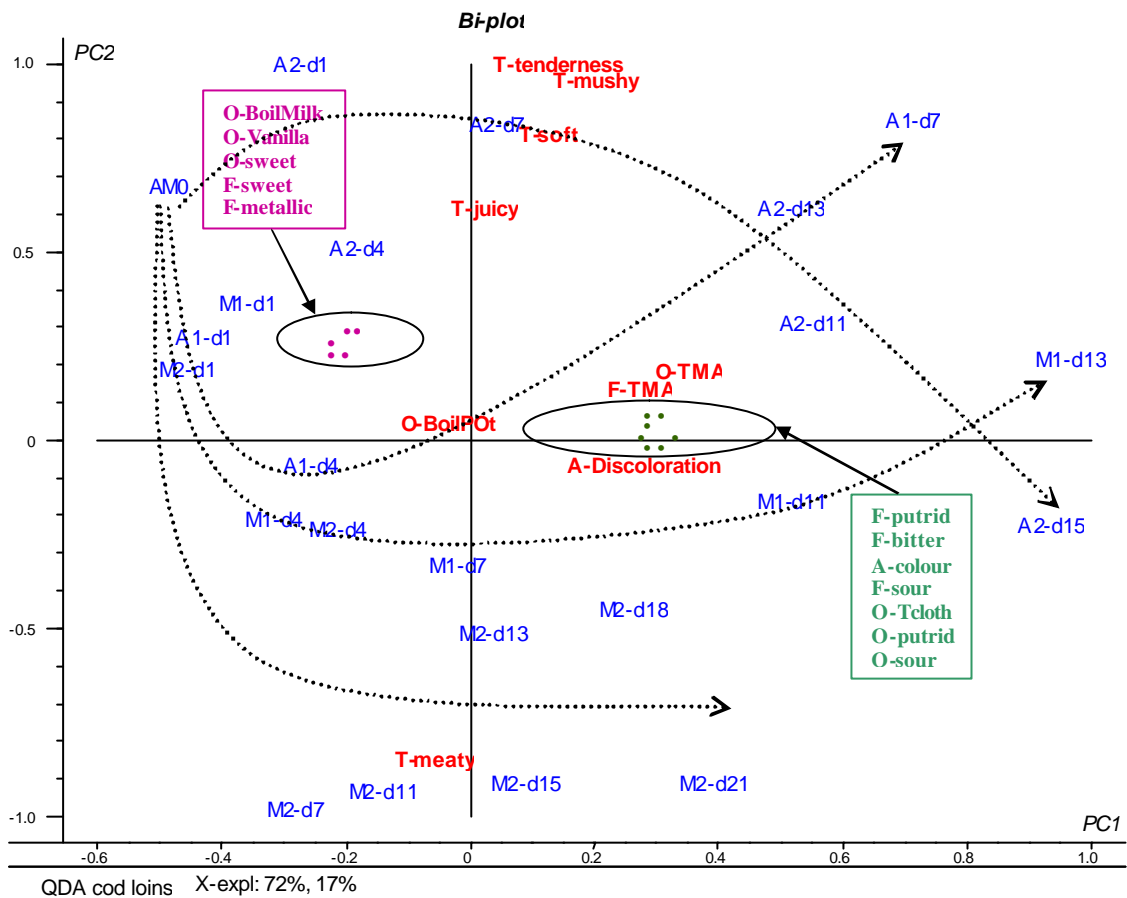
Figure 12: Bi-plot of scores (samples) and loadings (including all quality attributes assessed with QDA of cooked salmon in the shelf-life study) in PCA analysis. F = flavour, O = odour, A = appearance, T = texture attributes.

Figure 12 shows how the different sample groups of cod loins were described by the QDA attributes. Principal Component 1 (PC1) and Principal Component 2 (PC2) accounted for 72% and 17% of the experimental variance between sample groups respectively. The variations between samples were mainly due to the difference in odour and flavour attributes along the PC1 and appeared to be substantially linked to the duration of storage.

Fresh sample (AM0) which received the highest scores for the positive attributes: sweet odour and flavour, metallic flavour, boiled milk and vanilla odour, juicy texture is located on the left side in the region corresponding to the negative values of PC1. At the beginning of storage, samples were more described by positive attributes. These characteristics became less evident as storage time progressed, but the least change was observed in group M2 because samples from M2 are all located in the left side of the plot before day 15. While, with increasing storage time, samples are more described by boiled potato odour, meaty, juicy, tenderness, soft texture, and then by bitter and sour flavours, mushy texture, discoloration appearance, putrid flavour, putrid and sour odours, table-cloth odour, TMA flavour and odour. These changes occurred over different time periods

for the four sample groups and corresponded well with the process of quality deterioration. It also needs to be noted that tenderness and soft texture contributed little to PC1 and therefore do not appear to change much with storage time.

PC2 primarily explained variation between samples with regard to texture parameters. Cod loins appeared to be juicier at the very beginning of storage as this attribute was grouped with the positive attributes on the left side of the PC1-axis. Sample group M2 showed a strong trend towards meaty texture because M2 samples were all located on the lower part of the plot after seven days of storage and were thus characterised by a meaty texture. To the contrary, M2 samples were less described by tenderness, mushy and soft textures due to the opposite location of these attributes along the PC2-axis. Sample group A2 showed some tendency to mushy and soft texture. These results were in accordance with those of statistic analysis by NCSS.

4.4 pH measurements

The increase of pH values during the storage period may be attributed to the production of basic compounds such as ammonia, trimethylamine (TMA) as well as other biogenic amines mainly derived from microbial action by fish spoilage bacteria (Hebard *et al.* 1982, Kyrana *et al.* 1997, Boskou and Debevere 2000, Ruiz-Capillas and Moral 2001b, Masniyom *et al.* 2002).
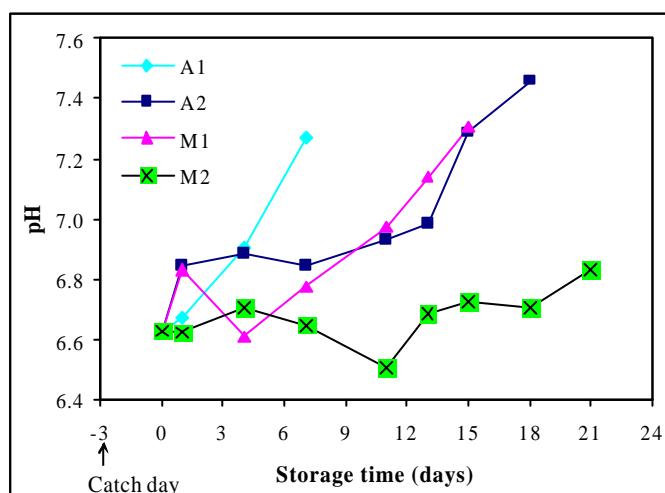
Figure 13: Changes in pH ($N = 3$) during storage of cod loins. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

Mean pH values over the period of four different storage conditions are shown in Figure 13. The initial pH of the cod loins was 6.63 upon arrival. The increases of pH value were rapid in the batch stored in polystyrene boxes under 1.5 °C (group A1), from the initial 6.63 to 7.27 after seven days of storage. Regarding group A2, pH value increased rapidly after one day storage and remained relatively stable until day 13, then increased sharply until the end of storage as a result of the production of basic compounds.

In our experiments, the strongest increase in pH was observed in group A1 and A2 after four and 15 days of storage, this coinciding with the peak production of TVB-N and TMA on those days.

A decreasing trend of pH values during early stages of storage was observed both in group M1 and M2 but in different patterns. The decrease in pH of cod muscle stored in the MAP group is in agreement with the findings of previous studies. Statham (1984), Sivertsvik *et al.* (2002) reported that pH values were slightly reduced in fish flesh with the dissociation of carbonic acid in general. It accorded with the CO_2 decrease in the headspace of MA packages observed at the early stage of storage. For group M1, the pH value increased steadily after seven days of storage due to the metabolism of microorganisms. This could also be linked to the results from TVB-N and TMA measurements. No marked increase in pH was noticed in group M2 except showing an increasing trend at the end of the storage period (day 21). Accordingly, in group M2 the pH value was always below 7.

4.5 Drip loss measurement

It has been previously suggested by several authors that MA packaging leads to increased drip loss, most likely due to dissolved CO_2 in the muscle which lowers the pH and thus results in a gradual loss in the ability of fish proteins to hold water as the storage time progresses (Davis 1998, Bøknæs *et al.* 2002, Masniyom *et al.* 2002, Sivertsvik *et al.* 2003). It has also been reported that the higher the level of CO_2, the higher the exudation losses (Dalgaard *et al.* 1993, Ruiz-Capillas and Moral 2001b, Masniyom *et al.* 2002, Pastoriza *et al.* 1998). The exudate loss of muscle contributed to the texture change, lower acceptability due to the fewer taste constituents remained as well as the shrinkage of the sample (Masniyom *et al.* 2005).

Drip loss from the samples increased with storage time for both MAP groups (Figure 14). No obvious differences were observed in drip loss between chilled and superchilled MAP cod loin samples (group M1 and M2) during the first seven days of storage. Exudation loss was higher in group M2 during the subsequent period of storage. However, no significant difference was observed between these two groups.

It has been reported that water binding capacity (WBC) variations are related to pH increases and muscle protein modifications (Pastoriza *et al.* 1998). The present study has also shown that high drip loss values in chilled MAP cod loins (group M1) correlate well with pH increases (over 7). This is suggested to be highly dependent upon bacterial growth and, consequently, spoilage of fish. Drip losses reached levels of 3.4-3.9% after 11 days storage which was in accordance with the 4-8% reported at the end of shelf-life of fresh MAP cod (Dalgaard *et al.* 1993, Guldager *et al.* 1998). The excessive drip loss in superchilled MAP cod loins during later stages of storage could be explained because partial freezing destructs the cells of fish muscle rather than pH increases caused by bacterial growth. According to Sivertsvik *et al* (2003), superchilled storage of fresh Atlantic salmon fillets at -2°C does not lead to excessive drip caused by cell destruction. Chilled and superchilled MAP salmon fillets had almost the same loss of water (about 3%)

while drip was highest in the chilled overwrap samples (4-5%) and lowest in the superchilled overwrap (about 2%).
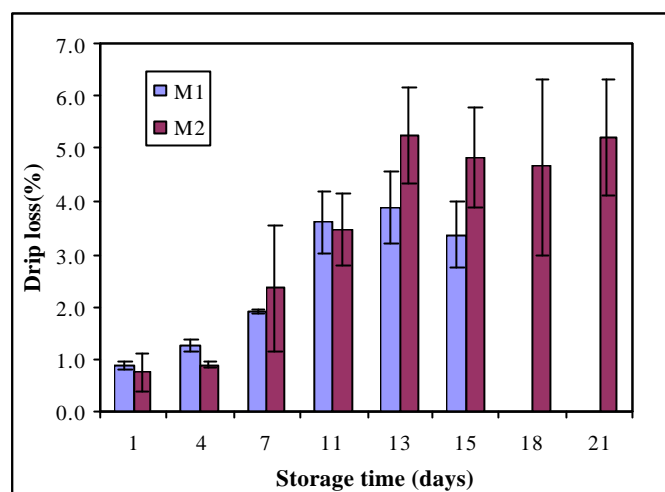
Figure 14: Changes in drip loss ($N = 3$) during storage of cod loins. Vertical bars represent SD. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

The results from the drip loss measurements had large standard deviations, suggesting that including more cod loins in the assessment of each batch might reduce this interval. The large variance in drip measurement was also observed by Sivertsvik *et al* (2003).

A variety of techniques adopted to minimise drip loss are discussed in detail by Church (1998). Pastoriza *et al.* (1998) reported that exudates from MAP fish can be reduced significantly by dipping fillets in NaCl solution prior to packaging. Alvarez *et al.* (1996) found that hake slices stored under MAP reduced drip loss when polyphosphate pretreatment was used. Moreover, texture of muscle tissue may also be improved by adding salt and/or cryoprotectants in a vacuum tumble for the incorporation of functional properties in fish products (Esaiassen *et al.* 2004). Masniyom *et al.* (2005) reported that pretreatment of seabass slices with pyrophosphate prior to keeping in MAP effectively retarded the exudates loss and improved the physicochemical and sensory properties of seabass slices during storage at 4°C.

4.6 TVB-N and TMA

The total volatile basic nitrogen (TVB-N) value was 10.8 mg/100 g in the fresh cod loins at the beginning of storage (Figure 15).

TVB-N values increased throughout the storage period for all groups. Generally, the increasing rate of TVB-N in cod loin samples stored at superchilled temperature (A2, M2) was always slower than in the other two groups stored at conventional chilled temperature (A1, M1). The initial lag phase for group A1 was four days, after this time the TVB-N content increased rapidly and reached 56.6 mg/100 g after seven days of storage. Much lower levels of TVB-N were observed in group A2 and M1 on day 7, which were 13.4 mg/100 g and 18.4 mg/100 g respectively. With regard to group M2, the production of TVB-N was effectively delayed and the evolution of the TVB-N content showed an almost horizontal profile up to day 13, followed by an increasing trend up to the end of the storage period. The final average TVB-N value reached 36 mg TVB-N/100 g muscle, indicating a significantly lower production of volatile bases in cod loin muscle stored in MAP at superchilled temperature.
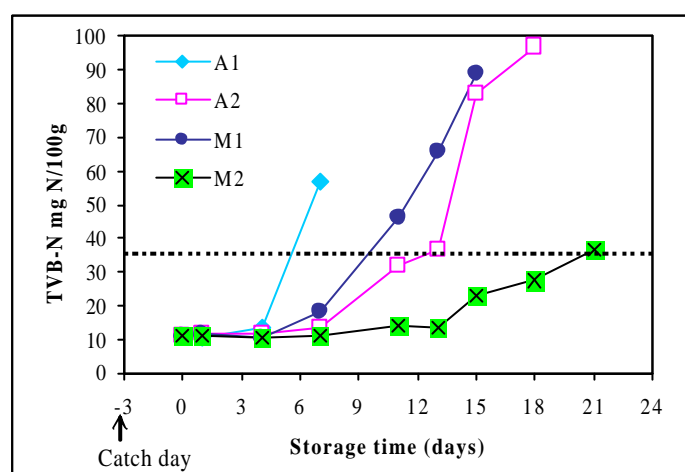
Figure 15: Total volatile basic nitrogen (TVB-N) mg/100 g formation of cod loins ($N = 3 \times 3$) during storage. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

For TMA, the changing pattern was similar to that of TVB-N. Initial trimethylamine (TMA) value of the sample was 0 mgN/100 g on day 0 before packaging (Figure 16). The results showed that MA packaging (group M1) and superchilled storage (A2) delayed the production of TMA at the early stage of storage. The initial lag phase for group A2 and M1 was around seven days. After this time, a sharp increase was observed in both groups up to the end of storage. But the increasing rate of TMA in group A2 was always slower than group M2 during the whole storage time. Additional lag phase extension in TMA values was obtained until day 13 by combining MAP with superchilled storage. The TMA value increased to 10.5, 23.9 mgN/100 g respectively after 15 and 21 days of storage.

TVB-N and TMA are products of bacterial spoilage and the content is often used as an index to assess the keeping quality and shelf-life of seafood products (Lannelongue *et al.* 1982b). TMA concentration is generally used to limit the acceptability of fish (Masniyom *et al.* 2005). Fresh cod normally has less than 20 mg TVB-N /l00 g and 3 mg TMA-N /l00 g. When the level of TVB and TMA exceeds 35 mg N/l00 g and 15 mg N/l00 g respectively, the fish is considered spoiled (Connell and Shewan 1980, Huss 1988a, b). However, Dalgaard *et al.* (1993) stated that the level of TMA was typically around 10–15 mg TMA-N/100 g in aerobically stored fresh fish rejected by sensory panels, but could reach up to 30 mg TMA-N/100 g in packed cod.
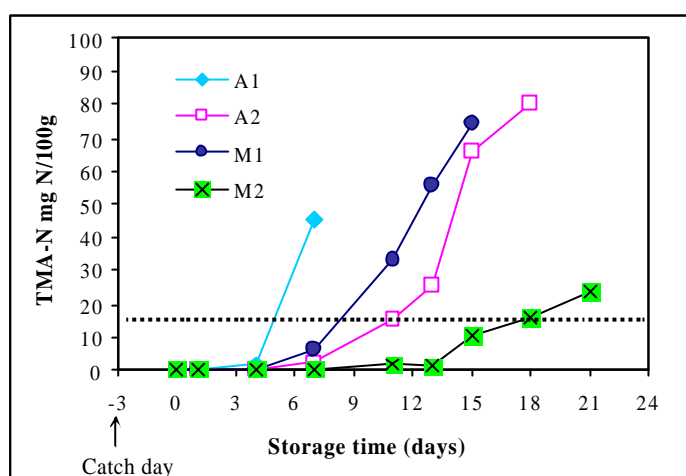
Figure 16: Trimethylamine (TMA) mg N/100 g formation of cod loins ($N = 3\times3$) during storage. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

Pastoriza *et al.* (1996) reported that TMA production in iced hake slices was significantly ($P<0.05$) reduced by storage under MA packaging. Ordonez *et al.* (2000) found that TMA formation was inhibited when hake steaks were stored in CO_2-enriched atmospheres. In our study, the relatively higher rate of TMA production in MA packed samples at 1.5°C was most likely due to an increasing growth of TMA producing microorganisms, including *P. phosphoruem* and *S. putrefaciens.*

It also needs to be noted that the TVB-N content in the samples correlated well with the increase in pH, especially the samples stored in air (groups A1 and A2). Furthermore, high TVB-N production was generally correlated with high TMA production in groups A1, A2 and M1. The TMA accounts for 70-80% TVB-N at sensory rejection time. This is in agreement with previous studies. Huss (1995) reported that in cod and other gadoid fishes, TMA constitutes most of the total volatile bases until spoilage.

4.7 Microbial analysis

Microbial analysis of the raw material at the onset of the experiment showed that the microbial quality of the fresh cod loins was high (5.3×10^4 colony forming units CFU/g). According to guidelines from the Icelandic Fisheries Laboratories (IFL) in fish processing, fish fillets with plate counts $< 2.5 \times 10^5$ CFU/g incubated at 22°C are considered 'good' while $2.5 \times 10^5 - 5 \times 10^5$ CFU/g and $> 5 \times 10^5$ CFU/g are considered 'fair' and 'poor'.

The total viable psychrotrophic counts (TVC) were always higher in cod loins exposed to air than those stored in MA under the same temperature conditions and for the same storage time (Figure 17). For group A1, the TVC increased from an initial level of 5.3×10^4 CFU/g to 9.6×10^7 CFU/g on day 7 when the fish reached the limit of acceptability. Among the four groups, samples stored in MAP under superchilled temperature had the lowest psychrotrophic bacterial count during the whole storage period ($P < 0.05$) and reached 2.6×10^7 CFU/g at the end of the storage trial (on day 22). It needs to be noted that there was no marked difference between bacterial numbers on LH agar and those on IA when all colonies were counted in the latter medium.
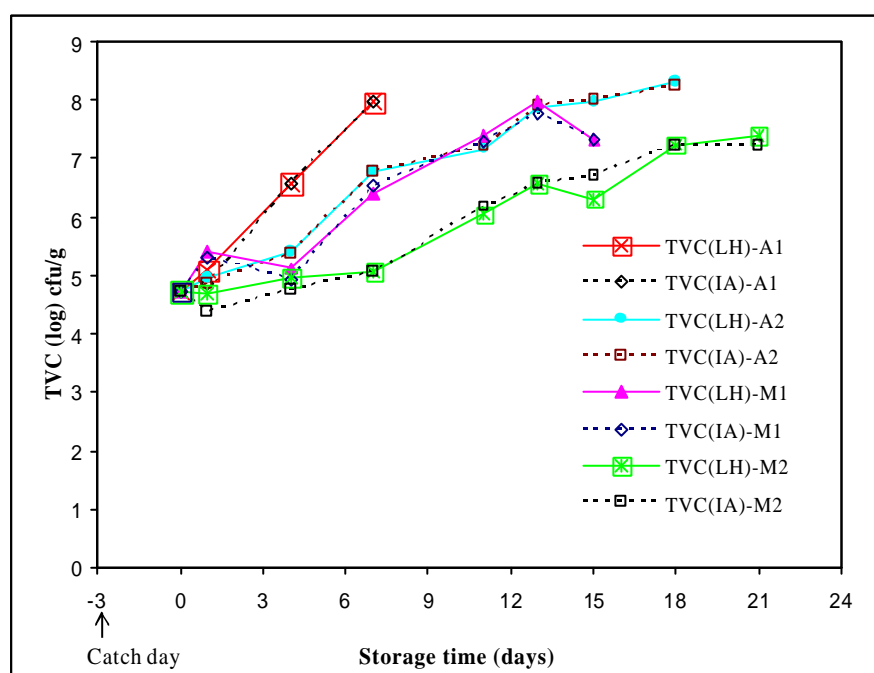
Figure 17: Total viable psychrotrophic bacteria counts (TVC) on LH and iron agar at 15°C ($N = 3$) in cod loins during storage. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

The H_2S-producing bacterial count was 3.7×10^2 CFU/g at the beginning of storage, then it grew faster in the cod loins stored in polystyrene boxes under 1.5 °C (group A1) and was generally higher than in the other groups ($P < 0.05$), it reached the spoilage level of

2.2×10^7 CFU/g on day 7 (Figure 18). The rapid increase of H$_2$S-producing bacteria may possibly be explained by the microaerophilic conditions developed in plastic covered cod loins in the polystyrene boxes (containing 7-10 loins, approximately 3 kg) that probably favored its growth. However, this can not be confirmed since the gas composition in the headspace of the boxes was not measured. *S. putrefaciens* and *Vibrionaceae* are able to use TMAO as final acceptor of electrons (Huss *et al.* 1988a, Joffraud *et al.* 2001) and produce TMA (Dalgaard 1995b). This reaction determines that these microorganisms can grow in microaerophilic or anaerobic conditions when the oxygen is poor or absent in muscle (Hobbs and Hodgkiss 1982).

The growth rate of H$_2$S-producing bacteria under superchilled temperature was delayed only at early stages of storage, after four days of storage an exponential increase of the H$_2$S-producing bacterial count was observed and subsequently on the 18th day it almost reached the same levels as for the total viable count, hence dominating the spoilage microflora. The obviously inhibitive effect of superchilling on *S. putrefaciens* was previously observed by Sivertsvik *et al.* (2003) who reported that superchilled salmon exposed to air had relatively lower counts of H$_2$S-producing bacteria when compared with aerobic plate counts (APC) than chilled salmon exposed to air. But according to Ólafsdóttir *et al.* (2005), H$_2$S-producing bacteria seem to tolerate better the superchilling conditions and reached 10^7 CFU/g in superchilled cod fillets at sensory rejection.

At the beginning of storage, H$_2$S-producing bacteria constituted only a small portion of the total microbial population (0.7%). These count values reached 23% (log 7.3 cfu/g) and 45% (log 7.6 cfu/g) from the total count in aerobically chilled and superchilled cod loins after seven and 13 days of storage. This fact suggests that these bacteria may contribute considerably to the overall spoilage of aerobically stored cod loins although the growth rate was slower in the superchilled group (A2).
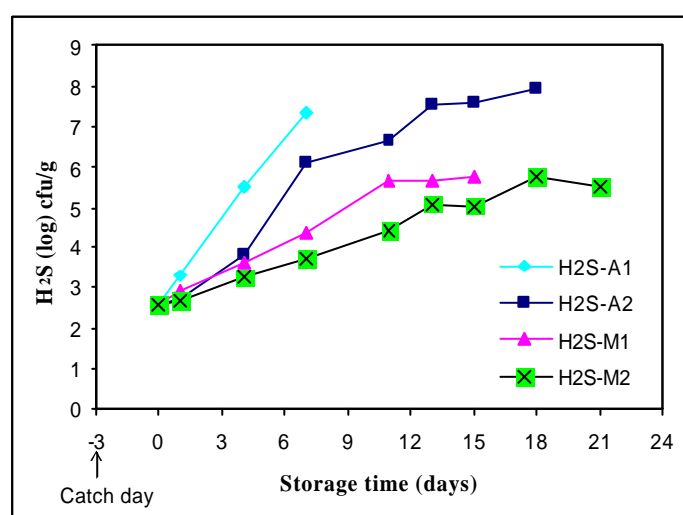
Figure 18: Growth of H$_2$S-producing bacteria on iron agar at 15°C (*N* = 3) during storage of cod loins. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

The effects of MA packaging alone was effective in slowing down the growth rate of H_2S-producing bacteria. H_2S-producing bacteria reached their maximum number after 11 days (4.7×10^5 CFU/g) and then remained quite stable. CO_2 is recognised to inhibit most of the Gram-negative bacteria (Banks *et al.* 1980, Gill and Tan 1980, Lannelongue *et al.* 1982a) and particularly respiratory organisms such as *Pseudomonas* spp. and *S. putrefaciens* (Gram and Huss 1996). According to Debevere and Boskou (1996), packaging of cod fillets in modified atmosphere, containing 60% CO_2 and 40% O_2 and storing at 6°C had an inhibiting effect on the growth of the normal TMA- and H_2S-producing flora *(Shewanella putrefaciens).* They found that hydrogen sulfide producing bacteria represented only a small part of the total flora in cod fillets packed in CO_2-atmosphere. When the total aerobic plate count reached log 6 cfu/g, the number of H_2S-producing bacteria was only log 3 cfu/g.

The combination of MAP and superchilled storage had a synergistic effect on the inhibition of H_2S-producing bacterial growth compared with groups A2 and M1 ($P<0.05$), reaching 3.1×10^5 CFU/g at the end of the trials (day 21).

Photobacterium phosphoreum was slightly above the detection limit (log 1.3 CFU/g) on the first day of storage (Figure 19). No lag phases were observed for any of the groups. After one day of storage they all reached the same level. For samples stored in polystyrene boxes at 1.5°C (group A1), this growth pattern is not surprising because the similar exponential growth of *P. phosphoreum* with no significant lag phase has been observed in fresh MAP cod (Dalgaard *et al.* 1997a, Guldager *et al.* 1998). For superchilled groups, the probable reason might be the slow cooling rate of the cod loins stored in the superchilling chamber (groups A2 and M2).
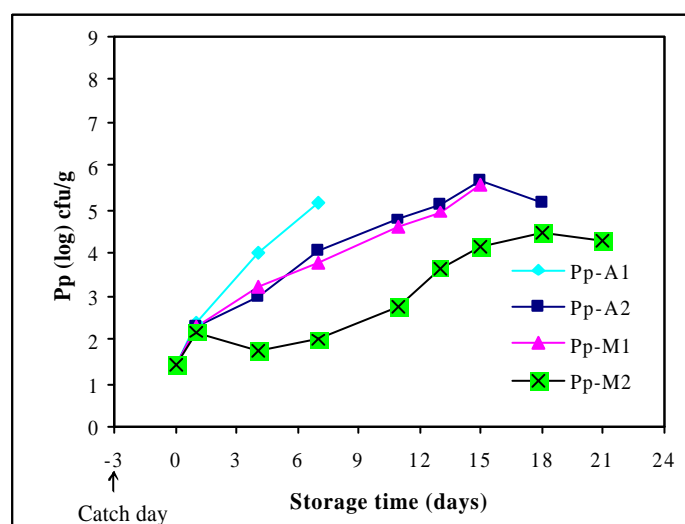
Figure 19: Growth of *Photobacterium phosphoreum* incubated at 22°C during storage of cod loins. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C.

Photobacterium phosphoreum grew most quickly in cod loins kept in polystyrene boxes under conventional chilled temperature (1.5°C, group A1) (P<0.05). This is in agreement with the previous studies by Dalgaard, *et al.* (1997b) who stated that *P. phosphoreum* can grow in all aerobically stored products and reached 10^5 cfu/g in spoiled gutted whole cod. However, due to rapid growth of other organisms, *P. phosphoreum* did not dominate the microflora of the aerobically stored fish.

For those stored in polystyrene boxes in superchilled temperature (-1°C, group A2) and chilled MA packing (group M1), an inhibitory effect was found on the growth rate of *P. phosphoreum*.

Lowest counts were with superchilled MA packing (group M2) where the lag phase was apparently extended (until day 7). After 21 days of storage, the level was only around log 4.3 CFU/g which indicated that the combined effects of MAP and superchilled storage had a retarding effect on the growth of *P. phosphoreum* (P<0.05).

The results presented showed that superchilled storage retarded *P. phosphoreum* growth while H_2S-producing bacteria appeared to grow steadily and was less affected. Similar results were reported by Lauzon and Martinsdóttir (2005) for cod fillets stored in EPS (expanded polystyrene) boxes at -1.0±0.4°C.

The specific spoilage organism for MA-packaged cod and salmon has been found to be *Photobacterium phosphoreum* (Dalgaard *et al.* 1993, Emborg *et al.* 2002). *P. phosphoreum* is more CO_2-tolerant than the specific spoilage bacteria observed for air stored temperate fish, *Shewanella putrefaciens* and the spoilage potential of this species is mainly due to the production of TMA from TMAO. It has been isolated from modified-atmosphere-packed cod fillets, saithe, plaice and marine trout, from Denmark, Greece or Iceland (Dalgaard *et al.* 1997b). The numbers of *P. phosphoreum* made up an important and often dominant part of the spoilage microflora in these products. For Danish and Icelandic modified atmosphere-packed (the atmosphere composition was 45% CO_2-50% N_2-5% O_2 and 60% CO_2-40% N_2-0% O_2 respectively) cod fillets stored at 0°C, the level reached 10^7-10^8 cells g^{-1} and made up 90% of TVC at sensory rejection time. Interestingly, the results from the present study are quite different from those of previous studies. In our experiment, the numbers of *P. phosphoreum* in the chilled MAP cod loins never reached 10^7 cells/g spoilage levels and did not dominate the microflora at sensory rejection. After 13 days of storage, the counts of *P. phosphoreum* only reached levels of log 4.9 CFU/g. The dissimilarity between our experiment and the published findings could be explained by the difference in experimental temperatures, gas composition used in MAP, initial bacterial population, freshness of the raw material and the handling method used before MAP packaging. Further investigations are needed for an accurate identification of the main spoilage organisms under different gas compositions and storage temperatures for MA packed fresh cod loins.

The interaction of the spoilage microflora has been studied by many researchers to understand better the spoilage development in fish (Gram *et al.* 2002a). Antagonism or symbiosis between different groups of microorganisms may influence their growth and

metabolism (Gram and Dalgaard 2002a). The part of the microflora which will ultimately grow on the products is determined by the intrinsic (postmortem pH in the flesh, the presence of trimethylamine oxide (TMAO) and other non-protein-nitrogen (NPN) components) and extrinsic parameters (temperature, processing and packaging atmosphere) (Huss *et al.* 1997). Based on the findings of Dalgaard (1995a) that *P. phosphoreum* was a 30 times more active TMA producer than *Shewanella putrefaciens* and cell counts of 10^7 CFU/g of *P. phosphoreum* corresponded to 30 mgN/100g TMA in packed cod fillets, it is likely that *P. phosphoreum* is very important in the formation of TVB-N and TMA in MAP fresh cod loins stored at 1.5°C. However, considering the H_2S-producer count (4.7×10 CFU/g), the role of the H_2S-producing bacteria in contributing to TVB-N and TMA can not be overlooked. The most likely explanation is that *P. phosphoreum* in combination with H_2S-producing bacteria accounted for the high levels of TVB-N and TMA measured in the chilled MAP group at sensory rejection.

Limited studies have shown that lactic acid bacteria (LAB) were of little importance for the sensory changes during storage of fresh fish and lightly preserved fish products and Leisner (1992) reported that no or very faint off-odours were produced by lactic acid bacteria compared to the very obnoxious off-odours produced by Gram-negative spoilers. A few studies have been published on the evolution of LAB under modified atmosphere and its relation to spoilage of MA packed fish. Stenstrom (1985) reported that CO_2 could favour the growth of some Gram-positive bacteria, i.e. lactobacillus, which would compete with spoilage bacteria. Conversely, Dalgaard *et al.* (1993) reported that low concentration of lactic acid bacteria was detected on NAP (about 10^4 CFU/g) at the time of rejection, indicating no quantitative importance for the spoilage process of MA packed cod fillets. Stammen *et al.* (1990) found that *Lactobacillus* spp. was an important spoilage bacterium of cod and sole together with *Shewanella putrefaciens* in MAP.

Counts of presumptive lactic acid bacteria (LAB) on NAP agar are shown in Figure 20. The number of LAB increased steadily for all groups during storage. From the first day on, an exponential increase was observed for groups A1, A2 and M1. There is no significant difference in the lactic acid bacterial plate counts between groups A1 and A2 during storage. This is not surprising because the growth rate of LAB was little influenced by superchilled temperature, as expected for a Gram-positive bacterium.
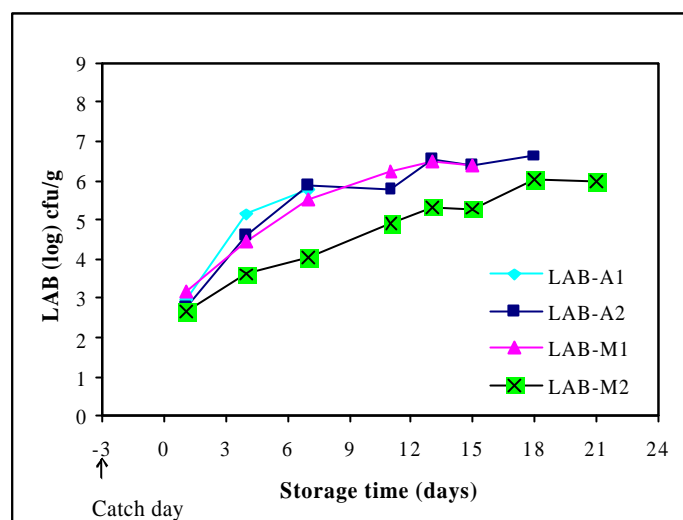
Figure 20: Growth of presumptive lactic acid bacteria (LAB) on NAP agar at 22°C (*N* = 3) during storage of cod loins. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at - 1.0°C.

Results of the present work show that the development of LAB for group M1 followed the same pattern as the air group. To our knowledge, lactic acid bacteria (LAB) are carbon dioxide tolerant and CO_2 packing inhibits respiratory organisms and selects for *Photobacterium phosphoreum* and facultative anaerobic bacterial species, i.e. LAB (Dalgaard 2000a). But referring to Hanna (1992) their growth in fish is limited for the first 14 days. This is in agreement with the results obtained by Masniyom *et al.* (2005) who found that LAB counts were generally higher in fresh seabass slices kept under MAP compared with those stored in air. The reason for the dissimilarity between our experiment and previous studies is not clear and further investigations are needed to better understand their role in spoilage of packed fish. It is likely that microaerophilic conditions developed in plastic covered cod loins in foam polystyrene boxes also favour the growth of LAB.

The lowest counts were with group M2 where the exponential phase was apparently extended. Even through superchillng and MAP alone have little effect on the growth of LAB, the combined effect was observed in superchilled MAP cod loins to restrain the growth of LAB.

4.8   Correlation between quality indicators

Table 5 shows the correlation coefficients between the parameters measured, i.e. pH, drip loss, TMA, TVB-N, TVC, H_2S-producing bacteria, *Photobacterium phosphoreum* and LAB counts, QI scores and all the QDA attributes. The shaded area highlights where high correlation was found.

The fact that there are fairly high correlations between parameters such as TMA, TVB-N and negative QDA attributes, i.e. TMA odour and flavour, sour odour and flavour, putrid odour and flavour, table-cloth odour and bitter flavour is not surprising, since the majority of these volatile bacterial metabolic products such as TMA contribute considerably to the sensory deterioration.

QI scores appeared to be more related with positive QDA parameters (negatively), i.e. sweet odour and flavour, boiled milk and vanilla odour, metallic flavour than negative QDA attributes. The Quality Index Method (QIM) has been demonstrated to be a rapid and reliable grading system for estimating the freshness and quality of seafood (Nielsen 1997, Sveinsdottir *et al.* 2002). Furthermore, it is easy to perform and can be used in the first part of the storage period where other instrumental methods are inaccurate (Nielsen *et al.* 1992). The results in this study are in agreement with these findings which suggest that the QIM method is a useful tool to evaluate the initial quality of fresh cod loins stored under either aerobic or modified atmosphere, chilled or superchilled temperature, although linear correlation was found for the whole storage period. Compared with other quality indicators, QI scores seem more realistic to evaluate the marketability of the fresh cod loins.

TVC, H_2S-producing bacteria, *Photobacterium phosphoreum* and LAB counts show some correlations with QI scores. They also negatively related to some QDA parameters- sweet odour and flavour, boiled milk odour and metallic flavour but less related to negative QDA parameters, pH, TVB-N and TMA.

It also needs to be noted that the texture parameters of QDA such as soft, juicy, tenderness, mushy and meaty did not show any high correlations to the other quality indicators measured.

Table 5: Correlation coefficients (r) between different quality parameters measured*

	Ph	drip	TVBN	TMA	QI	TVC(LH)	Black	LAB	Pp	O-sweet	O-BoilMilk	O-BoilPOt	O-Vanilla	O-Tcloth	O-TMA	O-sour	O-putrid	A-colour	A-Discoloration	F-sweet	F-metallic	F-sour	F-bitter	F-TMA	F-putrid	T-soft	T-juicy	T-tenderness	T-mushy	T-meaty
Ph	1.00																													
drip	0.20	1.00																												
TVBN	0.90	0.35	1.00																											
TMA	0.91	0.37	0.99	1.00																										
QI	0.54	0.88	0.76	0.74	1.00																									
TVC(LH)	0.76	0.77	0.80	0.79	0.87	1.00																								
Black	0.74	0.86	0.73	0.73	0.85	0.95	1.00																							
LAB	0.61	0.77	0.70	0.69	0.88	0.93	0.89	1.00																						
Pp	0.77	0.67	0.80	0.79	0.84	0.95	0.92	0.92	1.00																					
O-sweet	-0.64	-0.81	-0.86	-0.84	-0.92	-0.92	-0.86	-0.89	-0.89	1.00																				
O-BoilMilk	-0.62	-0.82	-0.82	-0.81	-0.93	-0.91	-0.85	-0.88	-0.88	0.97	1.00																			
O-BoilPOt	-0.76	-0.53	-0.91	-0.90	-0.83	-0.83	-0.75	-0.74	-0.80	0.90	0.90	1.00																		
O-Vanilla	-0.54	-0.88	-0.77	-0.75	-0.95	-0.88	-0.85	-0.88	-0.85	0.95	0.95	0.82	1.00																	
O-Tcloth	0.79	0.49	0.95	0.93	0.82	0.86	0.79	0.78	0.85	-0.92	-0.88	-0.93	-0.85	1.00																
O-TMA	0.84	0.37	0.95	0.97	0.73	0.79	0.71	0.65	0.76	-0.82	-0.80	-0.90	-0.73	0.93	1.00															
O-sour	0.82	0.50	0.98	0.97	0.77	0.78	0.72	0.66	0.76	-0.84	-0.81	-0.91	-0.76	0.92	0.93	1.00														
O-putrid	0.76	0.48	0.94	0.93	0.72	0.70	0.67	0.59	0.72	-0.77	-0.75	-0.87	-0.69	0.85	0.88	0.97	1.00													
A-colour	0.76	0.54	0.75	0.72	0.77	0.84	0.82	0.77	0.85	-0.81	-0.80	-0.74	-0.78	0.80	0.75	0.72	0.64	1.00												
A-Discoloration	0.74	0.61	0.83	0.79	0.82	0.86	0.84	0.80	0.88	-0.87	-0.83	-0.75	-0.85	0.84	0.77	0.81	0.76	0.94	1.00											
F-sweet	-0.68	-0.80	-0.86	-0.85	-0.91	-0.91	-0.84	-0.86	-0.87	0.97	0.95	0.90	0.91	-0.90	-0.83	-0.84	-0.78	-0.82	-0.85	1.00										
F-metallic	-0.66	-0.80	-0.79	-0.78	-0.93	-0.94	-0.89	-0.92	-0.90	0.95	0.96	0.87	0.94	-0.86	-0.79	-0.76	-0.68	-0.86	-0.86	0.96	1.00									
F-sour	0.80	0.58	0.94	0.93	0.72	0.76	0.73	0.63	0.74	-0.80	-0.77	-0.87	-0.71	0.86	0.86	0.96	0.93	0.67	0.75	-0.80	-0.71	1.00								
F-bitter	0.80	0.42	0.91	0.91	0.76	0.81	0.80	0.71	0.78	-0.85	-0.81	-0.85	-0.75	0.92	0.88	0.90	0.84	0.79	0.81	-0.86	-0.80	0.87	1.00							
F-TMA	0.85	0.43	0.95	0.97	0.76	0.85	0.76	0.70	0.80	-0.85	-0.83	-0.91	-0.75	0.94	0.98	0.91	0.85	0.78	0.77	-0.87	-0.83	0.87	0.92	1.00						
F-putrid	0.81	0.48	0.92	0.91	0.72	0.72	0.72	0.58	0.72	-0.76	-0.74	-0.85	-0.67	0.84	0.87	0.95	0.97	0.67	0.75	-0.78	-0.69	0.93	0.89	0.96	1.00					
T-soft	0.58	-0.11	0.29	0.30	0.01	0.32	0.34	0.17	0.35	-0.15	-0.05	-0.22	0.00	0.32	0.35	0.22	0.19	0.38	0.29	-0.18	-0.20	0.23	0.34	0.39	0.30	1.00				
T-juicy	-0.02	-0.72	-0.30	-0.28	-0.67	-0.48	-0.46	-0.58	-0.42	0.61	0.63	0.38	0.73	-0.37	-0.23	-0.31	-0.25	-0.40	-0.49	0.60	0.61	-0.30	-0.36	-0.25	-0.19	0.51	1.00			
T-tenderness	0.39	-0.44	0.08	0.10	-0.27	0.00	0.02	-0.09	0.00	0.13	0.22	-0.04	0.33	0.07	0.15	0.03	0.01	0.05	-0.08	0.10	0.11	0.07	0.12	0.18	0.13	0.89	0.75	1.00		
T-mushy	0.68	0.17	0.39	0.42	0.22	0.50	0.52	0.37	0.49	-0.35	-0.29	-0.39	-0.21	0.45	0.49	0.32	0.25	0.54	0.41	-0.42	-0.45	0.31	0.49	0.55	0.37	0.90	0.26	0.76	1.00	
T-meaty	-0.81	-0.17	-0.59	-0.61	-0.38	-0.62	-0.65	-0.51	-0.65	0.51	0.47	0.56	0.38	-0.63	-0.66	-0.55	-0.53	-0.66	-0.59	0.59	0.58	-0.53	-0.69	-0.69	-0.61	-0.80	-0.13	-0.61	-0.91	1.00

*When 25 measurements (pH, TVB-N, TMA, TVC, H_2S-producing bacteria, *Photobacterium phosphoreum*) were included in the correlation comparison, the correlation was significant if $r^2 > 0.39$. Similar, for 24 measurements (LAB counts): $r^2 > 0.40$, for 23 measurements (all the QDA attributes, QI scores): $r^2 > 0.41$ and for 14 measurements (drip loss): $r^2 > 0.53$.

4.9 Evaluation of multi-indicator criteria for the quality of cod loins by PCA (principal component analysis)

The first two principal components explained 90% of the variation in the data (Figure 21). The first principal component (PC1) explained 79% of the variation between the samples, which appeared to be expressing the spoilage level of the sample (increased values of QIM, negative QDA attributes, physical and chemical measurements and microbial counts) with the increasing storage time from left to right along PC1. Fresh cod loins (AM0) which received the highest scores for the attributes: sweet odour and flavour, boiled milk and vanilla odour, metallic flavour, juicy texture is located on the left side in the region corresponding to the negative values of the PC1, while, with increasing the storage time, all the samples from the four groups move from the left side to the right side of the plot, towards positive PC1 values which are negatively related to the freshness of the samples. Sample group A2 after 18 days and sample group M1 after 15 days of storage were located furthest to the right on the plot which is in line with their high spoilage levels. Samples within group M2 before day 15 are all located in the left side of the plot and were slightly described by negative QDA attributes, had lower values of TVB-N, TMA and microbial counts.

The second principal component (PC2) explained 11% of the variation of the data and contributed to the differentiation of the storage groups, related to QDA textural attributes such as meaty, tenderness, mushy, soft and juicy. Loading for meaty texture was situated on the lower part of the plot and contributed to the discrimination of the superchilled MAP group (M2) after seven days of storage with the higher score of meaty texture.

The microbial parameters have similar loading and the correlation of these variables is high. QIM is also located close to TVC, H_2S-producers, *P. phosphoreum* on the plot. This suggests that these microbial variables give similar information as QI scores.

Two chemical variables-TVB-N, TMA, all negative QDA odour and flavour attributes and pH are grouped in the right site of the loading plot indicating that these variables were co-linear. Their loadings were characteristic for the spoilage samples. This demonstrates that these indicators give more information about the onset of putridity (later stages of advanced spoilage). TMA determinations do not reflect the earlier stage of spoilage but often reflect more accurately the degree of spoilage (as evaluated by sensory evaluation) than do bacterial counts (Huss 1995).
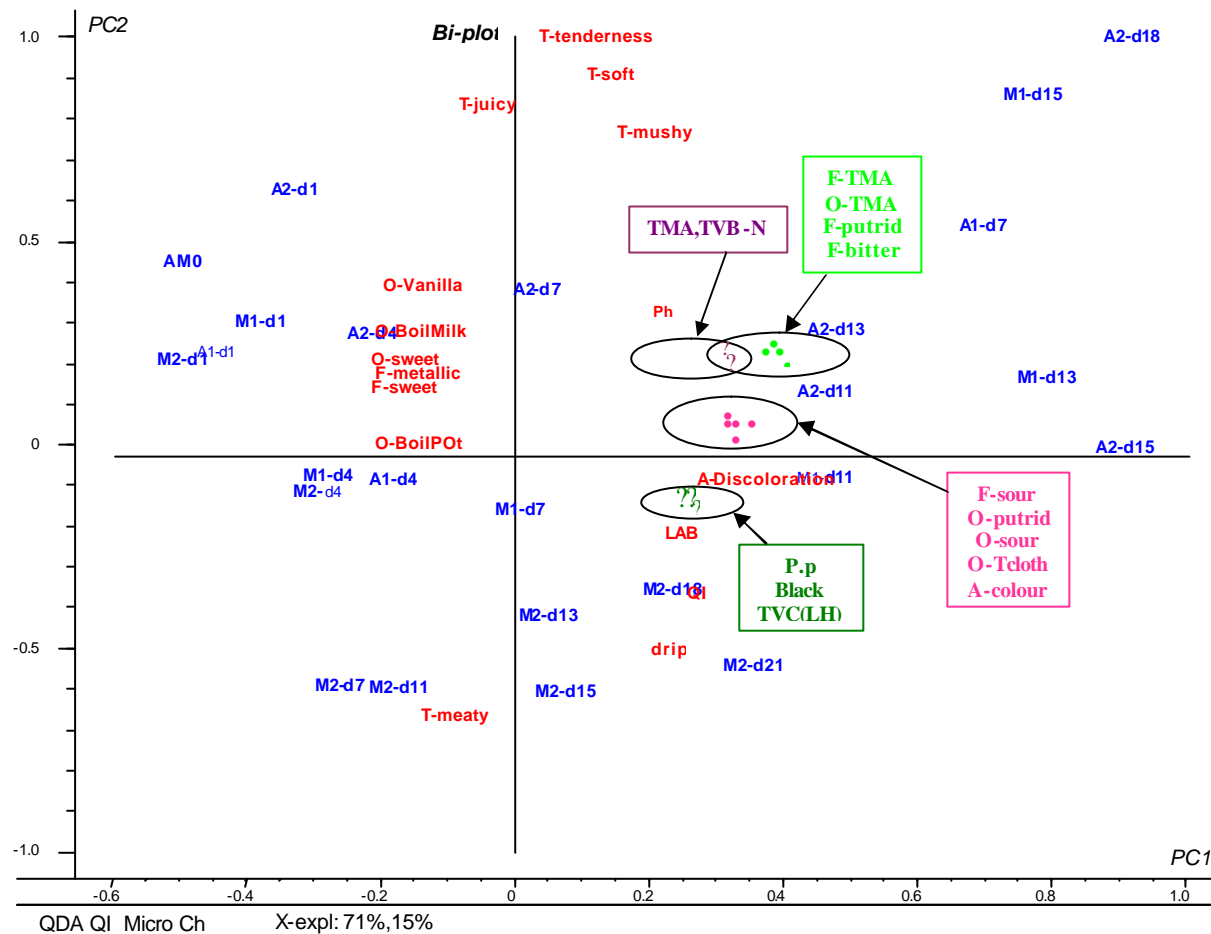
Figure 21: Bi-plot for the 1st and 2nd PCA of measured variables (pH, drip loss, TMA, TVB-N, TVC, H_2S-producing bacteria, *Photobacterium phosphoreum* and LAB counts, QI scores and all the QDA attributes) and sample groups. A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C. Letters indicate sample groups and numbers indicate storage days. F = flavour, O = odour, A = appearance, T = texture attributes.

4.10 Shelf-life determination

End of shelf-life is usually determined when sensory attributes related to spoilage such as TMA, sour, putrid odour and flavour become evident (Sveinsdottir *et al.* 2002, Bonilla *et al.* 2006, Magnússon *et al.* 2006). Those odours and flavours mainly have microbial origin (Huss 1995). When the average QDA scores for those attributes is above 20 (on the scale 0 to 100), most panelist detect those negative attributes which indicated that the sample is approaching the end of shelf-life. Based on this, the expected shelf-life should be around six days for A1 (stored in polystyrene boxes at 1.5°C), 13-14 days for A2 (stored in polystyrene boxes at -1.0°C), day 11 for M1 (MAP at 1.5°C), but M2 (MAP at -1.0°C) did not reach the end of shelf-life within the time span of the experiment (21 days). In short, the corresponding total shelf-life for A1, A2 and M1 is nine days, 16-17 days, and 14 days respectively and more than 24 days for M2 after catch.

For fresh cod loins stored in polystyrene boxes at 1.5°C, the shelf-life was shorter compared to what has earlier been reported for cod fillets. A shelf-life study of fresh cod fillets filleted one day after catch showed that the fillets had a shelf-life of around 10–12 days in ice (0 °C), or a total shelf-life of 11 to 13 days post catch according to Magnússon and Martinsdóttir (1995). Huss (1995) reported 14 days for packed cod at 0°C. Recently, Bonilla *et al.* (2006) reported that the maximum storage time of fresh cod fillets stored at 0–1 °C in ice was estimated to be eight days and concluded that the relatively shorter storage life might be due to the storage time of the whole fish from catch until filleting, which was from three up to five days. Regarding our experiment, the relatively shorter shelf-life of sample group M1 could be explained by the difference in experimental temperatures.

Total viable psychrotrophic counts (TVC) reached levels of log 7.4-7.9 at sensory rejection for the four storage groups. H_2S-producing bacteria represented about 15.9% and 43.7-44.7% total viable counts reaching loads of log 6.7 and log 7.52-7.54 respectively for group A1 and A2, indicating the importance of H_2S-producing bacteria in aerobically stored cod loins, while relatively lower levels were found for M1 and M2, reaching loads of log 5.67 and log 5.49, accounting for 1.8% and 1.2% of TVC respectively. Interestingly, the proportion of *P. phosphoreum* in TVC was similar (0.1-0.3%) for all groups and *P. phosphoreum* did not dominate the microflora in MA packed cod loins. The reasons are not clear and more studies are needed. Considering *P. phosphoreum* is a 30 times more active TMA producer than *Shewanella putrefaciens*, the most likely explanation is that *P. phosphoreum* in combination with H_2S-producing bacteria accounted for the spoilage of cod loins under all storage conditions.

At sensory rejection different levels of TVB-N and TMA were found in the experimental groups. It has been pointed out that often TVB-N and TMA give ambiguous information about the quality of the products and levels only increase at last storage when spoilage signs are obvious (Oehlenschläger 1997, Ólafsdóttir *et al.* 2005). TVB-N levels are also influenced by the storage method (Magnússon and Martinsdóttir 1995, Guldager *et al.* 1998).

Despite this controversy, fixed TVB-N limits (35 mgN/100 g) for acceptability of consumption for gadoids as a confirmation of a prior sensory assessment have been set in EU regulation (Anonymous 1995). Based on these limits the shelf-life of the experimental groups was evaluated. A slightly shorter shelf-life was estimated than when using the average QDA scores for attributes related to spoilage for group A1. The estimated shelf-life based on TVB-N criteria for group A2 was in agreement with shelf-life according to the sensory rejection limit. On the other hand, a relatively shorter shelf-life was estimated than when using sensory rejection limits for group M1.

The results of this study suggest that spoilage criteria previously suggested for fresh cod fillets cannot be applied uncritically and further research is needed to identify efficient and generally valid quality indices of spoilage for chilled and superchilled MAP cod loins.

Table 6: Overview of shelf-life estimation (based on the average QDA negative scores>20) and measured initial (first sampling day) values for the microbial, TVB-N, TMA, pH, and final estimated values for the experimental data at sensory rejection for all experimental groups of fresh cod loins stored in polystyrene boxes or MAP under chilled temperature and superchilled temperature.

	Initial values	Estimated values at sensory rejection			
		A1	A2	M1	M2
Estimated shelf-life (days)[a]		**9**	**16-17**	**14**	**>24**
Estimated shelf-life(days)[b]		8	16	12	24
QIM scores	1	4.5	7-8	7	6
TVC ($\log_{10}$CFU/g)	**4.72**	**7.50**	**7.87-7.9**	**7.42**	**7.42**
H_2S-producer counts	2.57	6.7	7.52-7.54	5.67	5.49
% H_2S-producer / TVC	**0.70**	**15.9**	**43.7-44.7**	**1.8**	**1.2**
P.phosphoreum counts	1.41	4.7	5.1-5.3	4.59	4.27
% *P.phosphoreum* / TVC	**0.05**	**0.2**	**0.2-0.3**	**0.2**	**0.1**
LAB counts		5.6	6.4-6.5	6.21	6.0
% LAB/ TVC		**1.3**	**3.2-4.3**	**6.2**	**3.8**
TVB-N (mg N / 100g)	10.8	42	37-57	46	36
TMA (mg N / 100g)	0	30	25-46	33	24
pH	6.63	7.15	6.99-7.15	6.97	6.84

a) Total shelf-life, including days from catch, based on the sensory evaluation of cooked loins (average QDA scores for attributes related to spoilage = 20)
b) Total shelf-life, including days from catch, based on TVB-N = 35 mgN/100g

5 CONCLUSIONS

Based primarily on sensory evaluation with QDA, but also on QIM, chemical and microbiological indices determination, MAP in combination with superchilled storage was the most effective way to extend the shelf-life of fresh cod loins.

Both modified atmosphere packaging and superchilled storage extended the shelf-life of fresh cod loins. MAP and superchilled storage alone increased the shelf-life from nine to 14 and 16-17 days, but when combined, a synergistic effect was observed and the shelf-life might be further extended to at least 24 days. Comparing the "marketable quality" (average score of the positive QDA odour and flavour attributes was above 20) of the cod loins of all storage groups revealed that the period of prime quality could be maintained up to 18 days. This could contribute to improved quality of fresh cod loins for consumers in distant markets.

QDA is an excellent indicator of freshness quality and may provide precise and detailed information on the time of storage under different storage conditions. But assessments by the quality index methods also give useful results, and in a more convenient and faster way. The use of the modified QIM scheme developed for fresh cod fillets in this study showed a clear linear relationship to storage time in polystyrene boxes under chilled and superchilled temperature conditions and in chilled MA packaging (A1, A2 and M1). The flesh gaping parameter did not adequately describe the freshness change during storage. The results of this study suggest that the category giving 3 points should be included in the 2 points category, but this modification will need additional trials. Relatively lower correlation (r^2=0.883) was found between the average QI and storage time in superchilled MA packaging. To better describe the sensory characteristics under superchilled MAP conditions, further investigations should include other attributes which reflect the endogenous enzymatic degradation when microbial growth is delayed.

No obvious texture difference was observed among chilled, superchilled and chilled MAP groups (A1, A2 and M1) during storage. However, MAP combined with superchilled storage might have an impact on the texture. There is a significant difference of meaty texture among superchilled MA packed cod loins and the other three groups after seven days of storage. This could be related to high drip loss caused by cell destruction due to slow freezing. New techniques such as CBC (Combined Blast and Contact), rapidly lowering the temperature of the fillets during processing, combined with MAP and subsequent superchilled storage may minimise ice crystal formation and further extend the shelf-life of fresh cod loins.

H_2S-producing bacteria contribute considerably to the overall spoilage of aerobically stored cod loins although the growth rate was slower in the superchilled group. Superchilled storage retarded *P. Phosphoreum* growth while H_2S-producing bacteria and LAB appeared to grow steadily and were less affected. MA packaging was effective in slowing down the growth rate of H_2S-producing bacteria. Neither *P. phosphoreum* nor H_2S-producing bacteria dominate the microflora at sensory rejection for the four storage groups. *P. phosphoreum* in combination with H_2S-producing bacteria accounted for the

high levels of TVB-N and TMA measured at sensory rejection time. It is noteworthy that the combination of MAP and superchilled storage had a synergistic effect on the inhibition of H_2S-producing bacteria, *P. Phosphoreum* and LAB growth.

QIM offers a fast and reliable procedure to evaluate the quality of fresh cod loins especially during the first period of storage. Compared with other quality indicators, QI scores seem more realistic to evaluate the marketability of the fresh cod loins, although some modifications are needed for superchilled MA packed cod loins. Results of Multi-indicator evaluation by PCA indicated that good correlation was found between QIM and TVC, H_2S-producer, *P. Phosphoreum*, demonstrating that these microbial variables gave similar information as QI scores, even though microbial action appeared to play a minor role in the deterioration of prime quality. On the other hand, TVB-N, TMA and pH gave more information about onset of putridity and values only increased at late stages when spoilage signs were obvious.

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APPENDIX I:

Experimental groups and sampling plan

	Whole cod		Loins		Sample group				Measurements			
Day	Storage time	Whole cod	Storage time	Loins	A1	A2	M1	M2	QIM-whole	QIM-loins	QDA/drip	Micro/chem
28 Nov	(Catch day)											
29 Nov	1											
30 Nov	2	x	Processing day: filleting						x	training	training	
1 Dec*	3		(0) packing in air/MA							x	x	x
2 Dec*	4		1	x	x	x	x	x		x	x	x
3 Dec	5		2									
4 Dec	6		3									
5 Dec	7		4	x	x	x	x	x		x	x	x
6 Dec	8	x	5						x			
7 Dec	9		6									
8 Dec	10		7	x	x	x	x	x		x	x	x
9 Dec	11	x	8						x			
10 Dec	12		9									
11 Dec	13		10									
12 Dec	14		11	x		x	x	x		x	x	x
13 Dec	15	x	12						x			
14 Dec	16		13	x		x	x	x		x	x	x
15 Dec	17	x	14						x			
16 Dec	18		15	x		x	x	x		x	x	x
17 Dec	19		16									
18 Dec	20		17									
19 Dec	21		18	x		x		x		x	x	x
20 Dec	22		19									
21 Dec	23		20									
22 Dec	24		21					x		x	x	x

APPENDIX II:

QDA attributes for cod products used for sensory evaluation of cooked loins with the QDA method

AttributeShort	AttributeName	Anchors on line scale:		Attributes Explained
		Left	**Right**	
O-Sweet	sweet odour	little	much	sweet odour
O-BoilMilk	odour of boiled milk	little	much	boiled milk, fruity/mushy odour
O-BoilPot	odour of boiled potatos	little	much	odour of boiled potatos
O-Vanilla	vanilla odour	little	much	vanilla odour, sawdust, timber
O-TCloth	table cloth odour	little	much	Reminds of a table cloth (damp cloth used to clean kitchen table, left for 36 hours on the table)
O-TMA	TMA odour	little	much	TMA odour, reminds of dried salted fish, amine
O-Sour	sour odour	little	much	sour odour, spoilage sour, acetic acid
O-Sulphur	sulphur odour	little	much	sulphur, matchstick
O-Putrid	putrid odour	little	much	putrid odour
A-Colour	colour	light	dark	light: light colour, white colour. Dark: yellowish, brownish, grey
A-Discol	discolouration	homogenous	heterogenous	Heterogenous: discoloured, e.g. Stains, discoloured to the ends
F-Sweet	sweet flavour	little	much	sweet flavour
F-Metallic	metallic flavour	little	much	metallic flavour
F-Sour	sour taste	little	much	sour taste, spoilage sour
F-Bitter	pungent flavour	little	much	pungent flavour, bitter
F-TMA	TMA flavour	little	much	TMA flavour, reminds of dried salted fish, amine
F-Putrid	Putrid flavour	little	much	Putrid flavour
T-Soft	softness	firm	soft	Evaluate how firm or soft the fish is during the first bite
T-Juicy	juiciness	dry	juicy	Evaluated after chewing several times: dry - pulls juice from the mouth
T-Tender	tenderness	tough	tender	Evaluated after chewing several times
T-Mushy	mushy texture	little	much	mushy texture
T-Meaty	meaty texture	little	much	meaty texture, meaty mouthfeel

Evaluated on a line scale approx 15cm (0% to 100%)

APPENDIX IIII:

Quality Index Method (QIM) scheme for whole cod (Gadus morhua)

Date: _____

Name: _____

Quality parameter		Description	Points	Score *codes:*			
Appearance	**Skin**	Bright, iridescent pigmentation	0				
		Rather dull, becoming discoloured	1				
		Dull	2				
	Stiffness	In rigor	0				
		Firm, elastic	1				
		Soft	2				
		Very soft	3				
Eyes	**Cornea**	Clear	0				
		Opalescent	1				
		Milky	2				
	Form	Convex	0				
		Flat, slightly sunken	1				
		Sunken, concave	2				
	Colour of pupil	Black	0				
		Opaque	1				
		Grey	2				
Gills	**Colour**	Bright	0				
		Less coloured, becoming discoloured	1				
		Discoloured, brown spots	2				
		Brown, discoloured	3				
	Smell	Fresh, seaweedy, metallic	0				
		Neutral, grassy, musty	1				
		Yeast, bread, beer, sour milk	2				
		Acetic acid, sulphuric, very sour	3				
	Mucus	Clear	0				
		Milky	1				
		Milky, dark, opaque	2				
Blood	**Colour**	Red	0				
		Dark red	1				
		Brown	2				
Fillets	**Colour**	Translucent, bluish	0				
		Waxy, milky	1				
		Opaque, yellow, brown spots	2				
Quality Index (0-23)			**Sum:**				

APPENDIX IV:
Quality Index Method Scheme for fresh cod loins - without skin

Name: _____   Date: _____

Quality parameter	Description	Score	Sample no			
Texture	Firm	0				
	Rather soft	1				
	Very soft	2				
Blood	Bright red, not present	0				
	Dull red	1				
	Shadowy, brown	2				
Odour	Fresh, neutral	0				
	Seaweedy, marine, grass	1				
	Sour milk	2				
	Acetic, ammonia	3				
Colour	White, greyish	0				
	Some yellowish, a little pinkish	1				
	Yellow, over all pink	2				
Bright	Transparent, bluish	0				
	Opaque	1				
	Milky	2				
Gaping	No gaping, one longitudinal gaping at the neck part of the fillet	0				
	Slight gaping less than 25% of the fillet	1				
	Slight gaping, 25-75% of the fillet	2				
	Deep gaping or slight gaping over 75% of the fillet	3				
Quality index (0-14)		**SUM**				

APPENDIX V:

Statistical analysis (ANOVA and Duncan's Multiple-Comparison Test) of microbial counts, values of physical and chemical parameters

Storage time (days)	group	TVC	H_2S-producer	Pp	LAB	Ph	TVB-N	TMA	drip
0	AM0								
1	A1	5.08	3.21	2.41	2.91	6.68[b]	10.52	0.00	
	A2	4.95	2.71	2.33	2.81	6.85[a]	11.45	0.09	
	M1	5.21	2.88	2.33	3.07	6.84[a]	11.57	0.09	0.87
	M2	4.68	2.64	2.19	2.63	6.62[b]	10.71	0.00	0.75
	p-value	0.2169	0.1000	0.9087	0.2174	0.0074	0.7255	0.5957	0.6948
4	A1	6.50[a]	5.48[a]	4.03[a]	5.09[a]	6.90[a]	13.53	1.75	
	A2	5.44[b]	3.84[b]	2.98[a]	4.59[b]	6.89[a]	11.54	0.00	
	M1	5.13[b]	3.64[b]	3.19[a]	4.41[b]	6.61[b]	10.43	0.00	1.26
	M2	4.91[b]	3.22[c]	1.74[b]	3.61[c]	6.70[b]	10.67	0.00	0.9
	p-value	0.0001	0.0000	0.0024	0.0002	0.0002	0.0693	0.1625	0.0520
7	A1	7.96[a]	7.32[a]	5.16[a]	5.79[a]	7.27[a]	56.62[a]	45.53[a]	
	A2	6.60[b]	5.85[b]	4.08[b]	5.53[a]	6.85[b]	13.40[b]	2.06[b]	
	M1	6.37[b]	4.35[c]	3.77[b]	5.50[a]	6.78[b]	18.44[b]	6.13[b]	1.91
	M2	5.06[c]	3.70[c]	2.00[c]	4.04[b]	6.65[b]	10.97[b]	0.00[b]	2.35
	p-value	0.0000	0.0000	0.0000	0.0031	0.0009	0.0000	0.0001	0.6564
11	A2	7.16[a]	6.60[a]	4.75[a]	5.79[a]	6.93[a]	31.45	15.23	
	M1	7.36[a]	5.52[b]	4.59[a]	6.15[a]	6.97[a]	46.12[a]	33.22[a]	3.62
	M2	5.53[b]	4.37[c]	2.76[b]	4.88[b]	6.51[b]	14.22[b]	1.91[b]	3.48
	p-value	0.0097	0.0006	0.0011	0.0006	0.0057	0.0275	0.0252	0.8440
13	A2	7.86[a]	7.49[a]	5.14	6.53[a]	6.99[a]	36.55[b]	25.47[b]	
	M1	7.91[a]	5.60[b]	4.93[a]	6.45[a]	7.14[a]	65.85[a]	56.01[a]	3.87
	M2	5.82[b]	4.90[b]	3.67[b]	5.30[b]	6.69[b]	13.28[c]	1.45[c]	5.27
	p-value	0.0128	0.0004	0.0560	0.0003	0.0087	0.0013	0.0010	0.2222
15	A2	7.97[a]	7.52[a]	5.68[a]	6.22[a]	7.29[a]	82.89[a]	65.76[a]	
	M1	7.32[b]	5.68[b]	5.58[a]	6.34[a]	7.31[a]	89.20[a]	74.22[a]	3.36
	M2	6.29[c]	5.02[c]	4.16[b]	5.27[b]	6.73[b]	22.90[b]	10.47[b]	4.83
	p-value	0.0001	0.0000	0.0038	0.0098	0.0001	0.0000	0.0001	0.2107
18	A2	8.29[a]	7.91[a]	5.17	6.59	7.46[a]	96.78[a]	80.15[a]	
	M2	7.21[b]	5.65[b]	4.47	5.97	6.70[b]	27.20[b]	15.74[b]	
	p-value	0.0054	0.0005	0.1542	0.0560	0.0021	0.0000	0.0001	

A1- stored in polystyrene boxes at 1.5°C, A2- stored in polystyrene boxes at -1.0°C, M1- stored in MAP at 1.5°C and M2- stored in MAP at -1.0°C. Values in the same column followed by different superscript letters are significantly different.

APPENDIX VI:

Comparison shelf-life study results between IFL and RIVO (SEAFOODplus project 2.2 seafoodsense)

<u>*Sensory evaluation of cod loins stored in polystyrene boxes with QIM*</u>

The results from the sensory evaluation of fresh cod loins stored under different conditions with QIM scheme are shown in Figure 22.

There was a linear relationship with high correlation between the average QI and storage time in polystyrene boxes at 1.5°C (IFL) (r^2=0.961) , -1°C (IFL) (r^2=0.986) and 0°C (RIVO) (r^2=0.988).

The slope of the regression line was 0.655, 0.523 and 0.432 respectively. The results apparently indicated that fresh cod lions stored at 1.5°C spoil faster than those stored at 0°C. It also need to be noted that cod loins stored at 0°C in RIVO received higher scores than those at 1.5°C in IFL after 1 day storage. The probable reason might be the temperature fluctuation during transport.
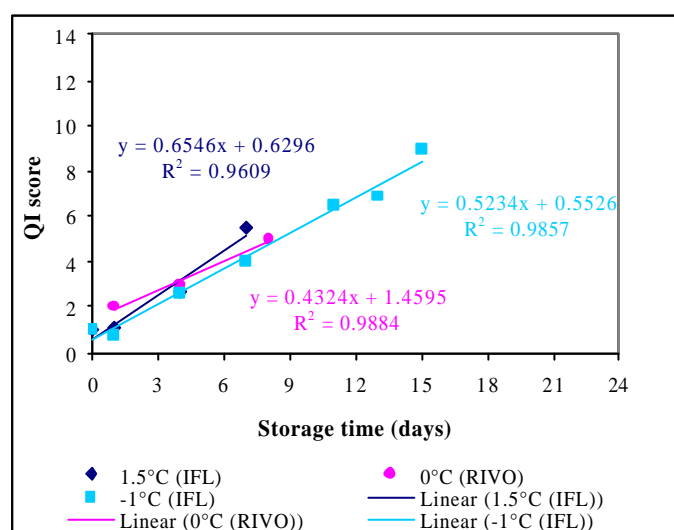

Figure 22: Average QI scores of each storage day analysed for fresh cod loins (*N* = 3) versus storage days.

<u>*Quantitative descriptive analysis*</u>

The scale used by IFL was 0-100, but the scale used at RIVO was 0-10. The scores from the RIVO panel were multiplied by 10 to make the results more comparable in Figure 23-Figure 26.

The comparison of the results showed that the IFL and RIVO panel appeared to use the scale differently for the attributes. Samples evaluated after 7 days at 1.5°C (IFL) received

high scores for attributes such as sour and TMA odour (Figure 23), but the sample evaluated after 8 days of storage at 0°C (RIVO) received lower scores for those attributes. However, the scores for the attribute sour odour were over 20 for both samples, indicating they were close to the end of shelf life. Similar results were observed for sour flavour (Figure 24).
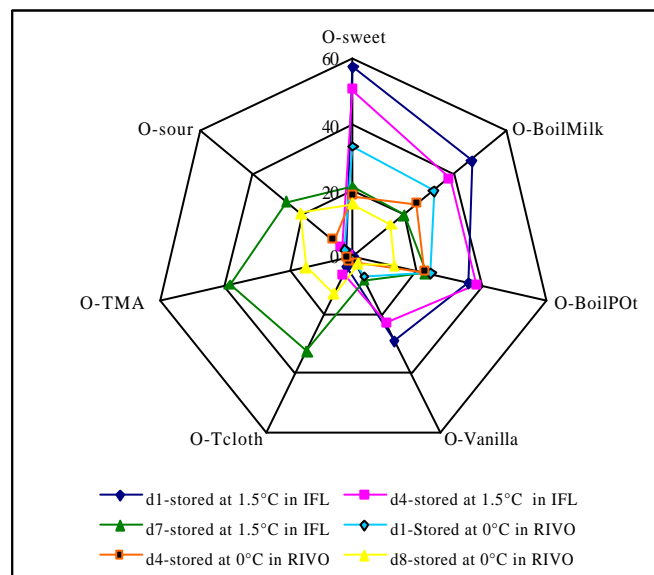

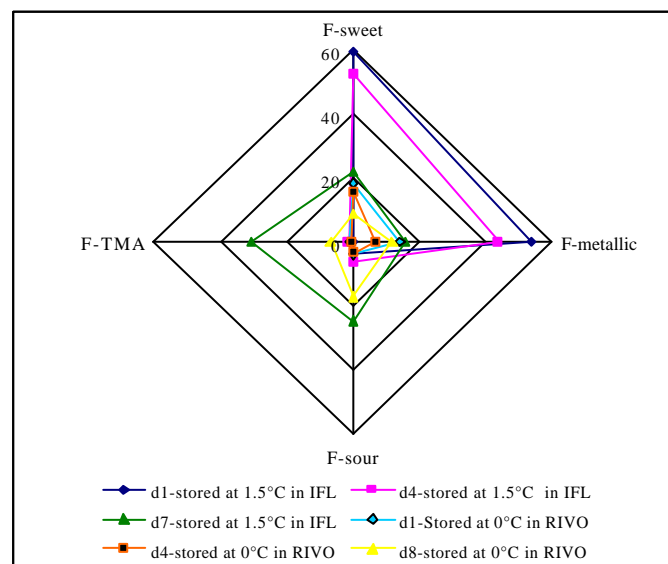Figure 23: QDA odour attributes (average scores, $N = 2$) of cooked cod loins versus storage days.

Figure 24: QDA flavour attributes (average scores, $N = 2$) of cooked cod loins versus storage days.
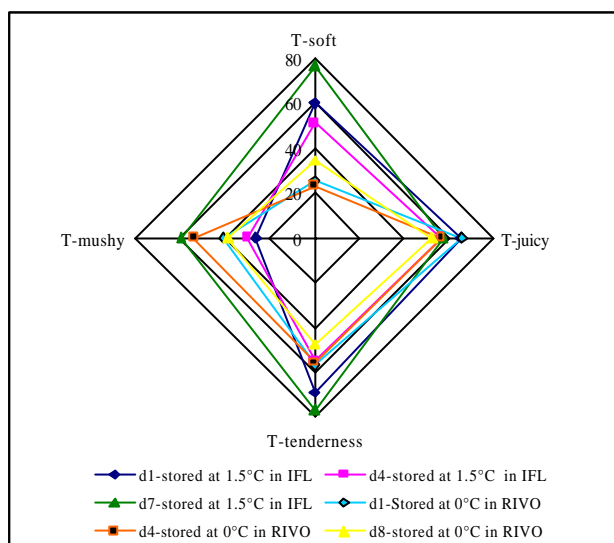
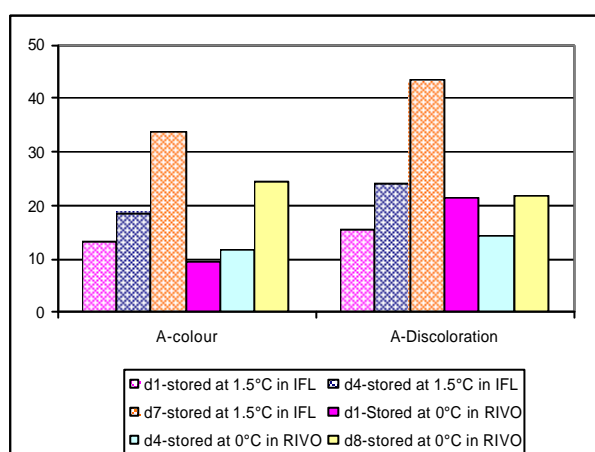Figure 25: QDA texture attributes (average scores, $N = 2$) of cooked cod loins versus storage days.

Figure 26: QDA appearance attributes (average scores, $N = 2$) of cooked cod loins versus storage days.

Sensory evaluation of cod loins stored in MAP with QIM

The MAP samples used for shelf-life study in RIVO were normal consumer MA packaging cod loins processed by Fjord Seafood Pieters in Belgium. The cod is caught in Iceland but not filleted by Nyfiskur (by another Icelandic supplier from FS Pieters).

It is different to compare the shelf-life of MA packed cod loins between the two trials carried out in IFL and RIVO, considering the different gas composition used, different storage temperature and storage time before packaging. Unfortunately, temperature loggers had not been used for consumer MAP packages. Information from RIVO

indicated that the temperature is about 2°C in distribution centers, while in supermarket stores the temperature is variable and maximal 7°C. The storage time of the cod loins from catch until packaging was around 3 to 5 days. Based on this, the maximum storage time of the uncontrolled consumer MA packed samples was less than 8 to 10 days. This is a shorter shelf life compared to the result from controlled experiment in IFL which was about 14d after catch for MA packed cod loins stored at 1.5°C. The reason might therefore be due to the high storage temperature and temperature fluctuation during the whole chain or delayed MA packaging. Previous studies showed that the effect of modified atmosphere packaging on the shelf-life extension of fish products is also influenced by the time of application. Exposure of susceptible organisms to CO_2 before growth begins prolongs the lag phase of spoilage bacteria (Gill and Tan, 1980). After bacteria enter the logarithmic phase of growth, the retarding effect of CO_2 is reduced. According to Lambert et al (1991), when MA packaging is delayed and bacteria growth has commenced, shelf-life of fresh meat can only be extended by 30%. However, if MA packaging is applied immediately while the spoilage bacteria are still in their lag phase, shelf-life extension can reach 50%. The results suggested that effective shelf-life extension could be obtained if MA packaging is applied immediately after filleting at the Icelandic fish production site.
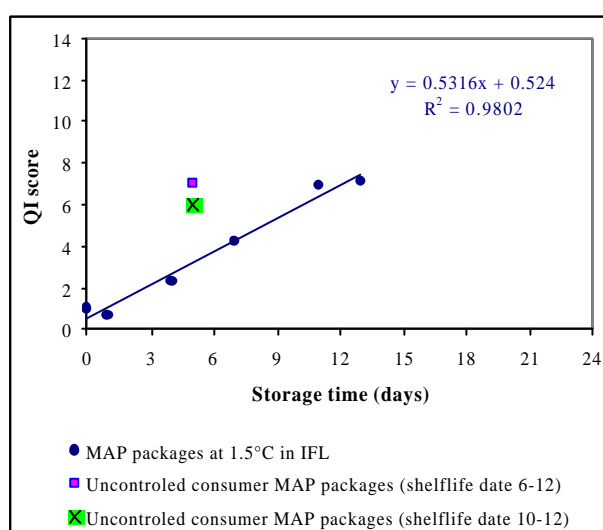

Figure 27: Average QI scores of each storage day analysed for fresh cod loins ($N = 3$) versus storage days.

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