Optimal storage conditions for fresh farmed tilapia (*Oreochromis niloticus*) fillets

By: CYPRIAN OGOMBE ODOLI

Supervisors: Sigurjón Arason and Emilía Martinsdóttir

MS committee: Hannes Magnússon, Hélène L. Lauzon and Kolbrún Sveinsdóttir

Thesis submitted in partial fulfilment of the requirements for the Degree of

MASTERS IN SCIENCE (60 ECTS units)

Department of Food Science and Nutrition

University of Iceland

May 2009

UNITED NATIONS UNIVERSITY
Fisheries Training Programme

Optimal storage conditions for fresh farmed tilapia (*Oreochromis niloticus*) fillets

Cyprian Ogombe Odoli

Supervisors: Sigurjón Arason and Emilía Martinsdóttir

MS committee: Hannes Magnússon, Hélène L. Lauzon and Kolbrún Sveinsdóttir

Thesis submitted in partial fulfilment of the requirements for the Degree of MASTERS IN SCIENCE (60 ECTS units)

Department of Food Science and Nutrition

Faculty of Science

University of Iceland

May 2009

Declaration

I wish to declare that this work has sole been done by me and has never been submitted at any other university before.

_____

Cyprian Ogombe Odoli

Dedication

I dedicate this thesis to my daughter Sharon for growing up at early age without seeing her father. More so, my beloved wife Lillian Awuor for accepting a lonesome life while I pursuit academic accomplishment.

Abstract

The main aim was to establish optimal storage conditions for fresh tilapia fillets, by determining its shelf life from the sensory and microbiological evaluation, as well as monitoring its physical-chemical properties. With this intent, Nile tilapia (*Oreochromis niloticus*) farmed in recirculation aquaculture system was filleted and packaged in 100% air and 50% CO_2: 50% N_2 MA prior to storage at different temperature; 1˚C and -1˚C. Initial samples from filleting (control d0) were also evaluated for comparison.

This report further describes the development of a Quality Index Method (QIM) scheme and a sensory vocabulary for fresh and cooked tilapia fillets accordingly and application in a shelf life study. The application of the QIM scheme for tilapia fillets showed a linear relationship between QIM scores and storage time with significant correlations ($r > 0.93$) for all sample groups experimented in the main study. The results from sensory analysis of cooked samples as well as microbial growth indicated fillets packaged in 100% air had a shelf life of 13-15 days during storage at 1˚C and 20 days during storage at -1˚C. At the end of shelf life in 100% air packaged groups, TVC and pseudomonads counts reached log 7 CFU/g in flesh. In MA packaged fillets, the lag phase and generation time of bacteria was extended and recorded counts below the limit for consumption (< log 4 CFU/g) up to 27 days of storage at both 1˚C and -1˚C. However, MA packaging affected negatively on fillets colour characteristics soon after packaging (as from d6) yet colour is an important indicator of quality and a major factor in influencing retail purchase decisions. Chemical analyses (TVB-N and TMA) were not good indicators of spoilage of tilapia fillets in the present study

Physical parameters (drip and water holding capacity) were observed to be a function of storage temperature and atmosphere, with storage at 1˚C as well MA recoding low quality (undesired) scores. 100% air packaged fillets stored at -1˚C recorded superior quality characteristics during storage as well as extended shelf life. For that reason, it is plausible that 100% air packaging at -1˚C storage temperature is the optimal storage conditions for fresh tilapia fillets.

Keywords: Shelf life, QIM scheme, tilapia fillets, air packaging, modified atmosphere

Table of contents

List of figures

List of tables

1. Introduction

1.1. General overview

The stagnation in the world's fisheries have provided impetus to domesticate fish species, just as overexploitation of land animals provided the impetus for the early domestication of land species. Aquaculture is one of the three major groups of activities that make human food available (Kinne, 1980). The other techniques agriculture and capture fisheries are rapidly nearing their inherent limit due to overexploitation and environmental degradation. World fish production increased from 40 million tonnes (t) in the early 60s to near 142 million t in recent years (FAO 2006a). During the last decade, this increase is mostly from growing aquaculture production amounting to 48 million t as wild fisheries have been essentially flat at about 97 million t (FAO 2006b).

The intensive farming of tilapia, *Oreochromis* sp. is rapidly expanding and tilapias (including all species) are the second most widely farmed fish in the world with annual production exceeding 2 million tons in 2005 (FAO, 2007). The six largest producing countries which, with the exception of Egypt, are all in Southeast Asia, account for nearly 90 per cent of total farmed tilapia production. China produces almost half of the worlds' tilapia, usually sold in the form of frozen products (FAO, 2006c; FAO, 2007). Several species of tilapia are cultured commercially, but Nile tilapia (*Oreochromis niloticus*) is the predominant cultured species worldwide. Nile tilapia is a tropical species native to Africa and one of the tilapia species with the Northern most range; it is more tolerant to lower temperatures than most of other tilapia species. The lower and upper lethal temperatures for Nile tilapia are 11-12 °C and 42 °C, respectively, while the preferred temperature ranges from 31 to 36 °C (FishBase, 2007).

Nile tilapia was introduced to developing countries and cultured at a subsistence level to meet local protein needs (FAO, 2007). As production techniques improved and off-flavours were controlled, tilapia moved into the mainstream seafood markets of these countries (FAO, 2006c). In highly industrialized countries, small markets for live local tilapia or frozen imports developed among immigrant communities (FAO 2002). Today, an important component of the growing tilapia industry is the proliferation of various product forms. Tilapia exports initially consisted of frozen whole fish from Taiwan Province of China, but the USA market preferred fillets, which were initially supplied by Jamaica, Colombia and Costa Rica as fresh products (FAO, 2006c). Today, fresh or frozen fillets are available in different sizes and packages, as skin-on, skin-off, deep skinned, individually quick frozen,

smoked, [or] 'sashimi' forms, and treated by carbon monoxide or ozone dipped (FAO, 2007). However, whole or gutted tilapia is still available but sold primarily in ethnic markets.

On world market, fishery products are among the most internationally traded food categories, in the sense that they have been widely crossing boarders (Moller, 2007). According to Moller (2007) thirty seven percent (live weight equivalent) of the total yearly production, estimated at around 130 million tonnes, enter international trade. Moreover, safety rules and regulations imposed by the consumer countries have continued to be more straight cut add challenges for the fish exporting countries. Fisheries business is therefore coherently becoming more concerned as regards quality and food safety.

The shelf life of fresh fishery products is usually limited by microbial activities that are influenced most importantly by storage temperature (Huss, 1995; Simpson *et al*., 2003). Typical shelf-life of fish fillets under icing and refrigerated storage conditions ranges from 2 to 14 days (depending on species, harvest location and season) and can result in heavy economic loss (Wilhelm, 1982; Reddy *et al.*, 1994; Sivertsvik *et al*., 2002). Furthermore, the consumers are increasingly demanding consistently high food quality, and have corresponding expectations that such quality will be maintained at a high level during the period between production and consumption (FAO, 2002; Creed and Pierson, 1999; Jones and Disney, 1996). This has made packaging an integral part of the food industry, as in addition to preservation function, it has several other important roles to play in delivering safe, wholesome and attractive foods to the market (Kilcast and Subramaniam, 2000).

1.2. State of the art

1.2.1. Changes during storage of chilled fish

Spoilage of fish is not clearly defined, but can be considered as any change that renders the product unacceptable for human consumption. Essentially, fish and seafood products spoilage is a consequence of various microbial, biochemical and chemical breakdown processes (Huis in't Veld, 1996). According to Sivertsvik *et al.* (2002), the degree of processing and preservation, together with storage temperature, will decide whether the fish undergoes microbial spoilage, biochemical spoilage or a combination of both. The initial quality loss is mainly due to the *post mortem* autolytic activity and chemical degradation processes, such as lipid oxidation. However, the most prevalent form in fresh fish chilled or not, is microbial spoilage (Bligh, 1980).

1.2.1.1. Microbial changes

Bacterial changes are considered the most important cause of fish spoilage (Gram and Dalgaard, 2002). This is because spoilage is often a result of off-odours and off-flavours caused by bacterial metabolism (Gram *et al*., 1990). Growth of a specific microorganism during storage depends on several factors, the most important being: the initial microbial loading at the start of storage; the physicochemical properties of food; the processing method used in the production of the food and the external environment of the food, such as the surrounding gas composition and storage temperature (Kilcast and Subramaniam, 2000).

Several authors have reported that microorganisms associated with most fish and fishery products reflect the microbial population in their environment (Adams and Moss, 2008; Liston, 1980; Gram and Huss, 1996; Shewan, 1977). However, the total number of organisms varies enormously depending on various factors. The wide range of environmental habitats (fresh water to salt water, tropical waters to arctic waters, pelagic swimmers to bottom dwellers and degree of pollution) and the variety of processing practices (iced to canned products), are factors determining the initial contamination of fish and fish products accordingly (Gram *et al*., 1996). The part of microflora which will ultimately grow on fish products will be determined by the intrinsic and extrinsic parameters. There are several intrinsic factors which greatly influence fish microbiology and spoilage, mainly: the poikilothermic (cold-blooded) nature of fish and its aquatic environment; a high post mortem pH in the flesh (usually > 6) and the presence of large amounts of non protein-nitrogen (NPN) (Huss *et al.,* 1997).

In general, fish caught in very cold, clean waters carry lower bacterial numbers compared with fish caught in warm waters (Gram and Huss, 1996). On live and newly caught fish, the microorganisms are found on the skin, gills and in the intestines. According to Adams and Moss (2008) and Huss (1995), the total numbers of organisms vary from 10^2-10^7cfu (colony forming units)/cm^2 on the skin surface and from 10^3-10^9cfu/g on the gills or intestines in fish. Bacteria from skin and gills are predominantly aerobic although facultative bacteria particularly *Vibrio* may occur in high numbers on pelagic fish. Obligatory anaerobic bacteria are common on the surface of fish but occur in significant numbers in the intestines. By contrast, the flesh of healthy live or newly caught fish is sterile. But when fish dies, the bacteria are allowed to proliferate in the beginning on the skin and during storage, they invade the flesh (Huss, 1988). Since fish are cold blooded, the temperature characteristics of the

associated flora will reflect the water temperatures in which the fish live (Adams and Moss, 2008).

It is well documented that bacteria in temperate regions are predominantly psychrotrophic, aerobic or facultative anaerobic Gram-negative, rod-shaped bacteria belonging to the genera *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrio*, *Photobacterium* and *Aeromonas* (Huss *et al*., 1997; Gram and Huss, 1996; Huis in't Veld, 1996). The microflora on fresh water fish is composed of similar genera with exception of *Vibrio* and *Photobacterium*. *Vibrio, Photobacterium* and *S. putrefaciens* which require sodium for growth and are typical of marine waters, whereas *Aeromonas* spp. is typical of fresh water fish (Sivertsvik *et al*., 2002). Although *S. putrefaciens* has been isolated from fresh water environment (Huss, 1995), it is not important in the spoilage of freshwater fish (Lima dos Santos, 1978; Gram *et al*., 1990).

The flora on tropical fish often carries a slightly higher load of bacteria predominantly Gram-positive and enteric bacteria (Gram and Huss, 1996; Liston, 1980). This suggests that fish from the tropics spoil much faster than fish from temperate waters by virtue of its high microbial load, if all other parameters are kept constant. When fish is held on ice however, the fish from warm waters keep longer than fish from cold waters (Gram *et al.,* 1990). This widely held view is apparently in relation to the relative proportions of psychrotrophic bacteria on fish from waters of different temperatures. Cold waters tend to favour proliferation of high numbers of psychrotrophs on fish, which in turn enhances spoilage at chilled condition and ultimately shortens the shelf life of fish (Karugi *et al.,* 2004). In addition, Huss (1995) reported bacteria on fish caught in temperate waters to enter the exponential growth phase almost immediately after the fish died, similar behaviour was observed even when fish were iced, and he explained it to probably the microflora having already adapted to the chill temperatures.

During fish storage, a characteristic flora develops but only a part of this flora contributes to spoilage (Huss, 1994). At chilled storage or low temperature (0-5°C), *Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas* spp. and *Pseudomonas* spp. have been reported, whereas at high temperature (15-30°C) different species of *Vibrionaceae, Enterobacteriaceae* and Gram-positive organisms were found to be responsible for spoilage (Liston, 1992; Gram *et al*., 1990; Gram *et al.* 1987). Gram *et al.* (1996) suggested that a clear distinction should be made between the terms 'bacterial numbers' (spoilage association) and 'spoilage organisms' since the first describes merely the bacteria present on the fish when it

spoils whereas the latter is the specific group that produces off-odours and off-flavours associated with spoilage. It is however very difficult to determine which of the bacteria isolated from the spoiling fish are responsible of causing spoilage. Spoilage bacteria are therefore characterised both by their dominance in the microflora of spoiling fish and their ability to produce spoilage compounds (Gram *et al.*, 1987). The bacteria most commonly identified with spoilage are species of *Shewanella* and *Pseudomonas* (Gram, 1992; Huss *et al.*, 1997). The latter has been found dominant in spoiling tropical or fresh water fish (Huss, 1994). Accumulated metabolic products of bacteria are the primary causes of the organoleptic spoilage of raw fish (Gram, 1992; Kraft, 1992; Gram and Huss, 1996).

The correlation between microbial growth and the development of chemical changes during spoilage has continuously been recognized as a means of revealing specific substrates and/or end products that may be useful for assessing food quality (Dainty, 1996; Huis in‘t Veld, 1996). However, the selection of the microbial association and the subsequent chemical changes during food spoilage depends not only on the imposed environmental conditions, as is well known, but also on microbial interaction (Lauzon, 2002). For example, although considerable data concerning the correlation between H_2S-producing bacteria (*S. putrefaciens*) and freshness have been collected, *Pseudomonas* spp. has not received the appropriate attention with regard to the effect of microbial interaction on spoilage. This may be important in understanding spoilage, since it has been found that there is an interaction between these bacteria. Indeed, *Pseudomonas* spp. can inhibit the growth of *S. putrefaciens* due to the ability of the former to produce siderophores, and this interaction can be the major factor governing the development of spoilage flora (Gram and Melchiorsen, 1992).

As aforementioned, the flesh of healthy live or newly-caught fish is sterile. This is due to the immune system of the fish which prevents the bacteria from growing in the flesh. When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely (Huss, 1988). The flesh becomes quickly contaminated by surface and intestinal bacteria and from equipments and humans during handling and processing. This is the case with fish fillets, most of the microbial contamination is found to occur during filleting and subsequent handling prior to packaging (Adams and Moss, 2008). Opening of the fish muscle by filleting creates conditions for the invasion of bacteria and oxidation in fatty fish since it increases the surface area to volume of the product (Adams and Moss, 2008). The experiment of Chytiri *et al.* (2004) reported changes in microbial flora of filleted rainbow trout during iced storage to be greater than those in whole rainbow trout. After contamination, the composition of wet fish

flesh makes it favourable to microbial growth. This unavoidable microbial contamination during processing of fillets makes fish fillets difficult to sustain prolonged storage even under refrigeration (Whittle *et al*., 1990; Duun and Rustad, 2007). There is however limited information of microbial composition of filleted tilapia in comparison to whole tilapia during storage.

1.2.1.2. Biochemical changes

The biochemical composition of fish or intrinsic factors and their interrelationships with post-mortem extrinsic factors, contribute substantially to the perishability of fish as a food commodity because they determine the initial contamination (Huss *et al.*, 1997). The physical, chemical and bacteriological characteristics of fish tend to vary with species, feeding habits, season (Smith *et al.*, 1980), spawning cycles, methods of catching, fishing ground (Whittle *et al.*, 1980) size, age, environment, initial microbiological load and possibly geographical location (Shewan, 1977). These factors are observed in wild free living fish in open sea and inland waters. According to Huss (1995), aquacultured fish may show variations in chemical composition, but in this case several factors are controlled, and thus chemical composition may be predicted. During chilled fish storage a number of inter-related systems take place. Among those factors are changes in protein and lipid fraction, degradation of nucleotides with the subsequent formation of amines (volatile and biogenic), hypoxanthine and action of certain bacteria (Surti *et al.*, 2001). As a consequence of these events, deterioration in sensory quality, a loss of nutritional value and negative modifications of physical properties of fish muscle has been reported (Olafsdottir *et al*., 1997).

The pH of muscle tissue of live fish is close to neutrality (Huss, 1995). During the later post-mortem changes, pH is more or less constant or slightly increased due to the formation of basic compounds (Shewan, 1977; Huss, 1988). Even though the changes in pH are generally rather small, they have great technological importance. The post-mortem pH is according to Huss (1988), the most significant factor influencing the texture of the meat and the degree of "gaping", for example the rupture of the connective tissue. The reasons for this is that even minor changes in pH drastically affect the properties of the connective tissue and the net surface charge on the muscle proteins is reduced causing them to partially denature and loose some of their water holding capacity (Huss, 1995).

One of the most important chemical spoilage processes are changes taking place in the lipid fraction of the fish. After death, the lipids in fish are subjected to two major changes, lipolysis

and auto-oxidation (Hardy, 1980) which constitute important chemical spoilage processes in fish (Huss, 1994). Fish fats (lipids) contain a high proportion of polyunsaturated fatty acids that react with atmospheric oxygen. The end products are aldehydes and ketones, which impart strong rancid flavour normally associated with spoilt fatty fish (Huss, 1994). In chilled fresh fish and even in fish stored at ambient temperatures, lipid oxidation does not seem to be a dominant spoilage process, though in the later stages of spoilage of fattier species such as trout, sardine, herring and mackerel, rancid flavours affect acceptability (Sikorski and Kolakoski, 2000; Whittle *et al*., 1990).

The high degree of perishability in fish is primarily due to the large amounts of non-protein nitrogen (NPN) such as free amino acids, volatile nitrogen bases (TVB-N) i.e. ammonia, trimethylamine (TMA), creatine, taurine, uric acid, carnosine and histamine (Malle and Poumeyrol, 1989). The readily available NPN compounds support the post-mortem bacterial growth (Connell, 1990; Huss *et al*., 1997). TVB-N, TMA and other volatile amines are commonly used as indicator for fish deterioration in fresh and lightly preserved seafood (Olafsdottir *et al*., 1997). Volatile bases result from degradation of proteins and non-protein nitrogenous compounds.

Changes in some of these compounds (Volatile nitrogen bases) are influenced most importantly by microorganisms. One example is the reduction of trimethylamine oxide (TMAO) in chilled marine fish by a bacterial process with the formation of TMA (Gram and Huss, 1996). TMAO constitutes a characteristic and important part of the non-protein nitrogen fraction (NPN-fraction). It is found in all marine fish species in quantities from 1 to 5 % of the muscle tissue (dry weight). Hebard *et al.* (1982) noted as with many earlier investigators that TMAO is virtually absent from freshwater species and terrestrial organisms, a fact that should be reviewed. Some studies have reported TMA in fresh water fish; tilapia (Waliszewski and Avalos 2001; Reddy *et al*., 1995) and Nile perch (Gram *et al*., 1987). TMA in farmed tilapia, according to Waliszewski and Avalos (2001) is due to marine fish meal fed to tilapia.

1.2.1.3. Sensory changes

Seafood products are perishable and their sensory characteristics depend on various factors, such as packaging methods, storage methods and time. The sensory characteristics of different species are very different, whether raw or cooked (Martinsdottir *et al.*, 2001). The first sensory changes of chilled fish during storage are concerned with appearance, texture and odour. Indeed, physical properties as firmness and appearance are strictly related to storage

days because of cellular flaking of autolytic and microbial changes (Fletcher and Statham, 1988). Freshly caught fish contains low levels of volatile compounds which contribute to the fresh like odours, whereas the characteristic sour and rotten odour at the end of shelf life originates from short chain fatty acids, alcohols, sulphur compounds and amines generated by microbial activity (Olafsdottir *et al.*, 1997). In cod fillets for example, at the beginning of storage time the skin is bright with iridescent pigmentation, the flesh texture firm with fresh or neutral odour (Bonilla *et al.*, 2007). Whereas, at the end of shelf life the skin becomes dull, the flesh texture very soft with sour, acetic or ammoniac odour. In cooked fish products, quality is directly related to the initial quality of fresh raw material (Olafsdottir *et al.*, 1997). Generally, the spoilage of marine temperate-water fish is characterised by development of offensive, fishy, rotten, H_2S off-odours and off-flavours. This behaviour is different from some tropical and fresh water fish, where fruity, sulphydryl off-odours and off–flavours are more typical (Gram and Huss, 1996).

The difficult in retaining the colour and appearance of the meat encountered during handling, processing and storage of fish and fish products has been reported to be the driving force behind increasing utilization of CO gas in US (Otwell *et al.*, 2003; Kristinsson *et al.*, 2005). This discoloration is highly undesirable as the product becomes less appealing to the consumer, thus leading to a lower price than if the product had a more favourable colour.

1.2.2. Fish chilling methods and applications

The most important factor influencing the composition of microflora is temperature. Use of temperature reduction as a means of preserving fish and fishery products is very important worldwide both for local and export markets. In the context to this study, chilling is defined as the process of cooling fish or fish products to a temperature close to its freezing point by means of heat withdrawal. There are other cooling agents used in fish chilling other than the traditional use of ice. Fish chilling constitutes of two major cooling methods namely conventional/traditional chilling and superchilling.

1.2.2.1. Conventional chilling and superchilling

Conventional chilling may be defined as the process of cooling fish or fish products to a temperature approaching that of melting ice. Superchilling on the other hand, has been defined differently by different authors. Duun and Rustad (2007) describe superchilling as a process where fish products are stored between the freezing point of the products and 1-2°C below it. Whereas, Ando *et al.* (2005) defines it as the temperature zone below 0°C but where

ice crystals are not generated. The aforementioned definitions and many more from other authors, positions superchilling between freezing and refrigeration (conventionally chilling), where the surrounding temperature is set below the initial freezing point of the product without freezing it. Frozen storage is very effective in extending storage life. However free drip loss occurs during thawing in addition to ice crystals damage to muscle cells consequently resulting into muscles softening. These unresolved problems which occur during frozen storage, have lead to new superchilling concept that poses the advantages of both freezing and refrigeration (Ando *et al.*, 2005). Chilling depresses the activity of putrefactive microorganisms and enzymes which cause undesired changes and the aging process of fish is slowed. Chilling time depends on the properties of both the cooling media and the product, such as thermal indices of the product, specific gravity, temperature of the surrounding media and heat transfer coefficient between the fish and the medium.

Rapid chilling of fish is achieved by the use of a chilling media with high thermal indices, maintenance of lowest possible temperatures of the media during the chilling process and the circulation of the liquid or gaseous media (LeBlanc and LeBlanc, 1992). Chilling is much more rapid in a liquid medium than a gaseous one. The freezing point for different fish species varies between -0.6 and - 2°C and depends entirely on the concentration of the cell fluids. Cooling agents used for the conventional chilling and superchilling of fish and fishery products are fresh water ice, liquid ice, ice packs, gel packs, refrigerated sea water (RSW) (Huss, 1995), refrigerated air, chilled sea water (CSW), brine, dry ice (solid CO_2) and liquid nitrogen (LeBlanc and LeBlanc, 1992). Each agent has its advantages and disadvantages.

In practical situation, Martinsdottir *et al.* (2002) applied a slurry ice system for the storage of sea bass a warm water fish species and contrarily to advantages of slurry ice over flake ice (LeBlanc and LeBlanc, 1992), the authors reported no significant differences in the spoilage rate observed between the flake ice batch and the slurry ice batch. However, in other studies a lower microbial development which correlated directly with production of TMA and TVB-N was observed in slurry system than in counterpart specimens stored under flake ice (Rodriguez *et al*., 2006; Rodriguez *et al* 2004). Quality changes during storage of fresh water Nile perch (*Lates niloticus*), showed fish stored at ambient temperature (20-30°C) spoiled rapidly and was unacceptable for human consumption after 11-17 hours, whereas icing ensured a storage life of 4 weeks (Gram *et al*., 1990).

It has however been observed that chilled warm water fish keeps longer than temperate species. Ola and Oladipo (2004) reported extended storage life of iced Croaker

(*Pseudotholitus senegalensis*) warm water fish compared to temperate fish species. Gram *et al.* (1990) similarly noted that chilled fish caught in temperate waters rarely keeps more than 2-3 weeks in ice whereas storage trials in the study confirmed that fish caught in warm waters often have extended chilled storage lives. Several proposals have been made to explain the long iced storage lives of warm water fish, among them the earlier mentioned lower initial number of psychrotrophic bacteria, a different flora or a non bacterial spoilage (Gram *et al*., 1990). This can in other words be explained by the flora found on warm water fish which are dominantly mesophilic and natural incidence of psychrotrophic bacteria is low. Therefore rapid chilling and sustained low temperature storage can result in long shelf life. On the other hand warm water fish may undergo rapid spoilage if chilling is delayed. The statement is in conformity with observations by Surti *et al.* (2001) who reported that in absence of ice, the warm water fish spoilt much faster than temperate water fish.

Superchilling is one of the few promising techniques with the potential to preserve prime quality of fresh fish when compared to conventional chilling (Einarsson, 1988; Duun and Rustad, 2007). The technique minimises microbial as well as autolytic reactions that tend to occur above and below the freezing point. Studies published on superchilled storage of fish have shown extended fish storage life (Olafsdottir et al., 2006; Lauzon and Martinsdottir, 2005; Ando *et al*., 2005). Olafsdottir *et al.* (2006) reported shelf-life of superchilled cod fillets based on Torry score and TVB-N to be 15 days at -1.5°C compared to 11 days for iced chilled cod fillets. Superchilled salmon fillets stored at -2°C had a 21 days sensory shelf-life, whereas stored at chilled conditions were spoiled after 7 days (Sivertsvik *et al*., 2003). In addition to extended storage life, superchilling under strictly controlled refrigerator, overcame unfavourable changes in fish quality such as discolouration compared to traditional refrigeration (Ando *et al*., 2005; Ando *et al*., 2004). For consumers, the colour of fresh foods provides an easily understood index of food freshness. However, the authors noted a problem with the technique that remains unsolved to be how to improve the strict temperature control system in practical situation since the effective temperature control for large storage rooms is difficult to achieve.

The challenge encountered with superchilling is that when some of the water freezes out, the concentration of solutes in unfrozen solution increases. This may lead to denaturation of the muscle proteins as well as structural damage of membranes, which can result in increased drip loss, loss of water holding capacity and textural changes (Duun and Rustad, 2007). Increased enzymatic activity has also been suggested during storage at subzero temperatures (Foegeding

et al., 1996). Despite this, superchilled products could be more attractive than frozen ones, since the amount of water frozen out is lower in superchilling compared to freezing. Less water frozen out will lead to less change in microstructure, which in turn may result in a lower degree of freeze denaturation and less drip loss (Einarsson, 1988).

1.2.3. Combination of chilling and packaging

In view of the increasing demand for fresh fish products, there is the need of shelf-life extension through a combination of methods in order to minimally expose the product to adverse conditions (Soccol and Oetterer, 2003). Such increase in shelf-life brings greater industrial advantages once it reduces losses in distribution and display of the product at retail stores, which may lead to improvements in marketing of fresh products and stabilize the supply at reasonable price (Lioutas, 1988). Promising results have been reported on combined chilling and different packaging methods such as modified atmosphere (Wang *et al*., 2008; Soccol and Oetter, 2003; Sivertsvik *et al*., 2002), vacuum packaging (Silliiker and Wolfe, 1980) and solid carbon dioxide (LeBlanc and LeBlanc, 1992).

1.2.3.1. Vacuum packaging and chilling

In this technology, the product is placed inside a type of packaging presenting low permeability to oxygen and the air is exhausted causing the bag to collapse around the product before it is sealed (Adams and Moss, 2008). The gaseous atmosphere of the vacuum packaging is reduced, but it is probably altered during storage, thus considered modified due to 10-20% increase in the CO_2 amount produced by microbial activity utilising residual oxygen. This CO_2 may inhibit the growth of aerobic microflora normally associated with the spoilage of fish and fish products (Silliker and wolfe, 1980). The microflora that develops is dominated by lactic acid bacteria which are metabolically less versatile. In marine vacuum packed fish spoilage is in most cases associated to *S. putrefaciens* which utilise oxygen atom donated by TMAO under anaerobic condition resulting in increased rate of TMA formation. However, with the expanding range of chilled foods stored under vacuum packed and availability of vacuum packing equipment for small scale catering and domestic use, there is increasing concern over the risk of psychrotrophic *Clostridium botulinum* occurrence (Adams and Moss, 2008). The toxin (botulism) formation in fresh fish appears to be dependent on the amount of oxygen available, as is also the case with smoked fish (Huss, 1981; ICMSF, 1980). Vacuum packaging fish in airtight bags considerably reduces oxygen pressure inside bags, but anaerobic conditions are established by oxygen exhaustion, following growth of the associated flora (Huss, 1981). Therefore storage of vacuum packed fresh and smoked fish at

chilled temperature may delay the onset of spoilage but permit toxic production by *C. botulinum* type E because of anaerobic condition and potential to grow at temperatures down to about 3°C.

1.2.3.2. Gas packaging (MAP) and chilling

MAP is defined by Soccol and Oetterer (2003) as a process where air inside the packaging is replaced by specific gas or a mixture of gases that differ from the air composition. The proportions of each gas are established, the mixture is introduced into the packaging and no further control is carried out during storage (Sillker and Wolfer, 1980). The use of modified atmosphere with enhanced carbon dioxide level has been shown to extend shelf-life of foods by retarding microbial growth. However, without proper storage temperature, the benefits of MAP may be lost (Sivertsvik *et. al.,* 2002) and hence the need for combination. Numerous studies have shown MAP to perform an effective synergy with superchilling in prolonging the shelf life of fresh cod loins and Atlantic salmon fillets (*Salmo salar*) fillets (Wang *et al.*, 2008; Duun and Rustad, 2008; Sivertsvik *et al*., 2003). Combined use of CBC (combined blast and contact), MAP and superchilled storage yielded an increased sensory shelf life of 3 to 4 days compared to superchilled aerobic storage of cod fillets (Lauzon and Martinsdottir, 2005). Recent study by Duun and Rustad (2008) reported superchilled salmon fillets stored at -2°C in combination with modified atmosphere packaging (MAP) maintained good quality with negligible microbial growth for more than 24 days based on both sensory and microbial analyses. Whereas, ice chilled reference fillets from farmed salmon of premium grade, maintained good quality up to 17 days. Combining MAP and superchilling produces effective synergy because the low temperatures inevitably lead to more dissolved CO_2 in the product and consequently greater inhibitory effects, which results in low microbial and enzymatic activity and thus uncertainties concerning the microbial safety as food-borne pathogens are minimised in the product. However, Fletcher and Statham (1988) noted that often it is only the period of moderate to low quality that is extended and not the initial period of prime quality.

The three main commercially used gases are carbon dioxide (CO_2), nitrogen (N_2) and oxygen (O_2). Carbon dioxide is the most important gas used in MAP of fish, because of its bacteriostatic and fungistatic properties (Soccol *et. al.*, 2005; Sivertsvik *et. al.*, 2002). It inhibits growth of many spoilage bacteria. Such an effect is influenced by the CO_2 concentration, initial bacterial population, storage temperature and species fat content/ product type (Reddy and Armstrong, 1992). CO_2 is highly soluble in water and fat being the

main responsible gas for the bacteriostatic effects (Soccol *et. al., 2003*), and the solubility increases greatly with decreased temperature (Sivertsvik *et. al.,* 2002). Therefore, the effectiveness of the gas is always conditioned by the storage temperature with increased inhibition of bacteria growth as temperature is decreased.

A drop in surface pH observed in MA products because of the acidic effect of dissolved CO_2, could not entirely explain all CO_2 bacteriostatic effects (Sivertsvik *et. al.,* 2002). Most microorganisms grow best at pH values around 7.0 (6.6 - 7.5), whereas few grow below 4.0 (Huss, 1995). According to Daniels *et al.* (1985), several theories can explain the ways that CO_2 influences bacterial cells, the most important being:

- Alteration in cell membranes functions, including effects on nutrient uptake and absorption;
- Direct inhibition of enzymes or decrease in the rate of enzymes reactions;
- Penetrations in the bacterial membranes, leading to intracellular pH changes;
- Direct alterations in physic-chemical properties of proteins.

Probably a combination of all these activities account for the bacteriostatic effect.

A certain amount of CO_2 has to dissolve into the product to inhibit bacterial growth. The ratio between the volume of gas and volume of fish or food product (G/P ratio) should usually be 2:1 or 3:1 respectively (Sivertsvik *et. al.,* 2002). In food presenting high moisture and/or fat amount such as fish, beef and poultry, the excessive absorption of CO_2 may lead to a phenomena known as 'packaging collapse' (Parry, 1993). The high G/P ratio is also necessary to prevent package collapse. Too high CO_2 concentrations in the atmosphere may also have negative impact on drip loss, colour, texture and flavour in the product. These can be explained by the pH drop caused by CO_2 dissolving in the muscle tissue, resulting in a decrease in water holding capacity of the proteins, the denaturation of the muscle and pigment protein, as well as development of sour odour and flavour (Huss, 1995).

Nitrogen can be used as an inert gas in smaller proportions than CO_2. N_2 is an insipid and inert gas, showing low solubility in water and lipids. It is used for displacing the oxygen from the packaging, decreasing oxidative rancidness and inhibiting the growth of aerobic microorganisms. Due to its low solubility, it is used as a filler gas preventing the possible packaging collapse caused by accumulation of CO_2. The use of oxygen in MAP is normally set as low as possible to inhibit the growth of aerobic spoilage bacteria. However, for some products oxygen could or should be used. High levels of oxygen have been used in red fish

meat (tunas and yellow tails, etc.) to maintain the red colour of the meat, reduce and retard browning caused by formation of metmyoglobin (Oka, 1989). However, MAP associated with high CO_2 levels improves the stability of fresh fish, increasing its shelf life (Soccol and Oetterer, 2003). Numerous articles on shelf life studies of fish under MAP has been written, some reporting a tremendous increase in shelf life, others reporting little or no shelf life extension. But more often an extension in range of 30-60% for fresh fish products using atmospheres with elevated levels of CO_2 has been observed (Sivertsvik *et. al.*, 2002).

Under MAP, the growth of common spoilage microorganisms is inhibited and microaerophilic strains of lactic acid bacteria become the dominant spoilage organisms (Soccol and Oetterer, 2003). The knowledge of SSOs of different fishes from various aquatic environments and under different MAP conditions is still limited (Sivertsvik *et. al*., 2003). In CO_2-packed fish, the growth of *Shewanella putrefaciens* and of many other microorganisms found on live fish is strongly inhibited. However for cod stored under MAP and vacuum packs at 0˚C, the Gram-negative organism *Photobacterium phosphoreum* has been identified as the main organism responsible for spoilage (Dalgaard *et al., 1997*). Dalgaard *et al.* (1997) studied the *P. phosphoreum* growth in fresh MAP fish by means of 20 experiments conducted in Denmark. The authors did not detect the presence of *P. phosphoreum* in fresh water fish, despite finding great growth (>10^7 CFU/g) in all marine species. *P. phosphoreum* reduces TMAO to TMA while very little H_2S is produced during growth in fish substrates. *Brochothrix thermosphacta* has also been found during spoilage of modified atmosphere packaged fish and meat (Lund et al., 2000).

The toxin production of *Clostridium botulinum* has been reported to increase for bacteria growing under anaerobic conditions, and this may be of importance for the safety of packed fish (Huss *et al.*, 1988; Reddy and Armstrong, 1992). Huss (1981) illustrated that packaging of fish in oxygen-permeable polyethylene with or without vacuum does not influence the botulinogenic properties. Therefore a potential botulism hazard created by packaging fresh fish in a controlled atmosphere is only related to the amount of oxygen present. Packaging fish in anaerobic atmosphere does not significantly influence the storage life of white fish, while that of fatty fish may be nearly doubled (Huss, 1981). This is because fish fats contain a high proportion of polyunsaturated fatty acids which react with atmosphere oxygen causing rancidity (Huss, 1995). It is therefore recommended to use oxygen when packaging white (lean) fish.

The colour of the fresh meat is an important indicator of quality and a major factor in influencing retail purchase decisions (Ross, 2000). With the introduction of modified atmospheric packaging (MAP) during the 1960's, it became more obvious that product exposure to gas blends with carbon monoxide (CO) yielded favourable colour development and retention (Otwell *et al.*, 2003). Studies have already shown the ability of CO to improve the colour and quality of fish (Kristinsson *et al.*, 2005; Chow *et al.*, 1998). When muscle foods are treated with CO, it binds with the ferrous iron of myoglobin and forms a stable complex. The stable CO-myoglobin complex provides the desirable red colour of meat. Usually, meat products are treated with CO as a part of modified atmospheric packaging (Hunt *et al.*, 2004). The use of CO has created controversy as it may mask spoilage because a stable cherry red colour can last beyond the microbial shelf life of the meat (Li et al., 2008) and more importantly could potentially hide underlying safety problems (Otwell *et al.*, 2003). Its use has therefore been banned in some countries such Japan, Canada, Singapore and the European Union (Li et al., 2008). In US, it has been observed that while there is a definite growing market for CO treated products based on product appeal and convenience, there is debate due to concerns for preserving, enhancing and masking of inferior products.

1.2.4. Methods for measuring fish freshness

The methods for evaluating wet fish quality may conveniently be divided into two categories: sensory and instrumental (or conventional) methods. Sensory evaluation is the most important method today for freshness evaluation in the fish sector, because the consumer is the ultimate judge of quality (Huss, 1988). However, sensory methods must be performed scientifically under carefully controlled conditions so that the effects of the test environment and personal bias may be reduced (Huss, 1988). Most sensory characteristics can only be measured meaningfully by humans. The sensory evaluation of raw fish is mostly done by scoring system based on changes taking place during fish storage. There are several methods used in sensory evaluation of seafood e.g. EU scheme, Quality Index Method (QIM), Torry scale, raw fillets grading method and Quantitative Descriptive Analysis (QDA).

The main advantages of the QIM method when compared to EU scheme is that QIM is specific for each species and confusion about attributes is minimized (Martinsdottir *et al*., 2001; Sveinsdottir *et al*., 2003). Each fish species has its own characteristic sensory attributes of flavour, appearance, odour and texture which change with time and temperature after harvest (Olafsdottir *et al*., 1997). The QIM schemes have been developed for species such as European cuttlefish (*Sepia officinalis*) (Sykes *et al.*, 2009), Arctic charr (*Salvelinus alpinus*)

(Cyprian *et al*., 2008), fresh cod (*Gadus morhua*) fillets (Bonilla *et al*., 2007), Mediterranean hake (*Merluccius merluccius*) (Baixa-Noguerras *et al*., 2003), farmed Atlantic salmon (Sveinsdottir *et al*., 2003) and frozen hake (*M. Capensis and M. Paradoxus*) (Herrero *et al*., 2003). It is equally important to have information about the maximum shelf life of the product and thus the need to use QDA to evaluate sensory attributes of cooked fillets, including spoilage attributes to estimate the maximum storage life. The success of a descriptive test depends largely on the sensory language describing the attributes of the products to be evaluated (Stone and Sidel, 1985).

On the other hand, instrumental (or conventional) methods namely; physical, microbiological and chemical are universally applied in food sector. Microbiological examination of fish evaluates the possible presence of bacteria or organisms of public health significance and gives an impression of the hygiene and quality of the fish including temperature abuse and hygiene during handling (Gram *et al*., 1987; Howgate, 1982). Chemical methods involve analysing a sample to determine the concentration of a specific chemical (Howgate, 1982). The concentration is used to indirectly measure or predict the level of a specific sensory attribute, which allows for the immediate determination of freshness (Olafsdottir *et al*., 1997).

1.3. Statement of the problem

With the alarming and logarithmically increasing human population and the continuing expectations of growth in the standards of living, demand for human food is becoming intense. However, in recent years the fishing sector has suffered from dwindling stocks of traditional species as a result of dramatic changes in availability. In Europe, cod catch has been declining and Iceland in particular, it's evident by the reduced annual quota allocation to fishers by the government (FAO, 2001). Fish farming may provide the opportunity to obtain a seasonally independent supply of fish to the market (Dunn and Rustad 2007) to meet the increasing demand. In recent years, an increased production of Nile tilapia as an aquaculture product has made it more available to consumers. Nile tilapia is produced most economically in tropical and subtropical countries, which have favourable temperatures for growth. These countries achieve low production costs, which facilitate trade with the leading importer, the USA (FAO 2006c). Imported products to USA consist of mainly frozen whole fish and fillets. Production costs in temperate countries are too high to compete in these markets. Therefore tilapia produced in temperate countries should generally be sold as live fish or fresh fillets to obtain premium prices that counter high production costs.

In Iceland, Nile tilapia farming could be economically feasible due to availability of warm water used in the cooling of geothermal power plants. It is therefore necessary to come up with clear market routes prior to advance in its production (business plan) as the target is export market. Due to Iceland´s location in Europe the main market target, export of fresh tilapia fillets to Europe at reduced cost can be achieved. However, the distance from the major markets in Europe requires fresh tilapia fillets to be transported by air, which adds to production costs. Transportation by sea (about 4-5 days) could be a realistic option if considerable increase in storage life of fresh tilapia fillets can be obtained comparable to 9 days shelf life at 4˚C (Reddy *et al.* 1995). Since fish fillets are highly perishable products, good handling conditions should be established to maintain quality and safety for the period of time needed in market distribution and display. In this regard, the technologies allowing for prolongation of the shelf life of fresh tilapia fillets are important to increase profitability as premium prices can be obtained in fresh market.

1.4. Aim of the project

The aim of the study was to develop a Quality Index Method (QIM) scheme and Quantitative Descriptive Analysis (QDA) vocabulary for tilapia fillets and test for application in shelf life studies of fillets stored at different temperatures. Further, the purpose was to propose optimal conditions that would allow for time needed in market distribution while maintaining tilapia (*Oreochromis niloticus*) fillets quality characteristics. Quality changes in tilapia fillets air and MA packaged stored at chilled and superchilled temperatures were studied using: sensory evaluation (QIM and QDA), microbiological and chemical analyses as well as physical changes (moisture content, water holding capacity and drip) during storage time. Optimal storage condition was established by comparing quality characteristics and shelf life of the experimented conditions (groups).

2. Materials and methods

2.1. Experimental design

The study was carried out in two phases at MATIS Reykjavik-Iceland between June 2008 and February 2009. Phase 1 henceforward referred to as pre-trials was carried out using two batches; tilapia (*Oreochromis niloticus*) farmed in Canada and Iceland, whereas phase 2 henceforward referred to as the main study was carried out on tilapia farmed in Iceland (Figure 1).

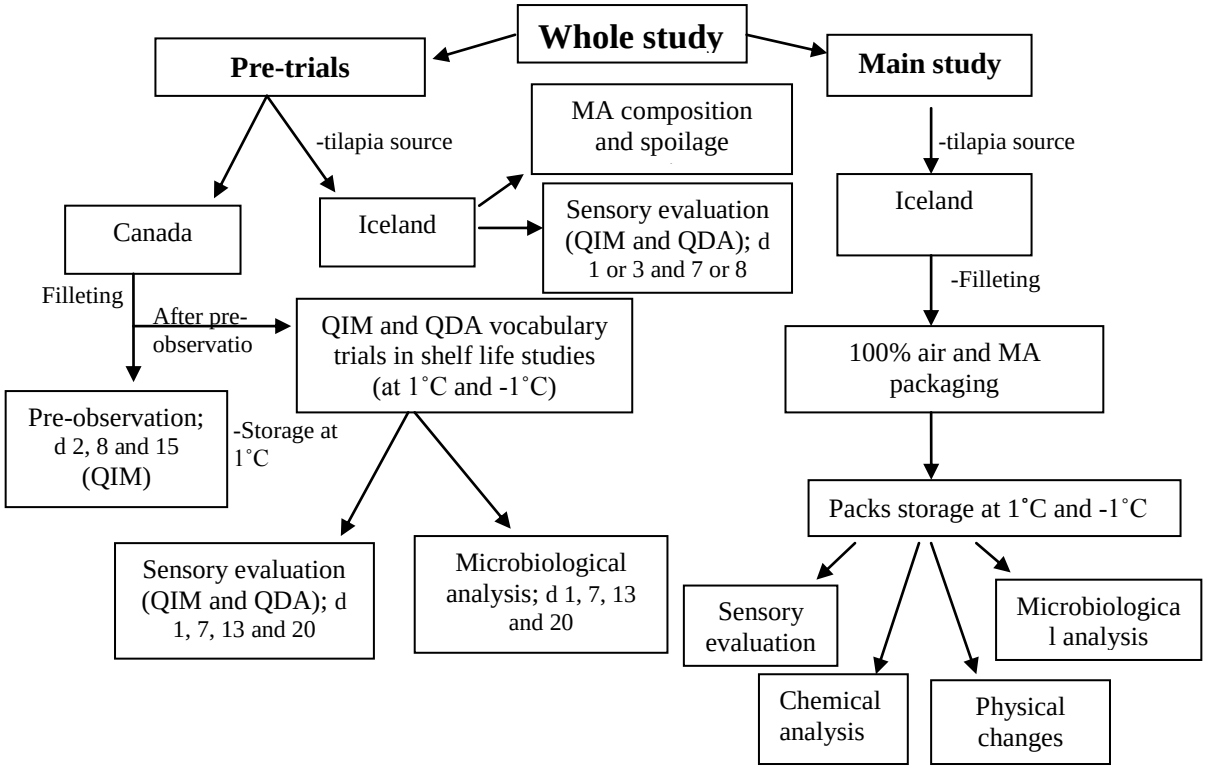


Figure 1: A chart showing experimental design for pre-trials and main study of fresh tilapia fillets.

2.1.1. Pre-trials design

During pre-trials, tilapia fillets were put into various groups based on where it was farmed, storage temperature, conditions to harvesting and mode of packaging (Table 1). Tilapia imported from Canada (transported by air; iced whole gutted) were filleted three days postharvest, whereas Icelandic fish were filleted the same day of harvesting.

Table 1: Definition of tilapia sample groups used in pre-trials. Sty box= Styrofoam box; RAS= recirculation aquaculture system; MA= modified atmosphere; d= day of evaluation; *=sampling days for pre-observation.

Sample group	Condition to harvesting	Origin	Packaging	Storage temperature
C	Unspecified	Canada	Sty box	1°C (d 1, 2*, 7, 8*, 13 15* and 20)
S (Sc)	Unspecified	Canada	Sty box	-1°C (d 7, 13 and 20)
IR	RAS	Iceland	Sty box	1°C (d 1 and 7)
IF	Fresh water for 10 days	Iceland	Sty box	1°C (d 3 and 8)
A, B, C and D	RAS	Iceland	Air and MA	2°C (d 0, 3 and 10)

2.1.1.1. Pre-trial 1: QIM scheme and QDA vocabulary development

Fish (C) were filleted immediately after arrival and fillets placed in polyethylene (PE) bags prior to re-icing in Styrofoam boxes with holes for drainage of melted ice and stored in a cold room (chamber) at 1°C. Six fillets were observed by three panellists, including two experts in sensory evaluation using QIM scheme under development on the first day of storage and approximately after every 6 days until the 15th day. In QDA vocabulary development that took place during fillets observation, samples were taken from loin part and cooked in a preheated electric oven Convostar (Convotherm GmbH, Eglfing, German) at 95-100°C for 6 minutes with air and steam circulation. The observations of tilapia fillets were always carried out in the same room with as little disruption as possible, at room temperature, under fluorescent light.

2.1.1.2. Pre-trial 2: Shelf life study trial

Tilapia imported from Canada was divided into two sample groups based on storage temperature after filleting and de-skinning; stored at 1°C (C) and -1°C (Sc) with thermometer loggers inside and outside the Styrofoam boxes. Both groups were sampled simultaneously approximately every 7 days until day 20 for sensory evaluation and microbiological analysis. Sample group IR fish was harvested directly from recirculation aquaculture system (RAS) after being starved for 5 days, whereas group IF fish was removed from RAS, kept in holding tank with fresh water and starved for 10 days prior to harvesting. After harvesting, fish (farmed in Iceland) was bled by cutting vertically across the gill arches and put in overflowing water for approximately 30 minutes (Figure 2 right) prior to transportation to the sensory laboratory where processing (filleting and de-skinning) was done for all groups. On arrival at Matis laboratory, IR fish (farmed in Iceland) was processed and stored chilled at 1°C, some of the fillets were evaluated the same day (1 d) in two sessions simultaneous to C and Sc on the

13th d of storage and thereafter until 20th d. IF fish was harvested later (to obtain fresh fillets) towards the end of study and compared to IR (old fillets) to verify the descriptions in scheme and make panellists more unanimous in tilapia evaluation (QIM and QDA).

Each day of sampling, three and six to seven fillets were taken randomly from each group for QIM and QDA evaluations, respectively. In addition, two fillets from each group (C and Sc) were taken for microbiological analysis.

Figure 2: Tilapia in tanks (RAS Iceland (left)) and bleeding of ungutted tilapia in overflowing water (right)

2.1.1.3. Pre-trial 3: Selection of gas composition and main spoilage organisms (SSO)

To establish the appropriate gas mixture to MA-package tilapia fillets, forty fish (un-starved) weighing between 450g and 700g were harvested from RAS and filleted on the same day pre-rigor. Fillets were divided into 4 groups of 20 fillets each; A (air), B (50% CO_2/50% N_2), C (70% CO_2/30% N_2) and D (90% CO_2/10% N_2). In all groups, three fillets were placed on a foam tray with a built-in absorption mat (expanded polystyrene, Linstar E 39-34, LINPAC packaging, West Yorkshire, UK) and put in a labelled high-barrier film bag (40PA/70LDPE, 250 mm × 400 mm × 0.120 mm, Plastprent Iceland). Packs for B, C and D groups were evacuated and packaged under respective MAs, whereas air packs (A) were left open. All the packs were stored chilled at 2°C up to 10 days. Samples were analysed for sensory (cooked sample), headspace gases and drip on days 3 and 10, and microbial load on day 10. During sensory evaluation sessions, 4 to 5 members of MATIS sensory panel made comments for the preferred group based on sensory attributes for cooked tilapia.

2.1.2. Main study design

Prior to main study, fish were starved and held in fresh water for 10 days. Three hundred and forty tilapia (approximately 6 months old) weighing between 450 and 700 g were harvested, bled and transported iced to Matis laboratory within three hours. 12 fish (control) were hand filleted on arrival (pre-rigor) and used for sensory, microbiological and chemical analyses. The rest were hand filleted on the following day (post-rigor) and fillets kept chilled at 0°C a waiting packaging. Four to five fillets weighing 250-290 g (altogether) were placed individually with ‘‘skin side’’ (red-coloured) up on a pre-weighed foam tray with a built-in absorption mat. Foam trays with fillets were put into a high-barrier film bag and divided into four treatments; T1, T2, T3 and T4 as elaborated in Table 2.

Table 2: Definition of sample groups used in the main study

Treatment codes	Atmosphere in packages	Storage temperature	Filleted and de-skinned	Sampling days [a]
Control	Not packaged	Iced	Pre-rigor	0
T1	100%Air	1°C	Post-rigor	2, 6, 9, 13, 16 and 20
T2	100%Air	-1°C	Post-rigor	2, 6, 9, 13, 16 and 20
T3	50%CO_2/50%N_2	1°C	Post-rigor	2, 6, 9, 13, 16, 20, 23 and 27[x]
T4	50%CO_2/50%N_2	-1°C	Post-rigor	2, 6, 9, 13, 16, 20, 23 and 27[x]

[a] Storage time was calculated from day of filleting (packaging). 1 day from harvesting and storage at 1°C

[x] Sensory evaluation not carried out

2.1.2.1. Packaging

In T1 and T2, each bag was sealed with 100% air using a Vacuum Packaging Machine (HENKOVAC Heavy duty 2000, Hertogenbosch, The Netherlands) equipped with a built-in vacuum pump and gas flushing modes. In T3 and T4 the bags were packaged under MA of 50%CO_2:50%N_2 instead of 100% air. CO_2 and N_2 was mixed using gas mixer MAP Mix 9000 (PBI-Dansensor, Ringsted, Denmark). The gas volume-to-product (G/P) ratio was approximated 5:1. Prior to sample packaging, gas composition was confirmed by packaging dummy packs and their headspace gas composition analysed using an oxygen and carbondioxide analyser (CheckMate 9900 Analyzer, PBI-Dansensor, Ringsted, Denmark). Following packaging half the number of packs per group, a package was analysed for CO_2 and O_2 concentrations to ensure the packages had the expected atmosphere.

After packaging, T1 and T3 packages were stored at 1°C whereas T2 and T4 were stored at -1°C throughout the study. Sampling was done twice a week until the end of shelf life for respective atmospheres. On each sampling day, five packages from each treatment (group)

were analysed for headspace gas composition. Two packages for each treatment were thereafter used for microbiological and chemical analyses while the remaining three were used for determining drip loss and subsequently sensory analysis.

2.2. Sensory evaluation of raw tilapia fillets (QIM)

2.2.1. Pre-trial 2: Shelf life study trial

Evaluation of raw fillets was done using preliminary QIM scheme for de-skinned tilapia fillets (Table 3) developed during pre-observation. Every sampling day, three raw deskinned fillets per group were placed randomly on a clean table at room temperature and under white fluorescent light. Each fillet was blind coded with a number consisting of three digits. Eight to twelve trained panellists, all members of the Matis sensory panel individually evaluated changes in colour, mucus, texture, appearance (blood and gaping) and odour. Panellists were encouraged during evaluation to note any other sensory parameters (observations) not included in the QIM scheme which could in addition be used to characterise changes in tilapia fillets with storage time. The QIM scheme (Table 12) was finalised after the end of preliminary studies.

Figure 3: Assessors evaluating deskinned tilapia fillets using QIM scheme (left) and tilapia loins in blind coded aluminium boxes before cooking for QDA

Table 3: A preliminary Quality Index Method scheme developed after pre-observations for de-skinned tilapia fillets (*Oreochromis niloticus*)

Quality parameter		Description	Score
Skin side	***Colour***	Dark red	0
		Red brown	1
		Light brown	2
Flesh	***Mucus***	Transparent, thin, little	0
		Opaque, a little thicker,	1
		Greyish, thick, clotted	2
	Colour	Light, beige , bluish transparent	0
		A little darker colour, a little brownish or greyish, yellowish	1
		Greyish and yellowish	2
	Texture	Firm, recovers quickly from pressure	0
		Rather soft recovers slowly from pressure	1
		Very soft doesn't recover from pressure	2
	Blood	Bright red, not present	0
		Dull red	1
		Shadowy, brown	2
	Odour	Fresh, neutral, trace of grass odour, pepper	0
		Seaweed, marine, grass	1
		Sour milk, silage	2
		Acetic, putrid	3
	Gaping	No gaping	0
		Slight gaping less than 25% of the fillet	1
		Some gaping, 25-75% of the fillet	2
Quality index (0-15)			

2.2.2. Main study

Sensory evaluation of raw tilapia fillets in the main study was done using final QIM scheme (Table 12). Every sampling day (0, 2, 6, 9, 13, 16, 20 and 23), three raw deskinned fillets per group (except d24 which only T3 and T4 were evaluated) were placed randomly on a clean table at room temperature and under white fluorescent light. Each fillet was blind coded with a number consisting of three digits. On each day, eight to twelve trained panellists, all members of the Matis sensory panel participated in fillets evaluation.

2.3. Sensory evaluation of cooked fillets

Twelve panellists all members of Matis sensory panel familiar with QDA method were trained during three sessions to evaluate cooked tilapia fillets. During pre-trial 2 (shelf life study trial) and main study, sensory evaluation of the cooked tilapia fillets was performed in parallel to the QIM evaluation. Fillets loins were cut into pieces of about 4-5 cm long and 3-4 cm wide. The pieces were placed in aluminium boxes coded with three digit random numbers and cooked in a preheated electric oven Convostar (Convotherm GmbH, Eglfing, German)

with circulation air and steam at 95-100°C for 6 minutes. Eight to ten panellists used unstructured scale from 0 to 100% (Stone and Sidel, 1985) to describe the intensity of sensory attributes for cooked tilapia fillets developed during training sessions as shown in Table 4 for shelf life study trial (pre-trial 2) and modified sensory attributes vocabulary (Table 5) in the main study. Each panellist evaluated duplicates of samples in a random order for each group. Additionally, in the main study panellists evaluated samples in two sessions per sampling day (four samples per session) unlike one session conducted in pre-trial 2, but in all cases same panellists attended both sessions. A computerised system (FIZZ, Version 2.0, 1994-200, Biosystemes, France) was used for data recording.

Table 4: Sensory attributes developed for cooked tilapia fillets

Odour	Flavour	Appearance	Texture
Earthy	Arctic charr	Colour on top (light-dark)	Softness
Boiled potatoes	Earthy	Underneath (brown-grey)	Juiciness
Hot milk	Sweet	Black threads	Fibre
Mouldy	Metallic		Flakes
Rancidity	Mouldy		Sticky
	Sour		
	Pungent		
	Rancidity		
	Rotten		

Table 5: Sensory attributes (n = 23) evaluated in cooked tilapia using an unstructured scale

Short name	Sensory attribute	Description of attribute
Odour		
O-Boilpot	Boiled potatoes	Whole newly-boiled potatoes or the stock of them
O-Boilmilk	Boiled milk	Hot milk, fruity odour
O-Earthy	Earth	Fresh earth
O-Musty	Musty	Mouldy
O-Rancid	Rancid	Rancidity
Appearance		
A-ColoT	Light/dark colour	Light, white or dark, yellowish colours
A-ColoU	Dark brown/light grey	Dark brownish or light grey colours
A-BlackThr	Black threads	Black threads in flesh
Flavour		
F-A.char	Arctic char	Arctic char, new trout
F-Sweet	Sweet	Typical sweetness flavour of fresh fish
F-Metallic	Metallic	Metallic flavour
F-Earthy	Earthy	Fresh earth
F-Musty	Musty	Mouldy flavour
F-Sour	Sour	Sour taste, spoilage sour
F-Pungent	Pungent	Pungent flavour, bitter
F-Rancid	Rancid	Rancidity, cod liver oil, reminds of paint or solvent/thinner.
F-Spoilage	Spoilage	spoilage, queasy sweet flavour
Texture		
T-Flakes	Flakiness	Fish portion slides into flakes when pressed with a fork
T-Soft	Firm/Soft	How firm or soft the fish is during the first bite
T-Juicy	Dry/Juicy	When chewed, dry: pulls liquid from mouth. juicy: gives liquid
T-Fibres	Fibre	Roughness of muscle fibre
T-Mushy	Mushy	Mushiness when chewed (mushy texture)
T-Sticky	Sticky	Glues together teeth when biting the fish

2.4. Microbiological analysis

2.4.1. Pre-trial 2 and 3: Shelf life study trial and selection of gas and SSO

In pre-trial 2 conducted to develop and test the QIM scheme, only total viable counts (TVC) and H_2S-producing bacteria were enumerated. Two fillets per group (C and Sc) were analysed separately observing strict hygiene to prevent cross contamination. Twenty five grams of mince flesh were mixed with 225 ml of cooled Maximum Recovery Diluent (MRD, Oxoid) in stomacher bag to obtain a 10-fold dilution. Blending was done in stomacher for 1 minute. Successive 10-fold dilutions were done as required. Aliquots were plated in triplicate on Iron Agar (IA) as described by Gram *et al.*, (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. In all counts spread-plating was used. Enumeration of TVC and H_2S-producing bacteria was performed after 4-5 days incubation at 17°C. Black colonies on IA produce H_2S from sodium thiossulphate and/or cysteine.

To find out the appropriate media and main flora developing in spoiling air- and MA-packaged tilapia fillets (A, B, C and D), various media were evaluated. On the sampling day (d 10), two packages per group were used. Sample preparation was done as aforementioned under this section. Total viable psychrotrophic counts (TVC) were enumerated by spread-plating of aliquots on modified Long and Hammer´s agar (mLH) (Van Spreekens, 1974) and Iron Agar (Gram *et al.*, 1987) both containing 1% NaCl (no overlay), with aerobic incubation at 17˚C for 4-5 days. H_2S-producers were also counted. Presumptive pseudomonad counts (22˚C, 3-4 days) were obtained using the modified Cephaloridine Fucidin Cetrimide (CFC) agar (Stanbridge and Board, 1994). Pseudomonas Agar Base (Oxoid) with CFC Selective Agar Supplement (Oxoid) was used. Pink colonies were counted. *Vibrio* counts were evaluated on Thiosulfate-Citrate-Bile-Sucrose Agar (TCBS, Difco), following incubation at 17°C for 4-5 days.

Nitrite-Actidione-Polymyxin (NAP) agar was used in comparison to MRS-S agar for counts of lactic acid bacteria (LAB) at 22˚C for 4 days under anaerobic conditions. LAB confirmation was done by assessing presence of catalase-negative colonies using 3% H_2O_2. Counts of *Brochothrix thermosphacta* were determined on STAA selective agar (CM0881 with SR0162, Oxoid) following aerobic incubation at 22°C for 4 days and colony confirmation done by catalase and oxidase tests, assessing 5 to 10 colonies per sample. Catalase-positive and oxidase-negative colonies were regarded as *Brochothrix thermosphacta*. In all analysis spread-plating was used and detection limit was 20 colony forming units (CFU)/g. Counts of *Photobacterium phosphoreum* (Pp) were estimated by the PPDM-Malthus conductance method (Dalgaard *et al.* 1996), as described by Lauzon (2003).

2.4.2. Main study

Samples (two packages of four to five fillets) were analysed in duplicate upon filleting (control d0) and per treatment on further sampling as storage progressed. Analysis of specific microflora in air- and MA-packaged tilapia fillets was as elaborated under section 2.4.1., except that aliquots were plated only on Iron agar and MRS-S agar for TVC and LAB respectively, evaluations twice a week. Furthermore, pseudomonads counts were obtained on CFC medium (twice a week) and using Real-time PCR analysis for pseudomonads (once a week) as described by Reynisson *et al*. (2008) for comparison on days both were analysed. Counts of *Photobacterium phosphoreum* (Pp) were evaluated once a week using a RT-PCR method under development at Matis.

2.5. Physical changes and chemical analyses

2.5.1. Headspace gas composition

The headspace gas composition in B, C and D groups (pre-trial 3) was determined on day 0, 3 and 10. Gas composition in 100% air and MA packages (main study) was determined upon packaging (day 0) and on every sampling day, using oxygen and carbondioxide analyser. The gas concentration measured in test packages in respect to treatments was averaged (n = 5).

2.5.2. Proximate analysis

A proximate analysis was performed during pre-trial 2 based on tilapia farmed in Canada and Iceland. The skinless muscle was ground and homogenised. The muscle composition from each group (Canadian and Icelandic) was determined as follows: protein, using the Kjeldahl method with a 6.25 nitrogen–to–protein conversion factor; fat, by petroleum ether extraction using the Soxhlet method and a SOXTEC System HT6 extractor; moisture by drying to constant weight at 105 ± 1 °C; and total ash by incineration to constant weight at 450 ± 2 °C in a muffle furnace.

2.5.3. pH measurement

pH was measured using the Radiometer PHM80, the glass calomel electrode was dipped into a mixture of 5g minced fish flesh and 5 mL of deionised water taken at room temperature and reading for each sample (n =2).

2.5.4. Moisture content and water holding capacity (WHC)

Samples were analysed for moisture content and WHC once a week (after every other sampling), by a centrifugation method (Eide *et al*., 1982). The tilapia samples (n = 2) were coarsely minced in a mixer (Braun Electronic, Type 4262, Kronberg, Germany) for approximately 20 s at speed 4. Approximately 2 g of the mince tilapia muscle was weighed accurately into sample glass with membrane on the bottom (height 62 mm, inner diameter 19 mm and outer diameter 25 mm) and immediately centrifuged at 210×g for 5 min, with temperature maintained at 2°C to 5°C in rotor SS-34 for Sorvall centrifuge type RC-5B (Dupoint, USA). The weight loss after centrifugation was divided by the moisture content of the fillet and expressed as %WHC.

$$\mathrm{WHC} = \frac{W1 - \Delta r}{W1} * 100(\%)$$

W1 is the water content of the sample before centrifugation (%).

Δr is the weight lost by centrifugation (%).

2.5.5. Drip loss

Drip loss was determined based on the initial weight of the fillets at packaging and on the respective sampling days. On each sampling day, packages for sensory evaluation (n = 3) were weighed (foam tray containing a soak pad and fillets = total weight), fillets hygienically removed and foam tray reweighed. Initial fillets weight was calculated by subtracting the pre-weighed foam tray containing a soak pad reading from the total weight. Drip on the other hand was obtained by subtracting the pre-weighed foam tray reading from the foam tray weight on the sampling day and expressed as a percentage of loss based on the initial sample weight. The drip loss of test packages per treatments was averaged.

2.5.6. Total volatile basic nitrogen (TVB-N) and Trimethylamine (TMA)

Duplicates of fillets mince for respective atmosphere (treatment) that remained after sampling for microbiological and WHC analyses was used for analysis of total volatile basic nitrogen (TVB-N) and trimethylamine (TMA). In TVB-N analysis, 200 ml of 7.5% trichloacetic acid solution was added to 100g of fish muscle (mince) and homogenised in a Waring blender. The mixture was filtered through a Whatman n°3 filter paper and steam entrainment was performed using a Kjeldahl-type distillator (Vapodest Gerhardt). A beaker containing 10 ml of 4% boric acid and 0.04 ml of methyl red and bromocresol green indicator was placed under the condensor for the titration of ammonia. Distillation was started and steam entrainment continued as described by Malle and Poumeyrol (1989). To determine TMA the same method was used but 20 ml of formaldehyde was added to the distillation flask to block the primary and secondary amines. The TVB-N content was calculated by the following formula:

$$\frac{14mg/mol \times a \times b \times 300}{25\ ml}(mgN/100g)$$

Where:

a = ml of sulphuric acid; b = nomality of sulphuric acid.

2.6. Data analysis

The mean values of QI, QDA attributes scores, microbial counts and physical-chemical parameter changes were plotted separately against storage time using Microsoft excel (2007). Correlation analysis of QIM data was performed in the SPSS statistic software (Version 8.0 Alibre Inc, Texas, USA). Panellists' performance was assessed using panelcheck program (Version 1.3.2, Norway) on QDA data for each sampling day and members who weren't using the scale well and unable to differentiate between samples omitted. Multivariate comparison of different sensory attributes and samples were performed with Principal Component Analysis (PCA) on mean sensory attribute values using full cross validation. The Analysis was performed using statistical program Unscrambler ® (Version 8.0 CAMO, Trondheim, Norway). Analysis of variance (ANOVA) carried out on the results were performed in the statistical program NCSS 2000 (NCSS, Utah, USA). The program (ANOVA) calculates multiple comparisons using Duncan's test to determine if sample groups are different. Significance level was defined at 0.05 (α=0.05).

3. RESULTS

3.1. Pre-trial studies

3.1.1. Pre-trial 1 (QIM scheme and QDA vocabulary development)

Remarks on appearance and odour of raw tilapia fillets and how parameters changed with storage time are listed in Table 3. Sensory vocabulary for cooked fillets developed concurrently to QIM scheme, are listed in Table 4.

3.1.2. Pre-trial 2 (Shelf life study trial)

3.1.2.1. Tilapia proximate composition

The macronutrient composition of tilapia fillets are as presented in Table 6. Among the analysed constituents, Icelandic tilapia recorded has high moisture content than Canadian.

Table 6: Muscle composition of Canadian and Icelandic farmed tilapia fillet samples. All values are presented as mean (%) and standard deviation based on the sensitivity of methods.

Muscle composition	Canadian (C and Sc)	Icelandic (IR and IF)
Moisture	76.5±0.4	77.8±0.4
Protein (N×6.25)	18.6±0.4	17.7±0.4
Fat (Soxhlet)	4.2±0.4	3.5±0.4
Ash	1.1±0.5	1.0±0.5

3.1.2.2. Temperature changes during fillets storage

The average temperature in styropore boxes with whole gutted tilapia transported by air from Canada was 2°C on arrival at MATIS laboratory. The results for temperature profile outside and inside the styropore boxes with deskinned fillets; sample groups Sc and C during storage are shown in Figure 4 and 5.

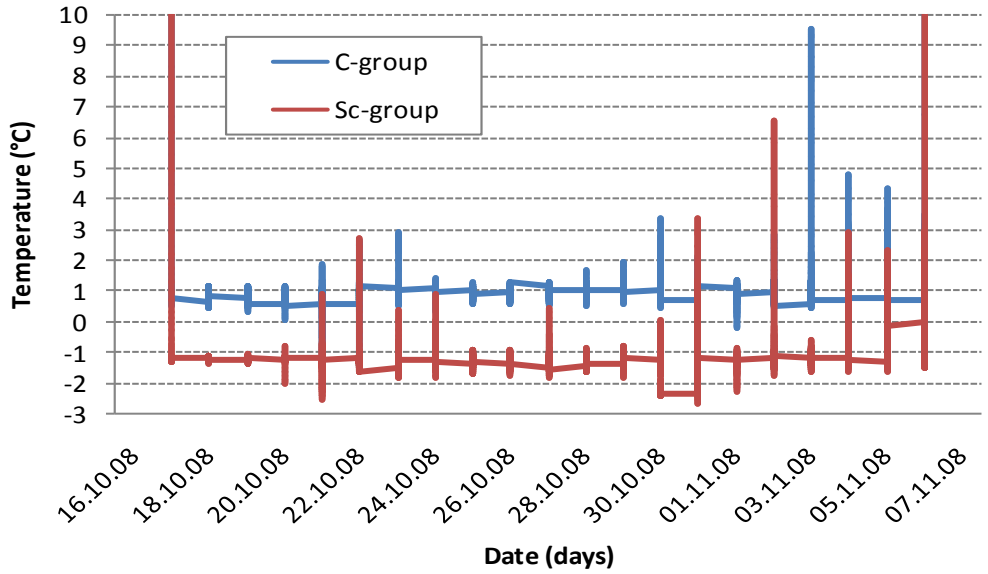


Figure 4: Ambient air temperature changes in controlled cooling cabinets holding Styrofoam boxes with tilapia fillets. C= chilled at 1˚C; Sc= superchilled at -1˚C.

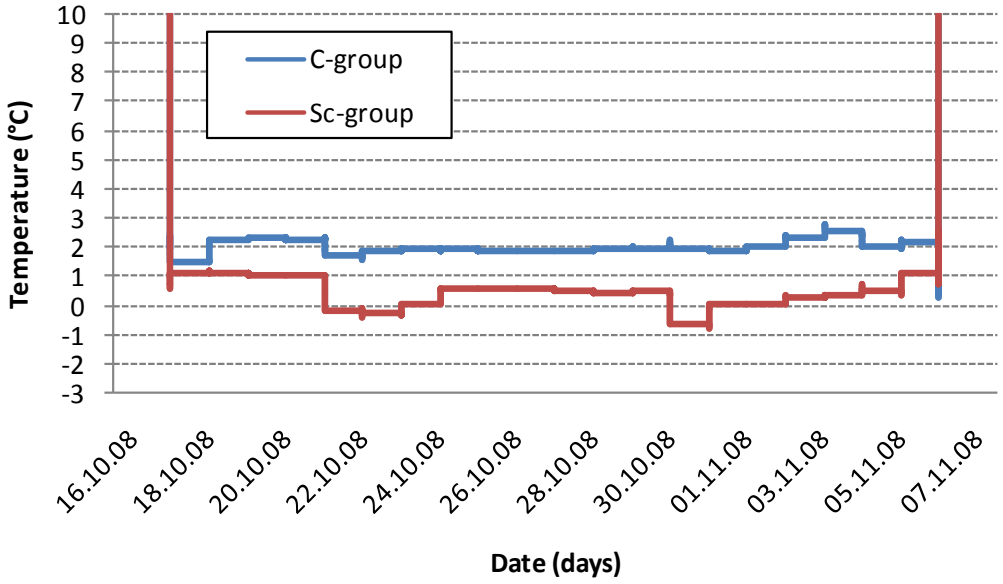


Figure 5: Temperature changes inside Styrofoam boxes with tilapia fillets (fillets contact temperature). C= chilled at 1˚C; Sc= superchilled at -1˚C.

According to figures 4 and 5, the fillets contact temperature inside Styrofoam boxes with sample group C stored at 1°C (chilled) ambient was on average 2±0.25°C. The temperature increased from approximately 1°C at the beginning of storage time to 2±0.25°C which was maintained throughout the study. The reverse trend was observed with fillets contact temperature for superchilled fillets (Sc), temperature reduced from the initial 1°C to averagely 0.5±0.5°C during the study.

3.1.2.3. Sensory evaluation using QIM (raw fillets)

Initial changes in the parameters describing raw de-skinned tilapia fillets were listed in preliminary scheme during the pre-observation: Colour skin side; flesh mucus, colour, texture, blood, odour and gaping (Table 3). The maximum sum of scores was 15. However, due to possible differences in culture conditions for tilapia farmed in Canada and Iceland which could have lead to differences in characteristics (Table 6), the developed scheme was tested and further modified towards the end of the study to suit both groups (Table 12). The sum of individual parameter scores evaluated according to the QIM scheme was presented as quality index (QI) based on average of three fillets per trial. The QI showed a linear relationship with the storage time (Figure 6) for both groups. A strong correlation of $R^2 = 0.943$ was found for chilled fillets (C) compared to $R^2 = 0.913$ for counterparts superchilled (Sc). At each trial, a difference of approximately 1.7 QI score was observed between the groups. Significant different was reported between the groups on 7, 13 and 20 d of storage ($P<0.05$), with sample group C recording higher index.

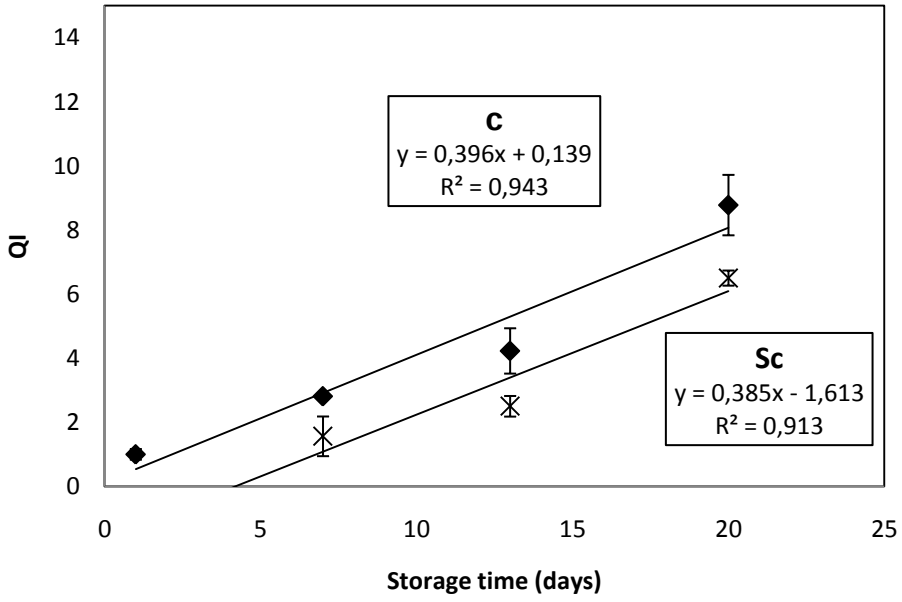


Figure 6: Quality index of de-skinned tilapia fillets using original scheme. Averages (±SD) over each day analysed (N=3 per group) during storage. ▲= chilled at 1˚C (C); ×= superchilled at -1˚C (Sc)

On applying the scheme to Icelandic tilapia fillets stored at 1°C (ambient temperature), higher QI was observed compared to Canadian counterparts (C) of corresponding storage day (data not provided). A QI score difference of 2.1 was noted on 1 d of storage when both batches were evaluated.

Changes in raw tilapia deskinned fillets stored for 1 and 20 d at 1°C and -1°C ambient temperature (2±0.25°C and 0.5±0.25°C fillets contact temperature accordingly) are shown in Figure 7 and 8. On day zero, the fillets had dark red colour on skin side and bluish transparent brightness on the inner/back born side. The flesh had a firm texture and if blood was present, the colour was bright red. On 20 d, both groups were characterized by light brown colour on skin side although Sc-group retained more of red brown colour. Whereas, on back born side the flesh colour was a little brownish for Sc-group with yellowish colour around the belly flaps; however counterparts C-group had pronounced yellow colour all over fillets surface

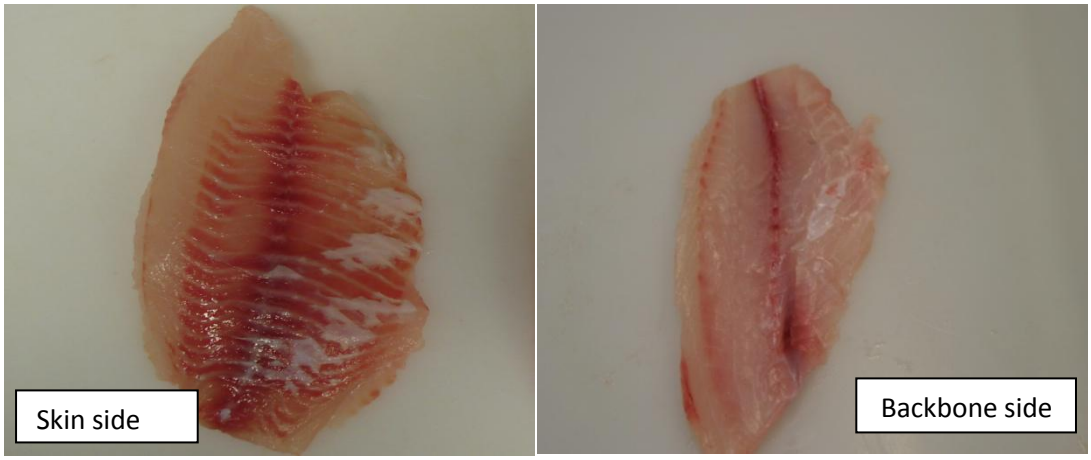


Figure 7: Appearance of de-skinned raw tilapia fillets at the beginning of storage time (d1), skin side (left) and inner side (right)

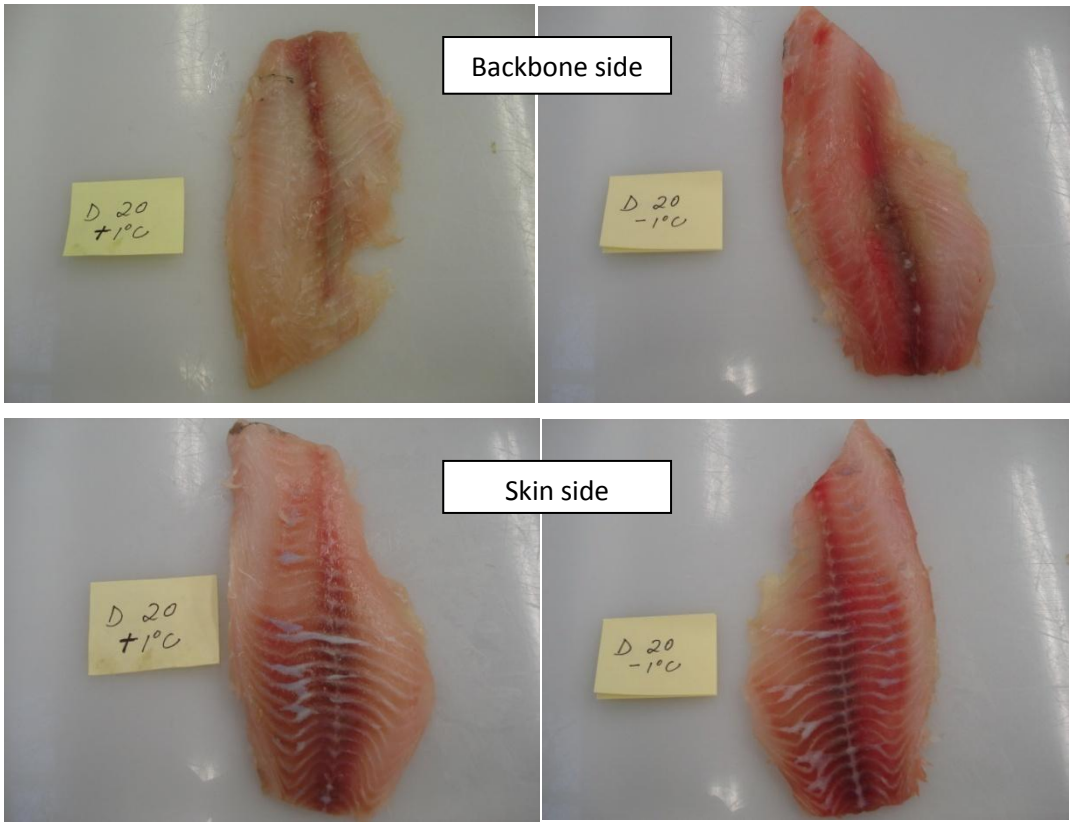


Figure 8: Appearance of raw de-skinned tilapia fillets stored at 1°C (C) and -1°C (Sc) ambient temperature at the end of storage time (20 d).

Changes in individual sensory parameters evaluated using QIM with storage time for both groups are shown in figure 9. The scores for all quality parameters increased with storage time in both groups. At the beginning of storage time (d1), all the evaluated attributes scored around zero. During storage, the attribute scores varied considerably within the groups with sample group C recording higher scores than group Sc as observed in QI. Score for mucus, flesh colour backbone side, texture, odour and gaping showed a rather sharp increase between 13 d and 20 d especially in group C. In both groups blood and flesh gaping attribute increased less with storage time compared to others, attaining a score of around 0.7 on d20 of the maximum possible 2 score units (Figure 9).

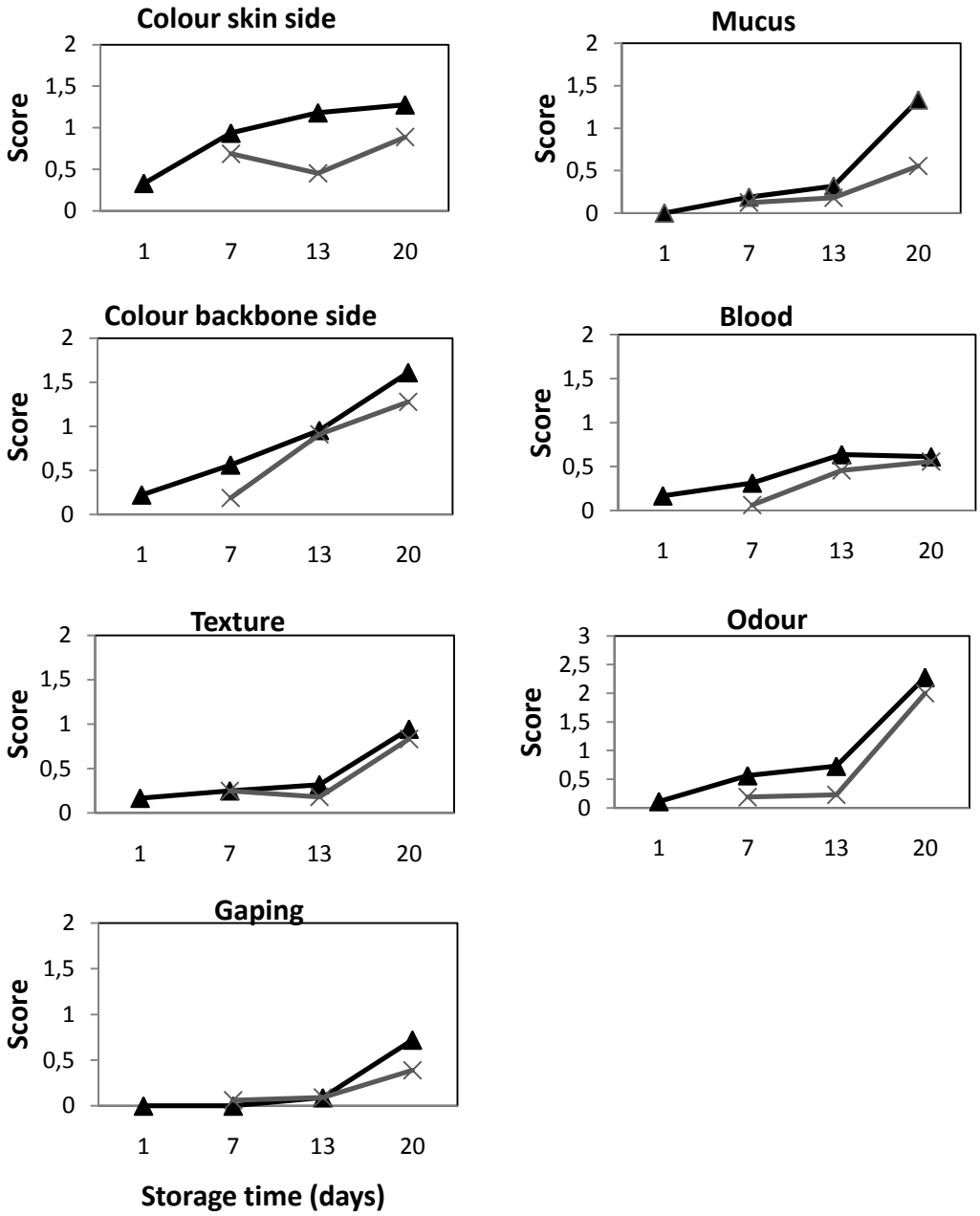


Figure 9: Average score for individual quality descriptors (attributes) evaluated with QIM scheme for de-skinned tilapia fillets (N=3 per group) during storage. ▲ = chilled at 1˚C (C); ×= superchilled at -1˚C (Sc)

3.1.2.4. Sensory evaluation using QDA

Panelists evaluated cooked samples based on sensory attributes for cooked tilapia developed during training/pre-observation (Table 4). The results for each attributes are presented as averages of the whole panel (7 to 10 panellists per trial). QDA attributes for cooked tilapia were described based on a scale ranging from 0 to 100%.

The positive odour attributes such arctic charr and boiled potatoes were very characteristic at the beginning of storage time for both groups, recording scores of between 30 and 50 until 13

d (Figure 10 and Table 7). These attributes became less evident (score of 10 to 30) towards the end of storage time, but similarly in both groups. However, odour of boiled potatoes appeared to be stronger compared to characteristic arctic charr throughout the storage time in both groups. More so, all positive attributes except characteristic arctic charr weren't significantly different between the groups and storage days (Table 7).

The negative sensory odour attributes that indicated spoilage of the samples, became prominent with increasing storage time. Specifically rancid odour increased sharply from a score below 10 for both groups to 15 and 30 between 13 d and 20 d for sample group Sc and C accordingly (Figure 10). The changes occurred differently for the sample groups. Sample groups differed mainly with regard to odour rancidity after 13 d of storage (Figure 10 B). On 20 d, odour rancidity for sample group C was significantly different from group Sc of the same storage day and both groups at preceding storage days (Table 7). On the other hand, group Sc on 20 d recorded odour rancidity mean score different from C on that day, but not different from both groups on 13 d of storage.

Earthy odour though considered negative attribute in the current study, recorded higher scores throughout the study. At the beginning of storage time it was characteristic with score of around 30, but became more pronounced with storage time attaining score of 40 at the end of storage time for both groups.

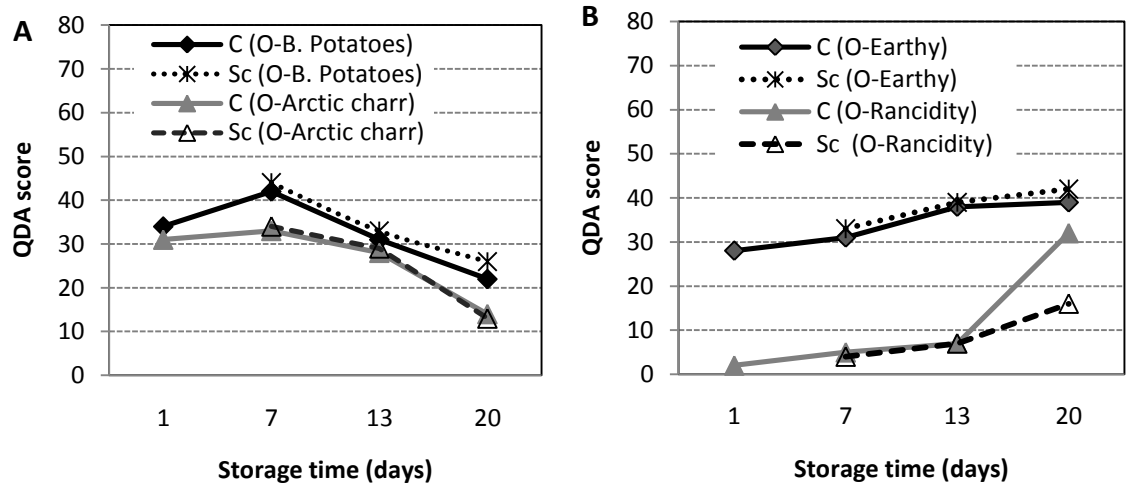


Figure 10: Changes in positive (A) and negative (B) odour attributes of cooked tilapia fillets during chilled storage at 1°C (C) and superchilled at -1°C (Sc), (average scores N = 2)

Table 7: Mean sensory scores of odour, flavour and appearance/texture attributes of cooked tilapia fillets (average scores N = 2). C = chilled at 1°C; S = superchilled at -1°C ambient temperature (1°C); D = storage days.

Groups:		D01c	D07c	D07s	D13c	D13s	D20c	D20s
Attributes	P- value							
Odour								
Boiled potatoes	*0.073*	34	42	44	31	33	22	26
Hot milk	*0.426*	26	29	32	26	25	21	21
Arctic charr	***0.002***	31 ^{a}	33 ^{a}	34 ^{a}	28	29	14 ^{b}	13 ^{b}
Metallic	*0.749*	25	24	26	18	20	19	20
Earthy	*0.368*	28	31	33	38	39	39	42
Algae	*0.994*	24	28	25	23	25	27	24
Rancidity	***<0.001***	2 ^{a}	5 ^{a}	4 ^{a}	7 ^{ac}	7 ^{ac}	32 ^{b}	16 ^{c}
Flavour								
Mould	*0.657*	48	45	47	56	54	53	51
Arctic charr	*0.101*	39	33	34	32	29	20	19
Sweet	*0.335*	37	29	30	28	31	22	20
Metallic	*0.562*	30	30	31	25	24	24	24
Sour	***<0.001***	3 ^{a}	10 ^{a}	6 ^{a}	12 ^{ab}	11 ^{a}	41 ^{c}	26 ^{b}
Pungent	***<0.001***	5 ^{a}	7 ^{a}	6 ^{a}	14 ^{a}	15 ^{a}	37 ^{b}	23 ^{c}
Rancidity	***<0.001***	3 ^{a}	7 ^{a}	5 ^{a}	9 ^{a}	9 ^{a}	46 ^{b}	25 ^{c}
Appearance/Texture								
Colour on top	***0.041***	32 ^{a}	41	41	57 ^{b}	50	44	45
Colour under	*0.519*	41	44	45	56	50	39	50
Black threads	*0.862*	21	18	20	24	23	22	19
Softness	*0.923*	69	68	65	64	64	71	65
Flakes	***0.048***	43 ^{a}	27 ^{b}	26 ^{b}	24 ^{b}	31	26 ^{b}	26 ^{b}

Significant difference in sensory attribute between sample groups was defined at P<0.05 (different letters indicate significant different values between samples within a row (a line)).

As with the odour attributes, flavour positive attributes did not show much change with storage time in both groups (Figure 11 and Table 7). However, the attributes were prominent at the beginning of storage but decreased with storage time in both groups despite recording no significant differences. For negative flavour attributes there was a sharp increase in the scores from around 10 to approximately 40 for sample group C and 25 for group Sc between 13 d and 20 d of storage. However, rancid flavour score increased above sour for C whereas in Sc both scored the same (Figure 11 B). The scores were higher and significantly different between the groups during the last day of storage and to sample groups of preceding storage days (Figure 11 and Table 7). Sour flavour on 20 d of storage for Sc was different from all other sample groups except C on the 13 d of storage.

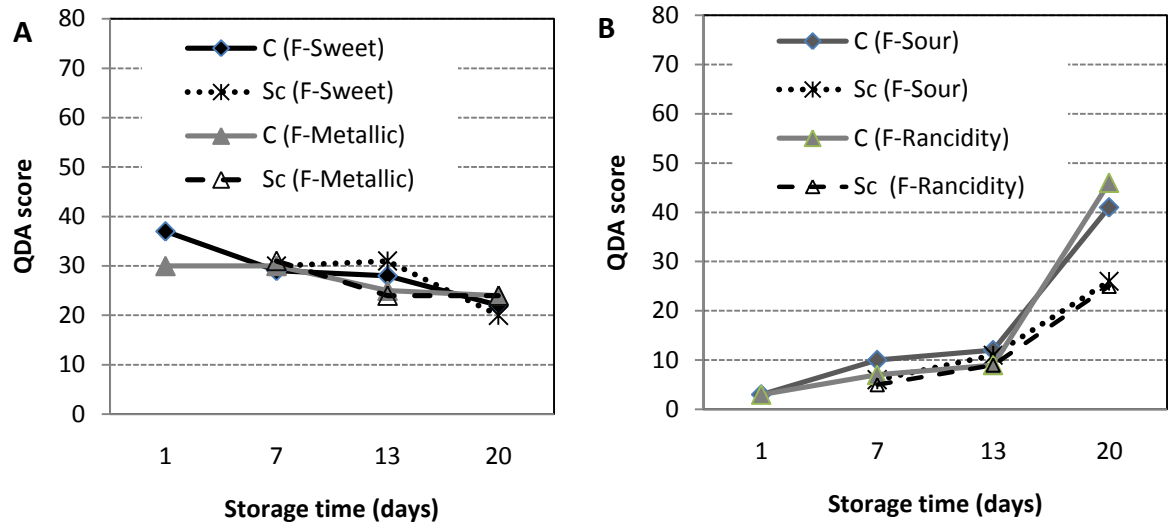


Figure 11: Changes in positive (A) and negative (B) flavour attributes of cooked tilapia fillets during chilled storage at 1°C (C) and superchilled at -1°C (Sc), (average scores N = 2)

Attributes describing appearance and texture; colour underneath the fillets, black threads and softness were not significantly different and showed no particular trend with storage time (Table 7). However, colour on top and flakiness (flakes) attributes depicted particular trends with storage time. Scores for fillets colour on top increased whereas flakiness scores decreased with storage. Both colour on top and flakiness were significantly different between Sc on 13 d (D13S) and C on 1 d (D01C) but not different for all other days.

In order to obtain a better understanding of how the sample groups C and Sc of tilapia fillets (stored at 1°C and -1°C respectively) were described by the sensory attributes with storage time, the QDA results were analysed with principal component analysis (PCA) (Figure 12). A clear grouping was evidenced on each side of the PC1-axis, indicating that fresh samples were easily distinguished from samples of deteriorated quality. The odour and flavour sensory attributes of cooked tilapia (arctic charr, boiled potatoes, metallic and sweet) detected at the beginning of shelf life on the left side of loadings plot along the 1st principal component (PC1) were considered positive attributes (Figure 12). Consequently, the sensory attributes detected closer to the end of shelf life describing spoilage (earthy, sour and rancid), were considered negative. The samples varied mainly with regard to differences in odour and flavour attributes along the 1st principal component (PC1), explaining 79% of the variation between the samples. The main difference occurred with the storage time, as the sample groups are located to the left side at the beginning of storage but on the right side after longer storage.

Samples also varied along the 2nd principal component (PC2) especially with regard to differences in appearance and texture attributes, explaining 13% of the variation between the samples. It's worth noting that up to 13 d of storage on scores mapping, both groups were more characterised by positive attributes on the left hand side than negative attributes located on the right hand side of correlation loadings (Figure 12).

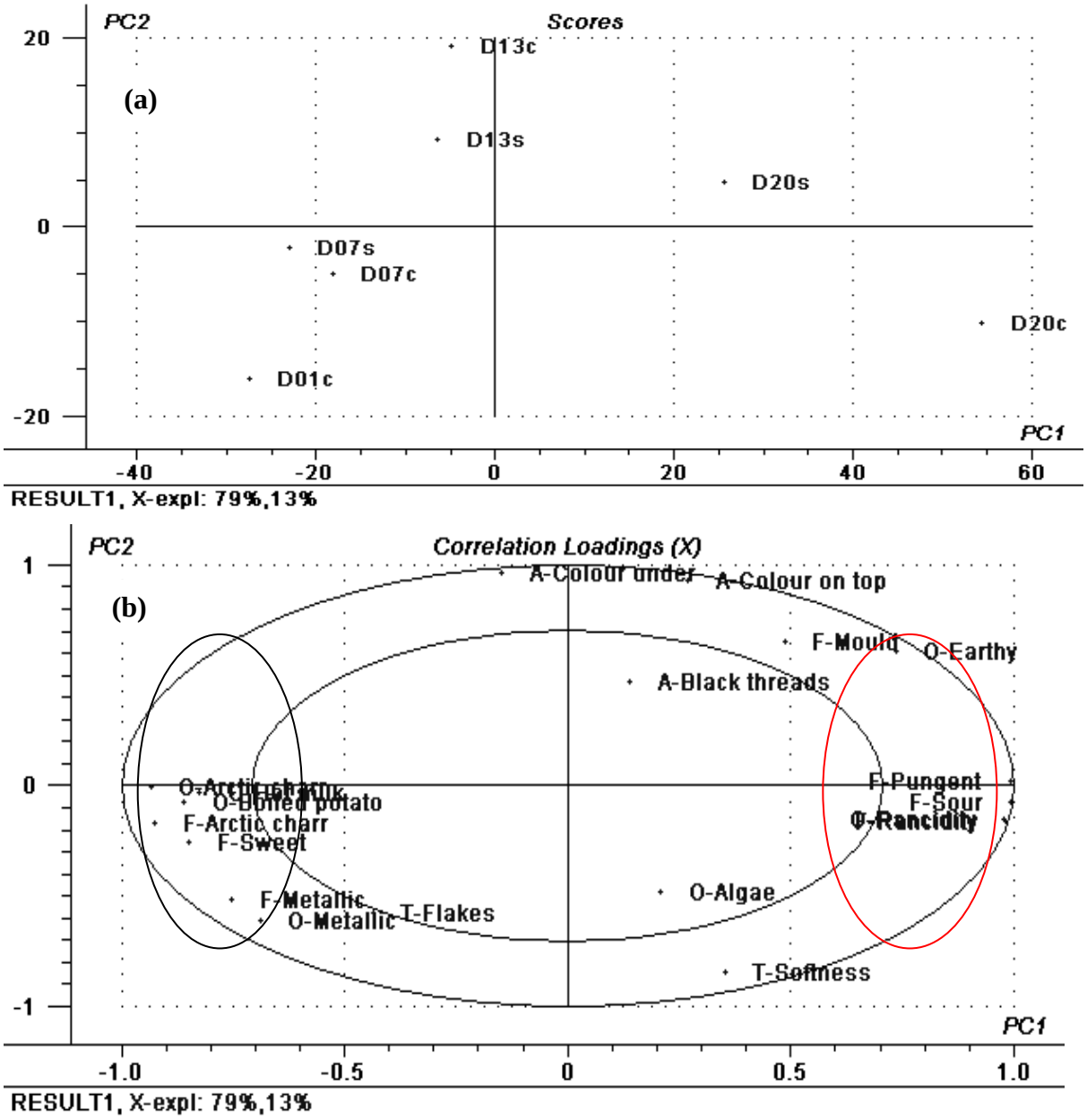


Figure 12: PCA describing sensory quality of cooked tilapia as evaluated by a trained sensory panel. Scores (a) and correlation loadings (b). PC 1 (79%) vs PC 2 (13%). D"xx" = days of storage; C= chilled (1°C); S = superchilled (-1°C); O = odour; F = flavour; T = texture; A = appearance (N = 2).

3.1.2.5. QDA in Icelandic tilapia trials

Mean sensory scores in odour, flavour, appearance/texture for Icelandic tilapia held in recirculation aquaculture system until harvesting day (IR) and counterparts held in fresh water for 10 days before harvesting (IF) were as presented in Table 8 . The odour and flavour positive sensory attributes of cooked tilapia were not significantly different between the groups although IF-group appeared to retain more of the characteristics. For negative sensory attributes a significance different was observed between the groups especially with regard to odour algae and flavour sour, pungent and rancid. In all cases IF-group recorded low negative attributes scores in comparison to IR.

Table 8: Mean sensory scores of odour, flavour and appearance/texture attributes of cooked tilapia fillets (average scores N = 2). Ir = Icelandic recirculating water (1°C); If = Icelandic fresh water (1°C); D = storage days.

Groups:		D01IR	D03IF	D07IR	D08IF
Attributes	P-value				
Odour					
Boiled potatoes	*0.479*	41	36	35	42
Hot milk	*0.070*	20	32	23	29
Arctic charr	*0.019*	28	24	18 ^{a}	35 ^{b}
Metallic	***<0.001***	23	20	16	43^{a}
Earthy	*0.871*	44	40	41	39
Algae	***0.001***	30^{a}	18^{b}	22^{b}	5^{c}
Rancidity	***<0.001***	9^{a}	2^{a}	10^{a}	51 ^{b}
Flavour					
Mould	*0.074*	52	48	54	38
Arctic charr	*0.160*	27	30	21	33
Sweet	*0.027*	31	39	20	30
Metallic	***<0.001***	26^{a}	33^{a}	22^{a}	5^{b}
Sour	***<0.001***	14^{a}	4^{a}	25^{b}	6^{a}
Pungent	*0.052*	10	9	19	7
Rancidity	***0.002***	8^{a}	7^{a}	24^{b}	6^{a}
Appearance/Texture					
Colour on top	***<0.001***	36 ^{ab}	29 ^{a}	38 ^{b}	14 ^{c}
Colour under	***0.001***	45 ^{a}	66 ^{b}	38 ^{a}	61 ^{b}
Black threads	***<0.001***	22^{a}	14^{a}	17^{a}	55^{b}
Softness	***<0.001***	66^{a}	78^{a}	60^{a}	24^{b}
Flakes	***0.011***	21^{a}	18^{a}	25	32^{b}

Significant difference in sensory attribute between sample groups was defined at P<0.05 (different letters indicate significant different values between samples within a row (a line).

3.1.2.6. Microbial counts

Changes in TVC and counts of H_2S-producing bacteria are shown in Figure 13. The results show a low initial psychrotrophic bacterial load (TVC) on day 1 followed by a steady development during the succeeding days in both groups. Growth curves for TVC and H_2S-producing bacteria had on average a similar shape, though the proportion of H_2S-producing bacteria to the TVC increased with storage time. Bacterial proliferation progressed rapidly in both groups although higher counts were observed in C throughout the study. It is interesting to note that H_2S-producing bacteria counts maintained low (<log 2/g) up to day 7 (when both C and SC were evaluated). The counts however increased exponentially in C between days 7 and 13. At the end of shelf life of C, TVC had surpassed log 8/g and H_2S-producing bacteria reached log 7/g, whereas in Sc, TVC was log 8/g and H_2S-producing bacteria counts were tenfold lower.

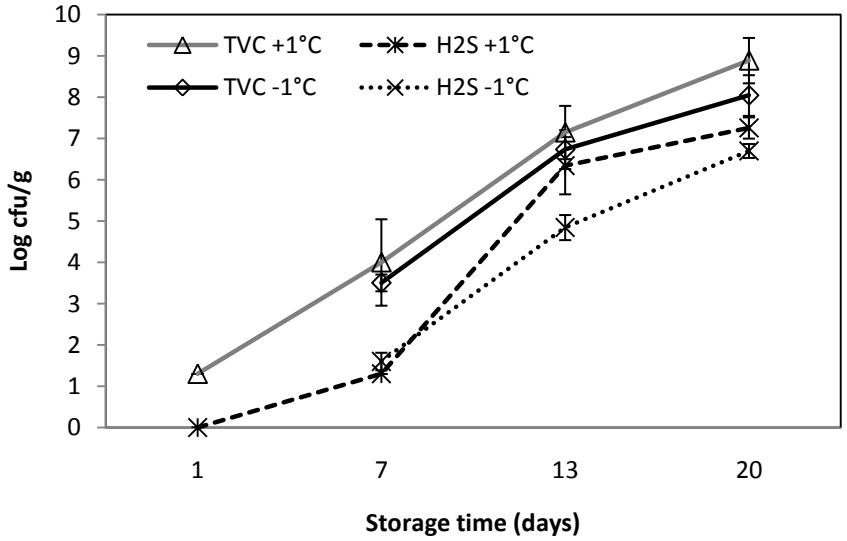


Figure 13: Total viable counts (TVC) and H_2S producing bacteria in tilapia fillets chilled at 1°C (C) and superchilled at -1°C (Sc) during storage (average scores N = 2). Detection limit log 1.3 CFU/g

3.1.3. Pre-trial 3: Selection of gas and main spoilage organisms

3.1.3.1. Headspace gas

The headspace composition of MA packages at the beginning of storage (d0) was as illustrated in Figure 14. Headspace carbondioxide concentration decreased at early storage (in average, 10%) but seemed to stabilise on days 3-10 for all MA groups. Oxygen level increased at the beginning and stabilised after day 3 to reach a level of almost 2% on day 10.

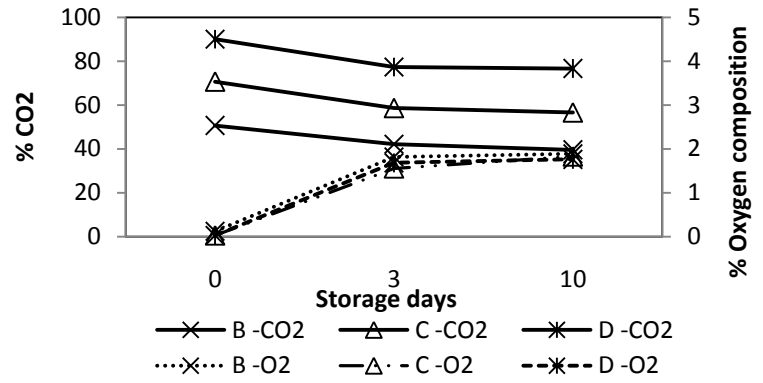


Figure 14: Gas changes in the headspace of tilapia fillets packaged under different gas mixtures. B= 50% CO_2/ 50%N_2; C= 70% CO_2/ 30% N_2; D= 90% CO_2/ 10N_2.

3.1.3.2. Drip loss

The percentage drip loss of fillets was higher in MA-packs with higher CO_2, but decreased with reducing levels of CO_2 in the headspace (Figure 15). Less drip was observed in fillets stored with air throughout the pre-trial storage time.

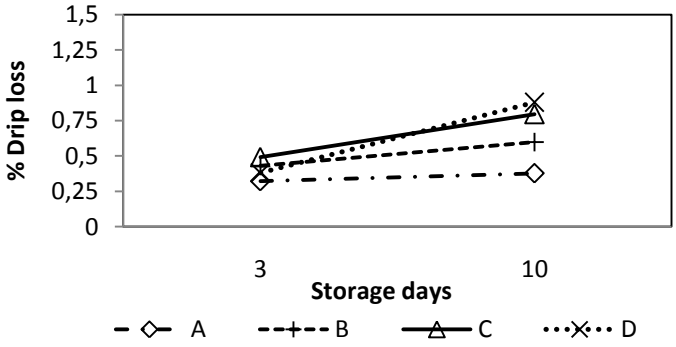


Figure 15: Drip changes during storage of chilled (2°C) tilapia fillets under different gas mixtures. B= 50% CO_2/ 50%N_2,; C= 70% CO_2/ 30% N_2 ; D= 90% CO_2/ 10N_2.

3.1.3.3. Microbiological analyses

Figure 16 shows the microbial profile of air- and MA-packaged tilapia fillets in different CO_2 levels. Similar patterns (log number) were observed for the microflora developing on TVC media, mLH and IA. TVC decreased considerably in presence of CO_2. All presumed specific spoilage organisms enumerated were present except H_2S-producers and *Vibrio* spp. Lactic acid bacteria (LAB) counts were tenfold higher on MRS-S than NAP agar. Pseudomonads were apparently dominating the microflora in air-stored fillets, while found at lower levels in MAP fillets. Among the presumed SSO investigated, *Brochothrix thermosphacta* ranked

second in importance followed by LAB while *Photobacterium phosphoreum* was just at the detection level after 10 days at 2°C. Few SSO tolerated 70% or higher CO_2 concentration.

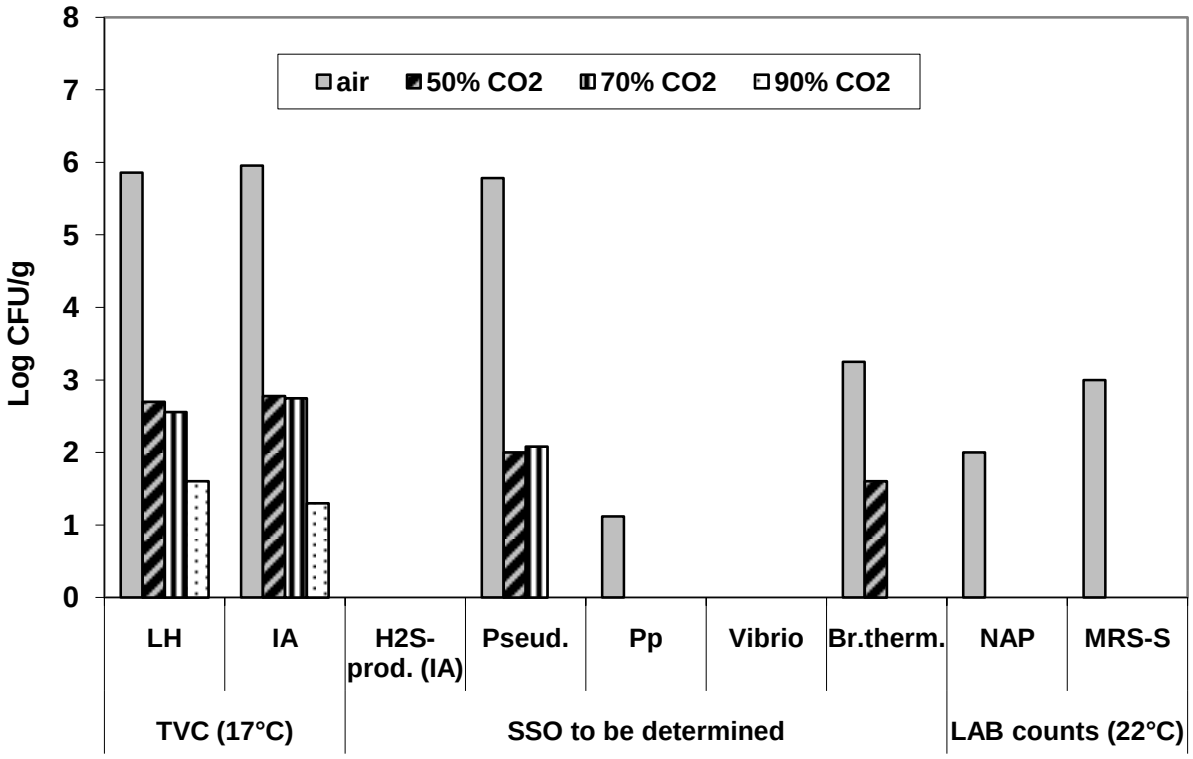


Figure 16: Microbial load in tilapia fillets air- and MA-packaged stored 10 days at 2˚C. LH=Long and Hammer's Agar; IA=Iron Agar; NAP=Nitrite-Actidione-Polymyxin agar; SSO=specific spoilage organisms.

3.2. Main study

3.2.1. Temperature profiles during storage

The average ambient and fillets temperature in the cold chamber was 1.0 ± 0.5˚C (T1 and T3 groups) and -1.0 ± 0.5˚C (T2 and T4 groups) throughout the storage (Appendix 1).

3.2.2. Headspace gas composition

The average headspace volume to fillets ratio (gas product ratio) in packages was 5:1. On packaging, 100% air (T1 and T2) had approximately 21% O_2 and 0% CO_2 (comparable to 20.95 ambient O_2) as shown in Figure 17 A. There was a decline in mean O_2 levels with storage time whereas CO_2 increased in air packages. In T1 O_2 and CO_2 levels reached an equilibrium point of around 10% between d16 and d20, thereafter CO_2 surpassed O_2 attaining a maximum level of about 15% on d23 when O_2 was around 0.5% (Figure 17 A). A significant decline ($p < 0.05$) in O_2 level of 13.5% to 1.5% was observed between d16 and

d20. Although O_2 levels in T2 decreased with storage time, the lowest concentration reached was 17.2% when CO_2 was 4.2% on d23. The initial headspace of MA packages contained low levels of O_2 (<1%). After day 2 of storage at 1˚C and -1˚C (T3 and T4), oxygen levels increased to 2.5% and thereafter, its concentration in the headspace gradually decreased as storage progressed (Figure 17 B). Initial CO_2 levels in headspace were 50% (MA packages). On the second day of storage (d2), its concentration had reduced to around 39%. However, the concentration in headspace (CO_2) remained on average at 39% in both groups during later storage days.

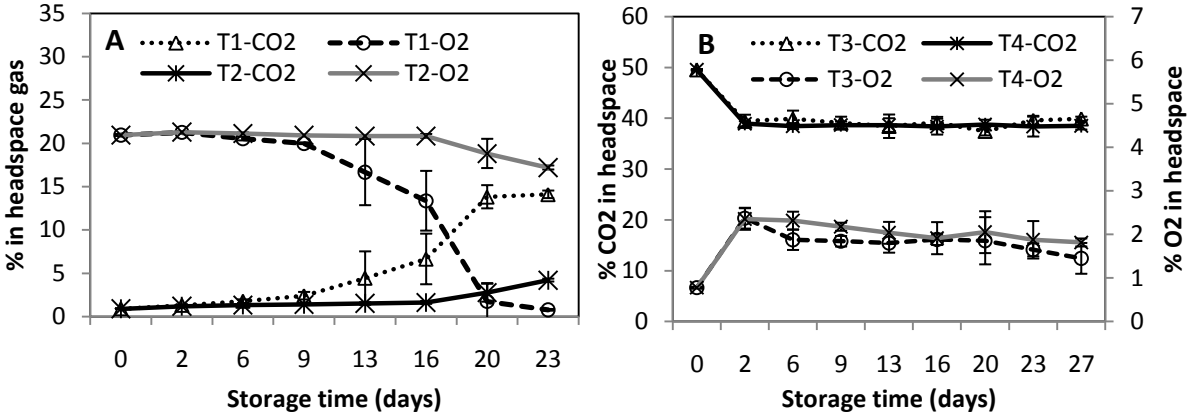


Figure 17: Gas composition changes in headspace of 100% air (A) and MA (B) packages of tilapia fillets during storage (average score N = 5). T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C.

3.2.3. Sensory evaluation using QIM (raw fillets)

The sum of individual parameter scores evaluated according to QIM scheme was presented as quality index (QI) based on average of three fillets per trial and whole panel (8-12 panellists). The QI showed a linear relationship with the storage time (Figure 18 and Appendix 2) for all the groups. A correlation of $R^2 = 0.942$ was observed for air packaged fillets stored at 1°C (T1) noted as reference under this section as the QIM scheme used was developed based on tilapia fillets air stored at 1°C. MA packaged fillets (T3 and T4) recorded high QI scores that were not significantly different ($p > 0.05$) except on d9 ($p < 0.05$) despite T3 scoring higher than T4 during the storage time. On the other hand, air packaged fillets recorded significantly different scores ($p < 0.05$) after d9 of storage, with T1 receiving higher QI. The QI of T1 increased significantly (sharply) after d13 of storage compared to counterparts stored at -1˚C (T2) and MA packaged, reaching a QI of 10 on d20 (Figure 18 and Appendix 2). The QI of T1 was significantly different after d13 (d16 and d20) from the preceding sampling day unlike observed in other sample groups (Appendix 2). The QI of T2 fillets did not change much

during storage reaching a maximum score of 4 on d20, however significant difference was observed between earlier and late storage days in the group (Appendix 2).

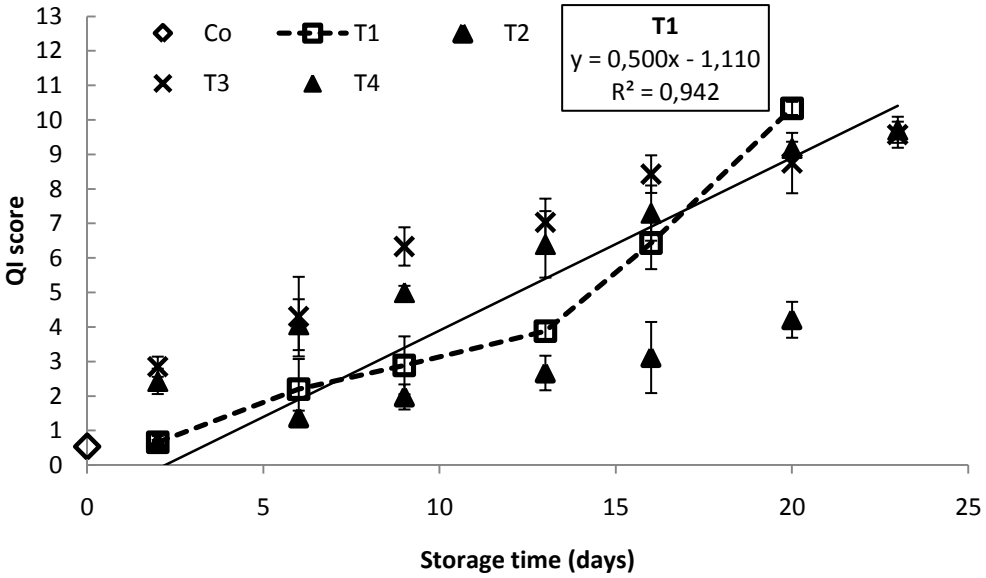


Figure 18: Quality index of de-skinned tilapia fillets (Max QI=13). Averages (±SD) over each day analysed (average score N=3) during storage at 1˚C and -1˚C. Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C.

The average scores for the individual quality parameters increased with storage time (Figures 19 and 20), but differently for the four groups. MA packaged fillets (T3 and T4) recorded consistently higher scores throughout the storage time for parameters describing colour changes (Figure 19: A, B and C). At the beginning of storage time d0 (control), colour parameters (colour skin side, flesh colour loin and colour belly flap) scored around zero. However, on subsequent assessment on d2, MA groups (T3 and T4) received higher scores for colour whereas counterparts' air (T1 and T2) recorded scores close to control on d0. The score for air stored at 1˚C (T1) and MAP (T3 and T4) increased consistently reaching almost the maximum score at the end of storage time. However, in T2 (air superchilled) the colour parameters recorded less than 1 of the possible 2 score (Figure 19). It was noted that scores (T2) on d16 were considerably higher than on d20 for flesh colour loin (Figure 19 B).

For other quality parameters (none colour related) evaluated in QIM scheme (slime, texture and odour), the scores varied considerably within the groups until after d13 of storage (Figure 20: A, B and C). Upon subsequent assessments on d16, d20 for air packaged (T1 and T2) and on d23 in addition for MAP (T3 and T4) a clear difference emerged between the groups, with T1 scoring higher in slime and odour on d20 (Figure 20: A and C). There was higher variability in the scores for texture in all groups, although T3 and T4 (MAP) recorded fairly

higher scores (Figure 20: B). As with colour quality parameters, scores for slime, texture and odour were low for T2.

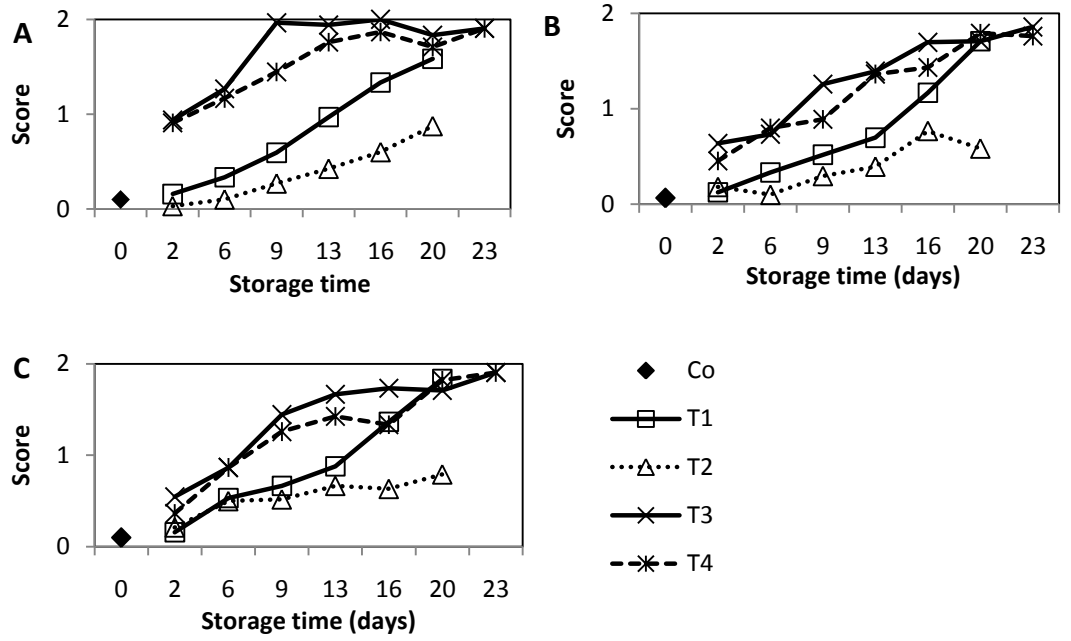


Figure 19: Averages scores (N=3) of colour changes (colour related parameters) as assessed in QIM scheme for de-skinned tilapia fillets during storage at 1˚C and -1˚C. A= colour skin side; B= flesh colour loin; C= colour belly flap; Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C.

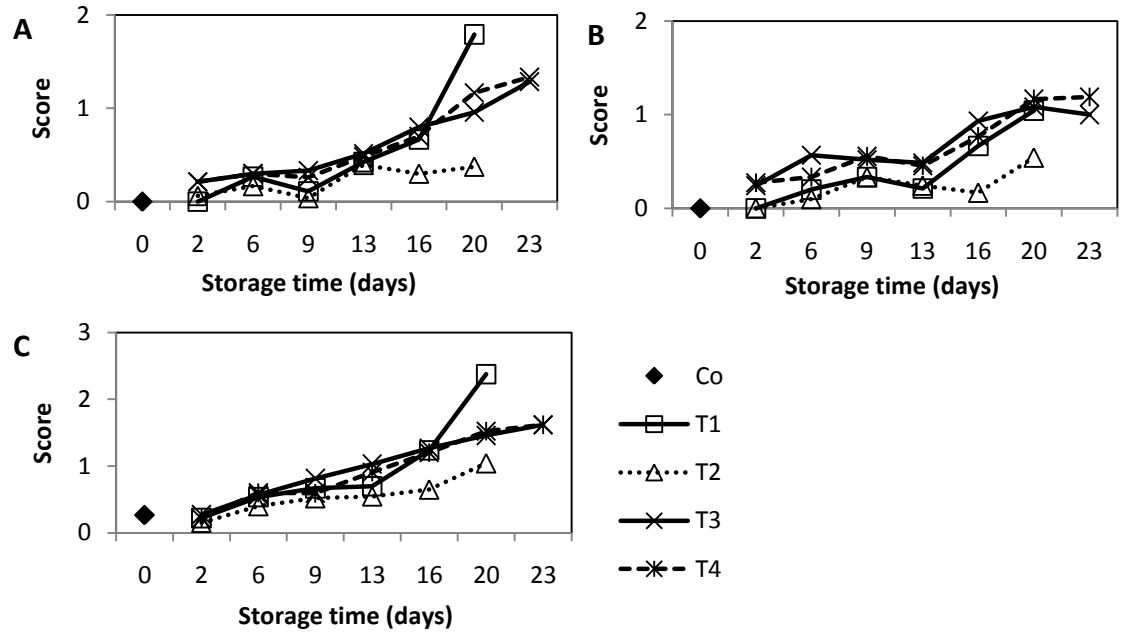


Figure 20: Averages scores (N=3) of other descriptors/parameters evaluated in QIM scheme for de-skinned tilapia fillets during storage at 1˚C and -1˚C. A= Slime (mucus); B= texture; C= odour; Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C.

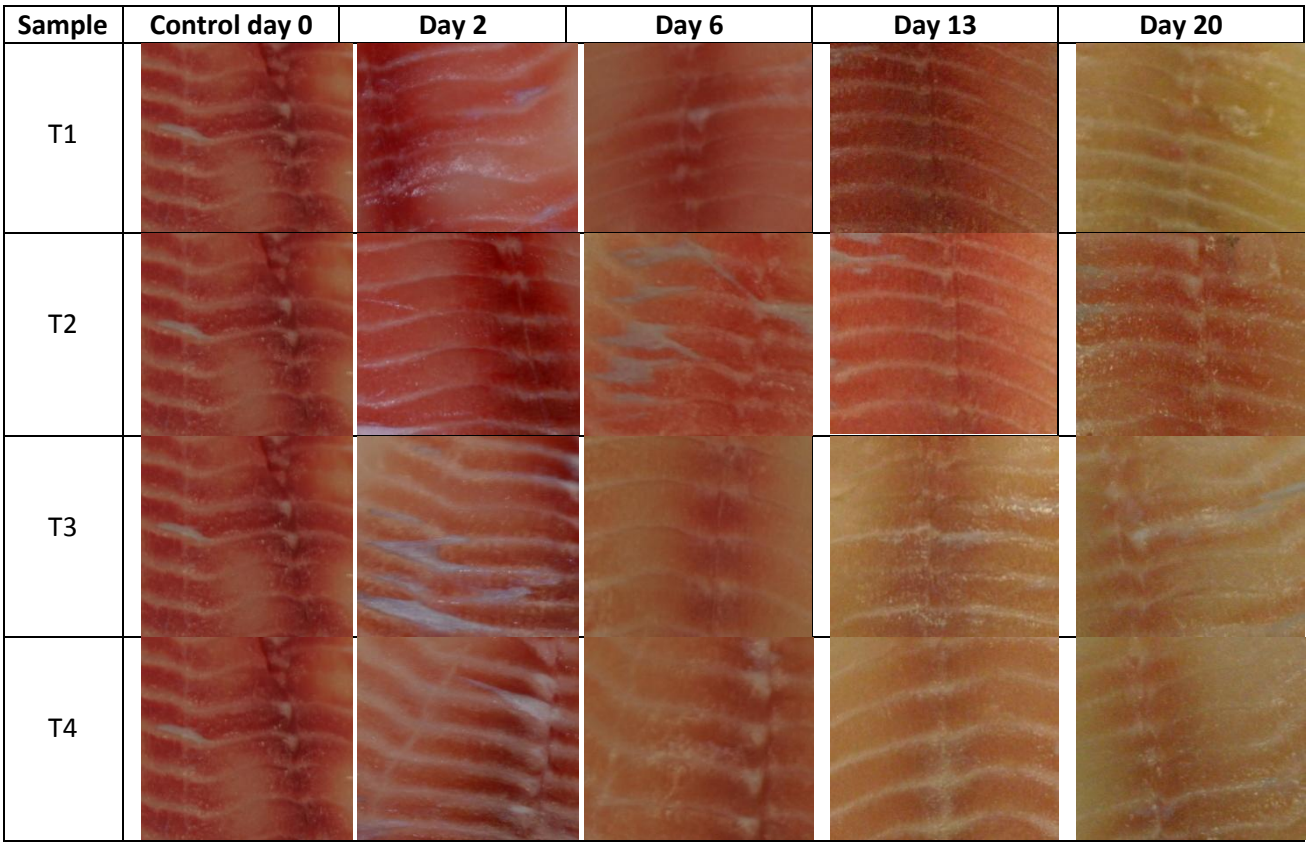


Figure 21: Skin side appearance of packaged deskinned tilapia fillets on selected days during storage. T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C.

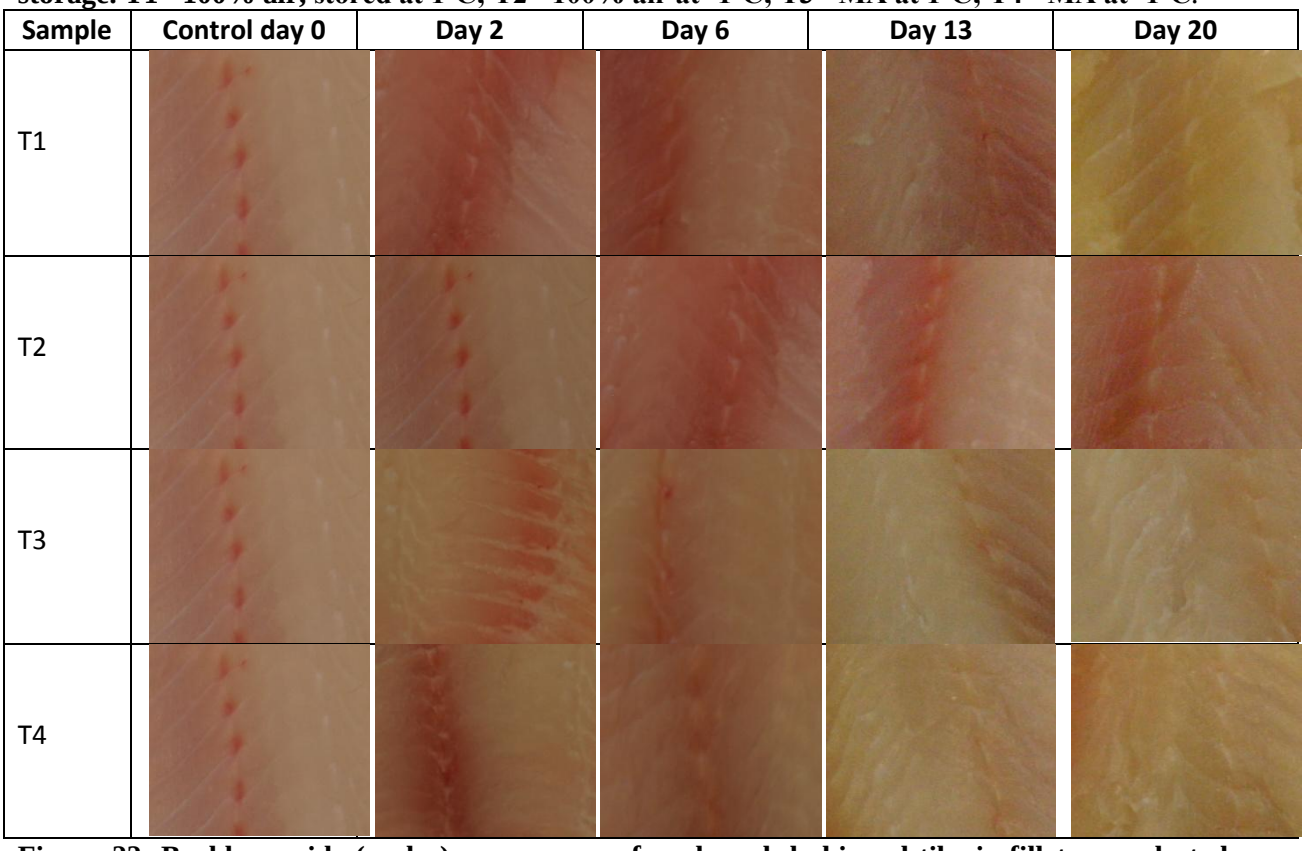


Figure 22: Backbone side (under) appearance of packaged deskinned tilapia fillets on selected days during storage. T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C.

Figures 21 and 22 show visual differences between the samples groups during storage time. A difference emerged between the groups especially with regard to atmosphere (air against MA) during early storage days and storage temperature later towards end of storage time. Air superchilled (T2) retained the initial characteristics longer than in other groups. However, during initial days of storage air packaged groups (T1 and T2) did not show much difference in appearance until after d13, whereas MAP (T3 and T4) showed similar characteristics throughout study period. Lightness showed a slight increase as the storage time progressed, while intensity of redness showed the opposed trend. MA fillets were characterised by light brown colour after packaging (d6) that became intense towards the end of storage time similarly noted with air chilled (T1) on d20. An additional illustration on visual characteristics of sample groups on d16 and d20 is as demonstrated in appendix 3 and MA packaged (T3 and T4) on d23 appendix 4.

3.2.4. Sensory evaluation of cooked samples (QDA)

Figure 23 shows how sample groups were described by sensory attributes of cooked tilapia fillets with storage time. Attributes detected at the beginning of shelf life along the 1st principal component (PC1) were considered to be positive attributes (sweet, metallic and arctic char). Consequently, the attributes detected closer to the end of shelf life (rancid, spoilage, musty and pungent) were considered to be negative attributes. The samples varied mainly with regard to differences in flavour and odour attributes along the 1st principal component (PC1), explaining 65% of the variation between the sample groups. Samples also varied with regard to differences in appearance and texture attributes, mainly ColorU along the 2nd principal component (PC2), explaining 10% of the variation. The main difference occurred with storage time, as the sample groups are located to the left side at the beginning of storage but on the right side towards end of storage time (Figure 23 A). At marginal quality (middle of storage time), samples were more characterised by attributes located along PC2 (especially appearance and texture) as they were located near the bottom or the centre on PC1, but the trend changed for air packaged (especially T1) towards the end of storage as they became more characterised by negative attributes and appearance colour. Air superchilled (T2) and MAP (T3 and T4) appeared to keep the positive attributes describing freshness longer than air chilled (T1). More so, at the end of storage time MA samples (T3 and T4) were more characterised by musty odour and flavour compared to air samples that were more characterised by pungent, spoilage and rancid flavour and rancid odour. Attributes located within the inner eclipse of loadings (Figure 23 B) are less important in describing the samples.

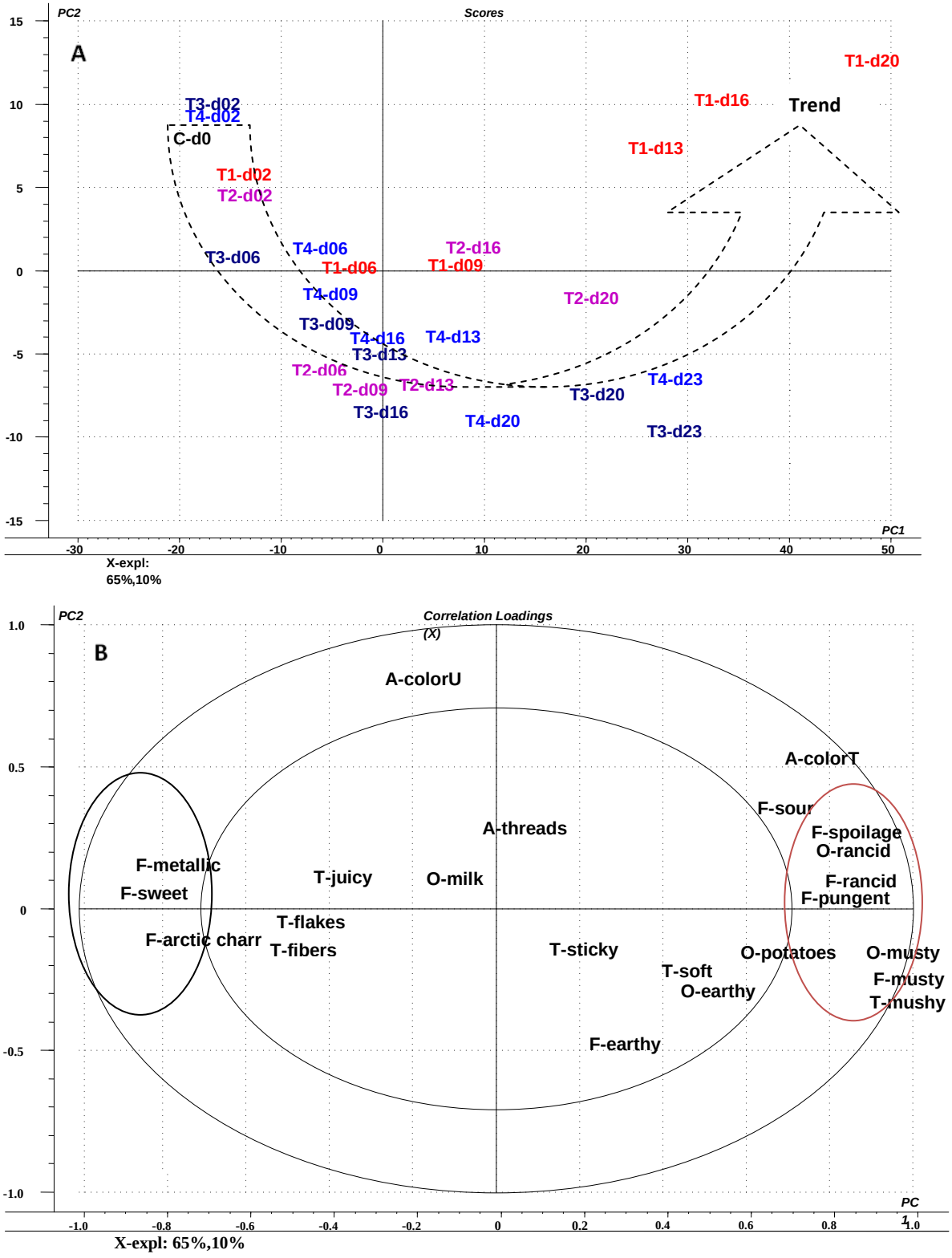


Figure 23: PCA: (a) Scores (sample groups) and (b) correlation loadings (attributes) describing sensory quality of cooked tilapia fillets as evaluated by a trained sensory panel. PC 1 (65%) vs. PC 2 (10%). d = storage time in days; Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C; F = flavour; O = odour; A = appearance; T = texture.

Average scores for sensory attributes of cooked tilapia and level of significance are shown in table 9 (A and B). Average scores for most positive attributes did not change much between sample groups and storage time (scores between 40 and 20) except scores for sweetness and metallic that dropped to below 10 for air chilled (T1) on d20. More so, no significance difference was observed in positive attributes between the sample groups on each sampling day except for flavour attributes (arctic char, sweet and metallic) on d 16 where air chilled was different from all other groups. On d 16 and d20 air chilled (T1) had less sweet flavour compared to the other three groups. In contrast to positive attributes, negative attributes scores (musty, sour, pungent, rancid and spoilage) were not detected in the groups at the beginning of storage (score of <10) except for musty odour and flavour (Table 9 A and B). The attributes (negative) that indicated spoilage of the samples (rancid, pungent and spoilage) became prominent with increasing storage time especially after d9, but differently by sample groups. In general, aforementioned negative attributes that indicated spoilage varied substantially as shown by numerous significant differences between sample groups. Scores for rancid odour and flavour and spoilage flavour increased to around 20 on d13 in air chilled (T1). Specifically, air chilled was different from other sample groups for rancid odour on d13, d16 and d20. Similar trend was noted for rancid and spoilage flavour.

Earthy odour and flavour appeared prominent throughout the storage period in all sample groups with scores of around 30 to 40 (Table 9 A and B).

Texture attributes did not change much with storage time (Table 9 A). The texture attributes fibres and mushy were not significantly different between sample groups on any sampling day. Neither did these attributes change much with storage time as the scores varied between 20 and 30; 40 and 65 throughout the study for texture fibres and mushy accordingly. Soft texture was significantly different between groups on d9 where air superchilled (T2) was softer than MAP superchilled (T4) (Table 9 B). On d20 chilled samples (T1 and T3) had more juicy texture than air superchilled (T2). The MA packaged groups (T3 and T4) had more sticky texture on d13, compared to air packaged groups (T1 and T2).

In appearance, colour on top (ColoT) showed much difference between sample groups than colour under (ColoU) and black threads (BlackThr) as shown in table 9 B. On d16 and d20, air chilled was different from other groups in ColoT. Air samples (T1 and T2) were different from MAP chilled (T3) on d16 in ColoU. MAP groups (T3 and T4) were observed to be different on d23 in regard to ColoT and BlackThr.

Table 9: Mean sensory scores of odour, flavour and appearance/texture attributes of cooked tilapia fillets (average scores N = 2). Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C

A		Odour					Texture					
Storage time (d)	**Group**	**Boilpot**	**Boilmilk**	**Earthy**	**Musty**	**Rancid**	**Flake**	**Soft**	**Juicy**	**Fibres**	**Mushy**	**Sticky**
0	Co	33	28	30	23	3	28	65	63	25	47	31[a]
2	T1	31	27	34	28	2	24	68	60	22	51	43[b]
	T2	30	27	36	25	2	25	69	63	25	45	34
	T3	27	22	29	24	3	26	65	62	26	41	45[b]
	T4	28	28	33	23	2	25	67	64	25	45	38
	P-value	*0.442*	*0.649*	*0.522*	*0.905*	*0.699*	*0.987*	*0.729*	*0.775*	*0.669*	*0.505*	***0.001***
6	T1	33	22	31	26	9	31	70	59	29	49	41
	T2	35	23	36	26	4	26	70	60	26	50	35
	T3	33	27	28	20	3	23	65	52	26	41	40
	T4	34	20	33	25	6	27	67	57	28	45	41
	P-value	*0.953*	*0.342*	*0.125*	*0.252*	*0.080*	*0.138*	*0.431*	*0.095*	*0.805*	*0.221*	*0.510*
9	T1	32	26	33	27	8	28	69	59	26	49	39
	T2	33	25	32	24	5	33	74[a]	60	23	52	37
	T3	31	27	30	23	6	31	67	57	28	46	42
	T4	29	26	28	25	5	33	65[b]	55	27	46	42
	P-value	*0.597*	*0.95*	*0.356*	*0.586*	*0.554*	*0.402*	***0.031***	*0.512*	*0.324*	*0.342*	*0.279*
13	T1	32	21	35	31[a]	15[a]	29	68	53	24	57	35[a]
	T2	30	21	34	30[a]	4[b]	26	71	61	22	55	35[a]
	T3	27	22	33	25[b]	5[b]	25	66	56	24	49	42[b]
	T4	27	21	30	24[b]	6[b]	23	65	54	23	50	41[b]
	P-value	*0.401*	*0.997*	*0.329*	***0.004***	***<0.001***	*0.332*	*0.142*	*0.122*	*0.743*	*0.595*	***0.020***
16	T1	38	24	31	29	20[a]	26	66	55	21	52	38
	T2	37	26	29	24	12[a]	28	65	60	23	50	36
	T3	36	28	27	25	8[b]	32	66	57	28	46	40
	T4	35	29	26	27	7[b]	29	63	54	27	43	39
	P-value	*0.759*	*0.428*	*0.506*	*0.328*	***0.014***	*0.250*	*0.467*	*0.450*	*0.084*	*0.065*	*0.705*
20	T1	35	26	27	32	25[a]	26	66	55[a]	23	55	34
	T2	40	30	28	26	14[b]	29	71	64[b]	23	57	37
	T3	37	25	32	28	14[b]	25	65	55[a]	24	55	38
	T4	36	26	33	28	10[b]	30	67	58	24	52	39
	P-value	*0.520*	*0.603*	*0.087*	*0.368*	***0.004***	*0.380*	*0.145*	***0.042***	*0.928*	*0.540*	*0.842*
23	T3	35	24	31	29	13	27	68	58	23	60	39
	T4	33	22	30	34	14	23	71	62	20	65	41
	P-value	*0.538*	*0.639*	*0.894*	*0.377*	*0.893*	*0.400*	*0.365*	*0.646*	*0.501*	*0.212*	*0.545*

B		Flavour									Appearance		
Storage time (d)	**Group**	**A.char**	**Sweet**	**Metallic**	**Earthy**	**Musty**	**Sour**	**Pungent**	**Rancid**	**Spoilage**	**ColoT**	**ColoU**	**BlackThr**
0	Co	33	33	28	38	23	4	7	4	3	28	54	12
2	T1	37	36	30	35	17	3	5	2	2	30	53	12
	T2	37	35	27	37	20	4	3	2	2	34	46	11
	T3	34	35	33	37	19	5	2	2	2	34	50	11
	T4	36	35	31	37	18	4	3	2	2	29	52	9
	P-value	*0.737*	*0.999*	*0.624*	*0.995*	*0.740*	*0.625*	*0.165*	*0.997*	*0.990*	*0.347*	*0.637*	*0.229*
6	T1	34	30	30	37	25^{a}	9	3	7	7	31	42	14
	T2	33	33	31	44	27^{a}	6	3	6	4	28	40	12
	T3	34	33	31	37	15^{b}	9	4	4	3	27	41	11
	T4	32	31	26	44	26^{a}	6	4	7	5	26	49	15
	P-value	*0.937*	*0.765*	*0.170*	*0.064*	***0.025***	*0.528*	*0.659*	*0.483*	*0.580*	*0.636*	*0.281*	*0.091*
9	T1	32	25	22	42	30	8	4	12^{a}	6	34	46	13
	T2	38	30	26	38	24	8	3	6^{b}	3	32	37	10
	T3	36	29	28	38	23	11	2	4^{b}	6	26	40	15
	T4	38	27	27	36	22	9	2	4^{b}	1	30	42	12
	P-value	*0.075*	*0.479*	*0.283*	*0.197*	*0.190*	*0.648*	*0.548*	***0.020***	*0.130*	*0.320*	*0.200*	*0.181*
13	T1	30	22	20	39	33	13	12	24^{a}	20^{a}	45^{a}	45	14
	T2	32	24	22	41	29	8	6	5^{b}	9^{b}	31^{b}	39	11
	T3	24	25	23	40	29	9	7	7^{b}	5^{b}	31^{b}	40	10
	T4	20	23	21	38	30	11	9	12^{b}	8^{b}	34^{b}	38	10
	P-value	*0.254*	*0.804*	*0.889*	*0.601*	*0.614*	*0.428*	*0.097*	***<0.001***	***0.002***	***<0.001***	*0.313*	*0.057*
16	T1	27^{a}	22^{a}	13^{a}	35	29	5	15	22^{a}	26^{a}	44^{a}	45^{a}	11
	T2	34^{b}	23^{a}	19	34	22	7	8	13^{b}	12^{b}	30^{b}	46^{a}	13
	T3	36^{b}	30^{b}	22^{b}	36	22	7	9	10^{b}	8^{b}	25^{b}	35^{b}	11
	T4	34^{b}	29^{b}	23^{b}	35	23	6	6	10^{b}	7^{b}	27^{b}	40	11
	P-value	***0.002***	***0.006***	***0.010***	*0.955*	*0.304*	*0.692*	*0.087*	***0.024***	***<0.001***	***<0.001***	***0.016***	*0.767*
20	T1	28	14^{a}	13	33	37	10	18	25^{a}	36^{a}	47^{a}	44	14
	T2	34	24^{b}	18	38	28	9	12	18	18^{b}	32^{b}	43	10
	T3	33	22^{b}	15	35	28	10	13	18	19^{b}	30^{b}	37	12
	T4	32	25^{b}	20	36	26	10	11	12^{b}	12^{b}	28^{b}	36	10
	P-value	*0.075*	***0.006***	*0.160*	*0.642*	*0.360*	*0.936*	*0.176*	***0.049***	***<0.001***	***<0.001***	*0.355*	*0.100*
23	T3	26	21	17	38	32	14	14	18	14	28^{a}	36	12^{a}
	T4	28	20	17	35	33	9	13	19	18	37^{b}	38	8^{b}
	P-value	*0.588*	*0.432*	*0.987*	*0.778*	*0.826*	*0.582*	*0.732*	*0.472*	*0.256*	***0.038***	*0.613*	***0.042***

Data within the same column in respect to storage time (d) with different letters are significantly ($p<0.05$) different.

3.2.5. Microbiological analyses

Microbiological quality of fillets packaged in 100% air and MA at the beginning of storage (d0) was generally good with total viable counts (TVC) and presumptive pseudomonads

counts of the flesh being slightly above the limit of detection (log 1.3 CFU/g), whereas H_2S-producers, *Brochothrix thermosphacta* and *Lactobacillus* counts were below the limit of detection (Figures 24-26).

3.2.5.1. TVC and Pseudomonads

Figure 24 shows growth curves for TVC and pseudomonads in air- and MA-packaged tilapia fillets stored at 1˚C and -1˚C. TVC and pseudomonads counts increased more rapidly in air packs than MA. More so, higher growth was evidenced in storage at 1˚C (chilled) than at -1˚C (superchilled). Counts of chilled air-packaged fillets (T1) reached on d16 log 8.6 CFU/g and log 8.4CFU/g for TVC and pseudomonads, respectively. However, the counts reduced slightly on the successive sampling d20. In air superchilled fillets, TVC and pseudomonads growth was moderate, reaching log 7 CFU/g at end of storage time d20. An extended lag phase (delayed growth) of TVC and pseudomonads was observed in MA packages at both storage temperatures. TVC counts reached log 4 CFU/g, though earlier under chilled (d14 for T3) than superchilled (d28 for T4) conditions. *Pseudomonas* spp. counts in superchilled MAP fillets fell below the detection limit after d9 but were detected towards the end of storage time while slight growth in chilled MAP fillets occurred from d16 after a lag phase. In general the correlation in growth curves observed with TVC and *Pseudomonas* spp. was very good especially in air packages, implying *Pseudomonas* spp. were the dominating microflora.

Pseudomonas spp. counts on CFC media (cultivation) and real-time PCR showed Pearson correlation coefficient of 0.842 (Figure 25), which indicates a high degree of correlation between the methods.

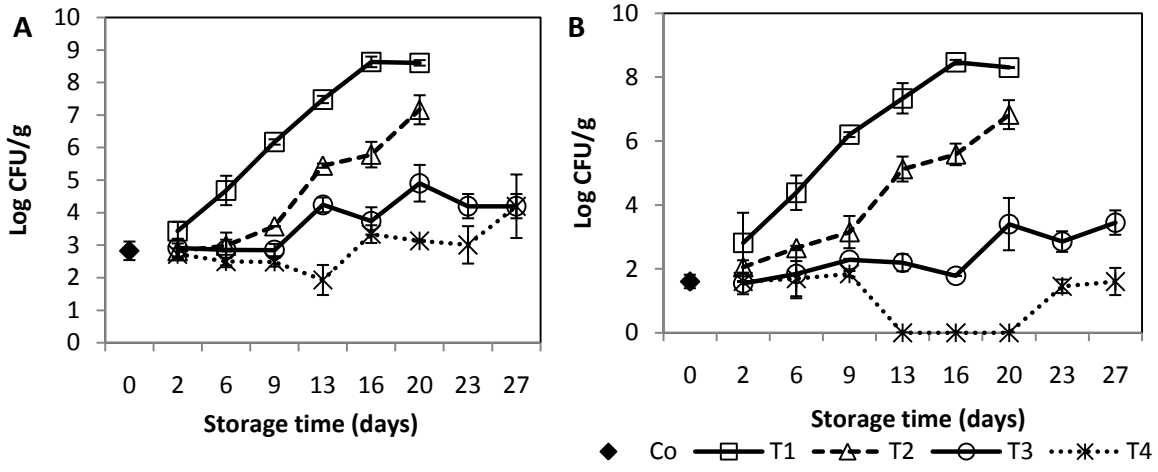


Figure 24: Total psychrotrophic viable counts (A) and presumptive pseudomonads (B) in packaged tilapia fillets during storage. Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C (average score N = 2).

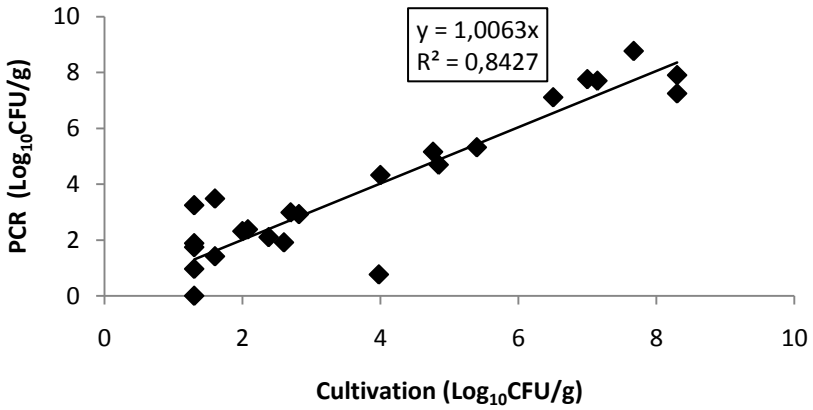


Figure 25: Correlation curve between pseudomonads enumeration using PCR (X-axis) and Cultivation on CFC media (Y-axis) on samples obtained (once a week) on shelf life study of 100% air and MA packaged tilapia fillets.

3.2.5.2. H_2S- producers

Counts of H_2S-producing bacteria varied considerably within the groups and storage time (Figure 26). The effects on growth of H_2S-producers was more evidenced with storage temperature than atmospheric condition with T1 and T3 (air chilled and MAP chilled) recording counts of log 3.1 and <1.3 CFU/g, respectively, on d20 and d27, at end of storage time for air packaged and MA. Low H_2S-producers' counts (< log 4 CFU/g) were observed in T1 and T3 fillets (chilled) at sensory rejection in comparison to other specific spoilage organisms evaluated. T2 and T4 (superchilled) counts were at or below the detection limit during the whole storage period.

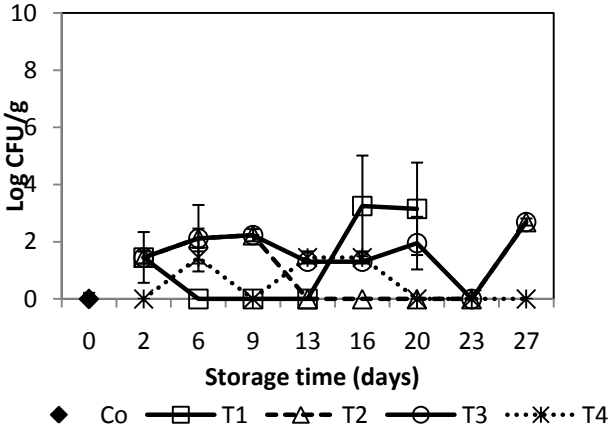


Figure 26: H_2S-producing bacteria in packaged tilapia fillets during storage. Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C (average score N = 2).

3.2.5.3. Brochothrix thermosphacta and Lactobacillus spp.

Brochothrix thermosphacta and lactobacilli developed significantly faster between days 2 and 6 at 1˚C in air packages (T1) compared to other groups, attaining log 6 CFU/g on d16 (Figure 27) after which no further growth was observed in the group. *Brochothrix thermosphacta* and lactobacilli counts in superchilled air-packaged fillets (T2) were log 5 and log 4 CFU/g, respectively, on d20 end of storage time for T2 and T1. In MA packages, a lag phase was evidenced for both bacterial groups, about 6-9 d for chilled but 13 d for superchilled fillets, after which growth occurred. Levels of lactobacilli reached in MAP fillets towards the end of storage were generally tenfold higher than those observed for *Brochothrix thermosphacta*. As observed with other bacterial groups, *Brochothrix thermosphacta* and lactobacilli counts were higher in fillets stored at 1˚C than at -1˚C in respect to atmospheric conditions.

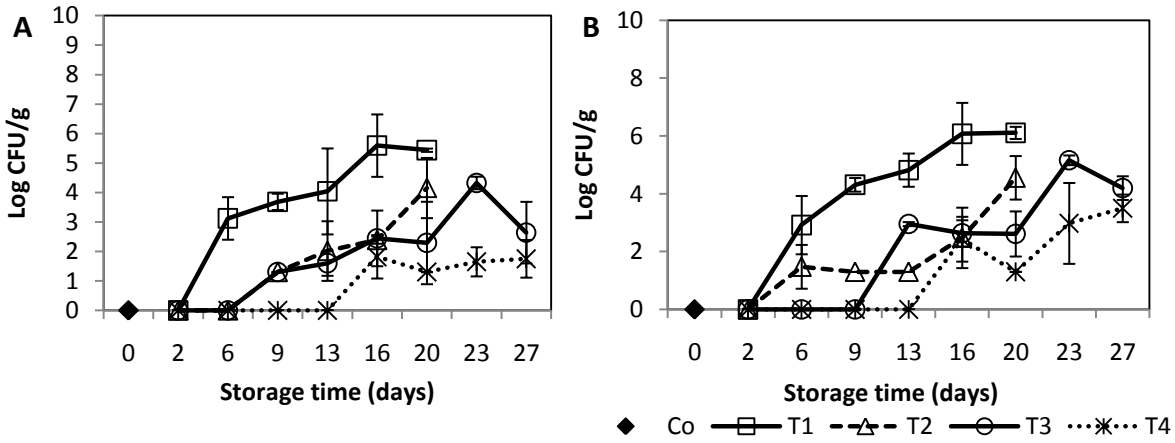


Figure 27: Counts of Brochothrix (A) and Lactobacillus in packaged tilapia fillets during storage. Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C (average score N = 2).

3.2.5.4. Photobacterium phosphoreum

Counts of *Photobacterium phosphoreum* were estimated using a newly developed RT-PCR method, calibrated earlier with samples analysed using the Malthus conductance method described in 2.4.1. As shown in Table 10, only few samples contained *Photobacterium phosphoreum* and at low levels (< log 3 CFU/g). Furthermore, the numbers did not reflect changes with respect to storage time but were only reported in 100% air packaged fillets (T1 and T2).

Table 10: Counts (log CFU/g) of Photobacterium phosphoreum in tilapia fillets during storage at 1˚C and -1˚C as enumerated by real time PCR. T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C

Storage time (d)	6	13	20
Sample group			
T1	2.62	0.01	ND
T2	ND	0.35	ND
T3	ND	ND	ND
T4	ND	ND	ND

Values reported as means of duplicate per group.
Initial count of product (control): log 0.47/g
ND: not detected

3.2.6. Physical changes and chemical analyses

3.2.6.1. pH changes

pH values were slightly higher in air than MA packaged tilapia fillets (Table 9). However, no significant differences were observed among values determined from different atmosphere and storage conditions (P>0.05) on each sampling day except MAP chilled (T3) that was different from others on d2. On comparing the values, it became obvious that no consistent trends for an increase or decrease in pH as a function of storage time and temperature were observed. pH varied between 6.5 and 6.7 during storage at 1˚C, and between 6.4 and 6.6 during storage at -1˚C for air packaged fillets (T1 and T2). In MA packages (T3 and T4), pH varied between 6.4 and 6.5 at both 1˚C and at -1˚C. In general therefore, higher pH was observed in air than MA packaged sample groups.

Table 11: pH changes in packaged tilapia fillet during storage. Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C (average score N = 2). - = not assessed on that day.

Day	0	2	6	9	13	16	20	23
Group								
C	6.5±0	-	-	-	-	-	-	-
T1		6.5±0	6.6±0	6.6±0.07	6.5±0.07	6.6±0.28	6.7±0	-
T2		6.6±0.07	6.7±0.07	6.4±0	6.6±0.07	6.4±0	6.5±0.07	-
T3		6.4±0^{c}	6.5±0.07	6.4±0	6.5±0.07	6.4±0.07	6.5±0.14	6.5±0
T4		6.5±0	6.5±0.07	6.5±0	6.4±0	6.4±0.14	6.5±0.07	6.5±0.07
P-value		***0.049***	*0.111*	*0.138*	*0.242*	*0.501*	*0.150*	*0.422*

Data within the same column with different letters are significantly (p<0.05) different.

3.2.6.2. Moisture content and Water holding capacity (WHC)

As shown in figure 28 A, moisture content remained apparently stable throughout storage time except samples stored at 1˚C which exhibited gradually decrease towards the end of storage. However, no significant difference was observed between sample groups (p > 0.05)

during storage time. Chilled samples (T1 and T3) recorded low moisture content of 74 and 75% on d20 and d27 accordingly, whereas counterparts stored at superchilled (-1˚C) recorded averagely 76% at the end of storage. On the other hand, WHC decreased during early in storage and increased later on d20 in all the groups, but the increase was less in air superchilled (Figure 28 B). Furthermore, a decrease in WHC was observed on d27 when only MA groups were evaluated. In general, WHC varied between 94 to 96% and no significant different was observed between the groups (p>0.05, data not provided).

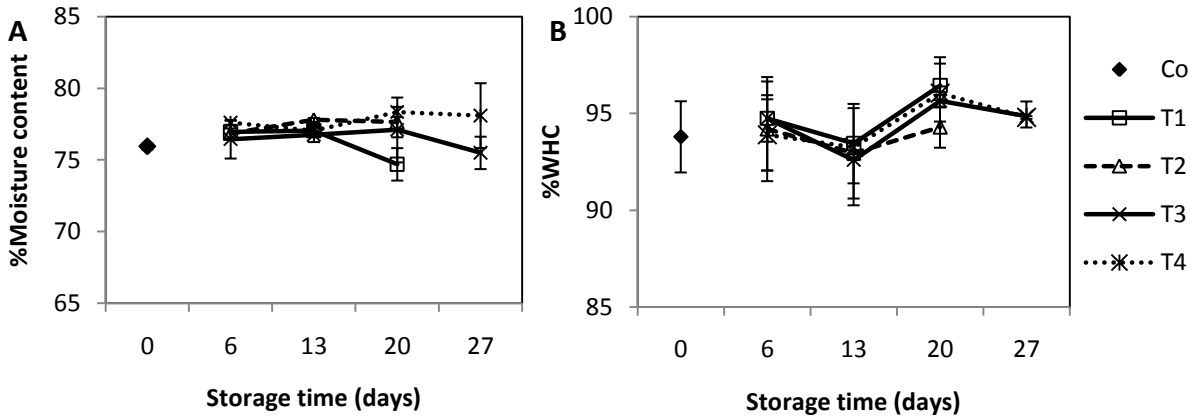


Figure 28: Changes in moisture content (A) and water holding capacity (WHC) (B) in packaged tilapia fillets during storage. Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C. Values (percentage of sample weight) are given as mean (N = 2).

3.2.6.3. Drip loss

Percentage drip loss (exudation) of fillets in all groups (air and MA) on d2 was not significantly different (p >0.05) irrespective of storage temperature and atmosphere (Figure 29). However, the amount of drip generally increased as storage time progressed. Effects of interaction between atmosphere condition in headspace and storage temperature were observed. Higher drip losses were recorded in MA packaged groups (T3 and T4), with chilled storage receiving significantly higher value (approx. 1.2%) than superchilled (approx. 1%) on d20 and d23 ($p < 0.05$). More so on d20 both MAP (T3 and T4) were different from air-packaged (T1 and T2) that were not different from each other. Drip loss of air packaged fillets increased gradually with storage time reaching approx. 0.8% at chilled and 0.6% at superchilling on d20 (Figure 29). Fewer changes in drip were observed in MAP after d20.

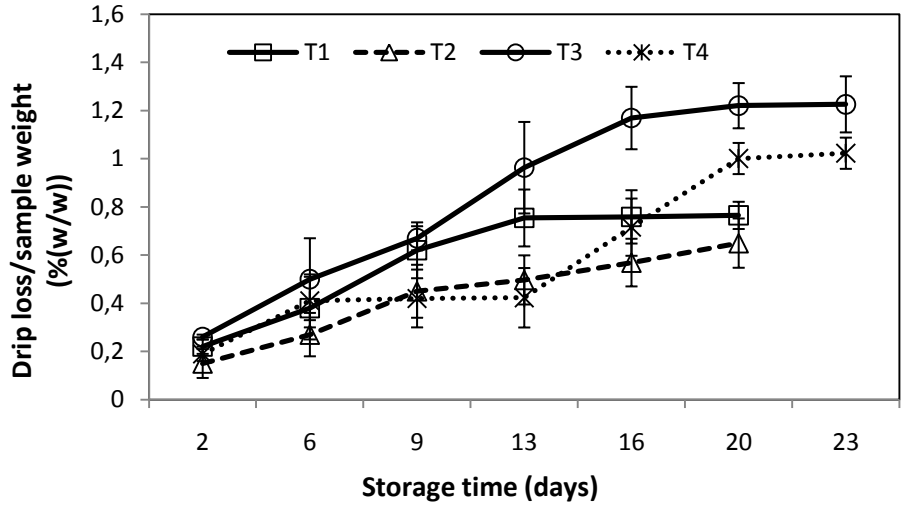


Figure 29: Relative quantity of drip loss in packaged tilapia fillets during storage. T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C. Values (percentage of initial sample weight) are given as mean ± SEM (N = 3).

3.2.6.4. TVBN and TMA

Figure 30 A, demonstrated on average TVBN values of 13.8–18.0 mg N/100 g that did not change much during storage period except for air chilled (T1) which increased above that level attaining 21.3 mg N/100 g on d20. On the other hand, TMA was not detected until after d16 in all the groups (Figure 30 B). Air chilled (T1) recorded 0.5 mg N/100 g on d20 whereas, air superchilled (T2) and MAP chilled (T3) recorded on average 0.07 mg N/100 g on the same day. However, on subsequent evaluation for MAP groups on d23, TMA value for chilled (T3) increased to 0.3 and was not detected on d27 whereas, it remained undetected in superchilled (T4) throughout the study period.

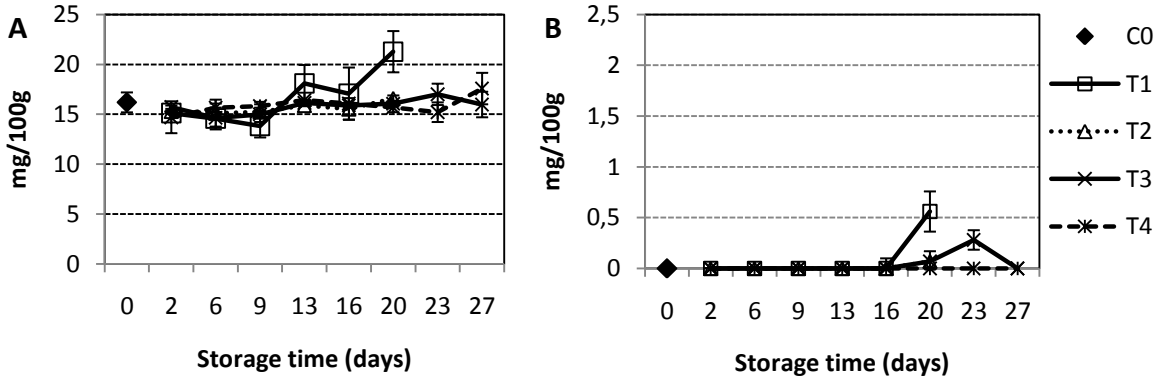


Figure 30: Changes in TVB-N (A) and TMA (B) content of packaged tilapia fillets during storage. Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C. Values (mg/100g) are given as mean ± air chilled SEM (N = 2).

4. DISCUSSION

4.1. Pre-trial studies

4.1.1. Pre-test 2: Shelf life study trial

4.1.1.1. Temperature changes during fillets storage

The reported average ambient temperature of -1°C and 1°C (outside Styrofoam boxes) designed for superchilling and chilling storage of tilapia fillets resulted to fillets contact temperature (inside Styrofoam boxes) of 0.5±0.5°C and 2±0.25°C respectively. The fillets contact temperature in the study showed conditions for both groups to fall under conventional chilling; which according to Duun and Rustad (2007) may be defined as the process of cooling fish or fish products to a temperature approaching that of melting ice. Conversely, superchilling may be defined as a process where the surrounding temperature is set below the initial freezing point of the product without freezing it (Ando *et al*, 2005). This was not the case with superchilling storage condition (S) reported in the current study. However, the difference of just 1.5°C between the groups led to a big difference in shelf life as discussed under evaluation methods.

4.1.1.2. Modification of QIM scheme

Scores for most parameters describing changes in fresh tilapia fillets listed in preliminary scheme during the pre-observation were well utilised, although in neither group was the maximum score reached at the end at storage time. This is in accordance with how the QIM scheme are constructed, where fish evaluated shortly after catch should be scored low and subsequently increase with storage time reaching close to maximum score at the end of shelf life (Martinsdottir *et al*., 2001). However, flesh blood and gaping parameters were omitted from the scheme as they were difficult to evaluate and recorded minor changes with storage time. Their scores were removed or added to other parameter in the modified QIM scheme at the end of study (Table 12). This is in agreement with Sveinsdottir *et al.* (2003) who reported removal from the scheme of parameters whose evaluation was destructive and difficult. The description of flesh colour in the scheme was modified into two parameters; colour loin and colour belly flap to describe changes more precisely. In addition, changes in selection of words were made for colour related parameters to describe the changes better. Modifications were necessary at the end of study to come up with an exclusive QIM scheme for farmed tilapia fillets based on Canadian and Icelandic tilapia. The maximum sum of points (QI) in the recommended scheme is 13 (Table 12).

Table 12: A final Quality Index Method scheme developed from preliminary scheme for de-skinned tilapia fillets (*Oreochromis niloticus*)

Quality parameter		Description	Score
Skin side	*Colour* *	Dark red, red brown	0
		Red brown, lighter colour	1
		Light brown	2
Flesh	*Colour, loin*	light, beige, trace of red or bluish	0
		A little darker colour, a little brownish or greyish	1
		Greyish, brownish, yellowish	2
	Colour, flap	bluish, transparent	0
		light, milky colour	1
		Greyish or brownish	2
	Mucus	Fresh shiny texture, thin clear mucus	0
		Trace of mucus, a little thick	1
		Milky or greenish mucus	2
	Texture	Firm	0
		Rather soft	1
		soft	2
	Odour	Fresh, neutral	0
		Seaweed, marine, grass	1
		Sour milk, silage	2
		Acetic, putrid	3
Quality index (0-13)			

* Lateral/longitudinal stripes at the middle of the loin

4.1.1.3. Sensory evaluation of raw fillets (QIM)

The linear relationship with a high correlation of $R^2 = 0.943$ at 2±0.25°C and $R^2 = 0.913$ at 0.5±0.5°C (fillets contact temperature) between QI and storage time reported in the study, showed that attributes gradually deteriorated with time as it is assumed in the Quality Index Method that the scores for all quality parameters increase with storage time (Martinsdottir *et al*., 2001). Due to individual variations present in samples of the same storage day (and conditions), three samples per group was used. According to the guidelines for freshness assessment of whole fish given by Martinsdottir *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 rate of fish. In all trials, a difference of approximately 1.7 QI score was observed between chilled (C) and superchilled (Sc) fillets (day 7, 13 and 20 of storage) having $P<0.05$, with chilled recording higher index. The difference in the QI corresponded to a 3-day longer shelf life of superchilled compared to chilled fillets.

4.1.1.4. Sensory evaluation of cooked fillets (QDA)

The sensory attributes sour and rancid odour and flavour were considered negative attributes, as they are indicators of spoilage (Cyprian *et al*., 2008 and Sveinsdottir *et al*., 2002). These attributes did not change significantly until after 13 d of storage for both groups, but

progressed rapidly thereafter. End of shelf life is usually determined when spoilage related sensory attributes become evident and most panellists detect them. The average QDA score of above 20 (on the scale 0 to 100) for these attributes (negative) indicates that sample is approaching the end of shelf life (Cyprian *et al*., 2008 and Bonilla *et al*., 2007). Based on this both groups were past shelf life after 20d. Sour and rancid flavour attributes were just within the unacceptable limits (20-30) for superchilled fillets (Sc). However, it was clear that sour and rancid flavour for chilled fillets (C) on 20 d was far above limit and thus past shelf life. These limits have been used in determination of maximum shelf life of farmed arctic charr (Cyprian *et al*., 2008), cod fillets (Bonilla *et al*., 2007), desalted cod (Magnusson *et al*., 2006) and farmed Atlantic salmon (Sveinsdottir *et al.,* 2002). Spoilage of chilled tilapia fillets during storage at 2±0.25°C and 0.5±0.5°C (contact temperature) might have been due to combined effects of chemical and bacterial activity, as sour and rancid flavour were both evidenced during evaluation of cooked samples at the end of storage time. The observation is similar to earlier study (Cyprian *et al*., 2008) who reported both chemical and bacterial spoilage in temperature abused prior to icing and iced arctic charr at the end of storage life.

Earthy and mould odour and flavour attribute recorded higher scores throughout the study. These could be characteristic of farmed tilapia, as off flavour and odour in tilapia are commonly observed under farmed condition. FAO (2002) reported farmed tilapia to be tested for flavour before they are accepted for processing and marketing in developed countries. If they have off-flavour, they are purged in clean water for 3-7 days in holding tanks or ponds. The procedure is generally not practised in developing countries where the use of spicy seasonings in the cooking process conceals any off-flavour (FAO, 2002).

Texture and appearance attributes did not change significantly, although gave information about the overall quality of tilapia fillets with storage time.

4.1.1.5. QDA in Icelandic tilapia trials

The difference in sensory characteristics of cooked (odour earthy and flavour mould) Icelandic tilapia harvested directly from RAS (IR) and the ones held in fresh water and starved prior to harvesting (IF) indicates that holding farmed tilapia in fresh water prior to harvesting could result to better sensory quality/eating quality. It is however noted that both groups should have been evaluated in parallel and over time to get to realistic conclusions.

Comparing Icelandic tilapia to Canadian (Tables 7 and 8), IR-group tends to be characterised more by negative attributes compared to counterparts C-group of the same storage day. This

could imply that Icelandic tilapia (IR) spoiled more rapidly than Canadian. In the study Icelandic tilapia had not attained table size as was with Canadian. The difference in quality yet same species could therefore be related to difference in culture conditions, size/age and geographical location. This is because the physical, chemical and bacteriological characteristics of fish tend to vary with species, feeding habits, seasonality (Smith *et al.*, 1980), spawning cycles, methods of catching, fishing ground (Whittle *et al.*, 1980) size, age, environment, initial microbiological load and possibly geographical location (Shewan, 1977).

4.1.1.6. Microbial counts

The low total counts reported at the beginning of storage time were due to the flesh of newly caught fish being sterile, since immune system of the fish prevents the bacteria from growing. However, when the fish dies, the immune system collapses and consequently during storage, bacteria invade the flesh (Huss, 1995). At the end of shelf life estimated to be about 16 d and 19 d for C (1°C ambient) and Sc (-1°C ambient) respectively, total viable counts (TVC) could be averaged to log 8/g. The counts are similar to previous study by Reddy *et al.* (1995) who reported TVC of 10^8cfu/g at rejection time (13 days) for tilapia fillets packaged under 100% air and stored at a temperature of 4°C.

At the end of storage time 20 d, both groups reported TVC counts of log 8/g although C appeared to have recorded higher counts throughout the study time. In addition, the composition of H_2S producing bacteria to TVC was similar in both groups. This may partly be attributed to both groups (C and SC) falling under conventional chilling instead of anticipated superchilling storage for Sc, as earlier studies reported superchilling technique to effectively delay bacterial growth and prolong the shelf life of chilled fish (Huss, 1995; Church *et al.*, 1998).

4.1.1.7. Shelf life

The QDA method is useful in shelf life studies, as can be used to provide information about maximum shelf life. The results from QDA and microbiological analysis indicated that the shelf life of sample group Sc (superchilled) and C (chilled) was approximately 19 and 16 days respectively. According to QDA, superchilled reached unacceptable sensory quality after around 18-20 d of storage, whereas chilled attained the limits approximately 15-17 d of storage, but was passed consumption limits on 18 d of storage.

4.1.2. Trial 3: Selection of gas composition (MA) and main spoilage organisms

Farmed tilapia used in the study was found to have 3.5-4.2% fat content (Table 6). However, when a product contains fat we can assume that oxygen barrier is required, although the nature and content of the fat, and whether or not any antioxidants are used will determine the degree of barrier (Kilcast and Subramaniam, 2000). To find an appropriate MA for fresh tilapia (*O. niloticus)* fillets, the component of the air in the packaging was altered without oxygen by adding CO_2 with different ratio of N_2. CO_2 composition in the packages reduced during storage because it's highly soluble in water and fat (Reddy and Armstrong, 1992; Sivertsvik *et. al.*, 2002). In terms of quality parameters MA packaged with 50%:CO_2:50% N_2 (B) showed good characteristics when cooked, were less dry and recorded less drip compared to other combinations of MA-packing. However, in comparison to control (air stored) it exhibited higher drip.

Iron agar was selected for enumeration of TVC in comparison to mLH agar, whereas for LAB MRSS was selected instead of NAP agar. This was due to higher counts recorded on MRSS agar, thereafter used in the study. Bacterial growth in tilapia fillets packaged in 50% CO_2 was not different to 70% CO_2 for all microbial groups evaluated except *Brochothrix thermosphacta*. It's therefore worth concluding that microbiologically 90% CO_2 packaged fillets offered better results.

On comparing results obtained (d10) based on quality parameters analysed (sensory, drip and microbial), the appropriate MA for storage of tilapia fillets was 50% CO_2: 50% N_2 (B). For TVC and pseudomonads that seem to be the main specific spoilage organism in tilapia, approximately log 4/g lower counts were recorded in 50% CO_2 packaged compared to control group (air stored). For that reason, the quality of the fillets remained good under such atmosphere due to low microbial load (food safety).

4.2. Main study

4.2.1. Headspace gas composition

Oxygen levels in all the groups (packs) showed similar trends of decline with storage time as those reported elsewhere (Reddy *et. al.*, 1995; Hovda *et. al.*, 2007). The largest decline occurred in 100% air packaged samples stored at 1˚C (T1) which corresponded with the occurrence of high bacterial numbers (> log 8 CFU/g for TVC and pseudomonads, Figure 24) suggesting that the oxygen was being consumed during microbial metabolism. In contrast oxygen did not decrease significantly in 100% air packaged stored at -1˚C (T2) which in

relation to the preceding statement was due to low bacterial numbers (< log 4 CFU/g) at the end of storage time. Similar trends in opposed direction were observed with CO_2 composition in headspace of 100% air packaged fillets (T1 and T2), CO_2 increased as secondary product of metabolism (Hovda *et. al.*, 2007).

Initially, the headspace of MA packaged fillets (T3 and T4) had low oxygen levels (<1%), which was observed to be higher on subsequent sampling d2 (>2%) and thereafter gradually declined (Figure 17). This as reported by Reddy *et al.* (1995) was as a result of oxygen leaching from the foam tray, soak pad, and fillet into the package headspace. In packages stored at 1˚C the decrease was more rapid than at -1˚C as microbial and post-mortem metabolic activity increased. However, O_2 did not get to below the initial 1% as less microbial growth was reported in MA packaged groups. On the other hand, CO_2 reduced from 50% at packaging to around 40% during early storage days up to d2 as it dissolved into fillets due to its high solubility in water and fat (Soccol and Oetterer, 2003). At later storage days, CO_2 showed insignificantly upward trend especially in MA packaged fillets stored at 1˚C which could be due to an increase in microbial activity.

4.2.2. Sensory evaluation of raw fillets (QIM)

The linear relationship observed between QI and storage time reported in all sample groups during chilling and superchilling storage at 1˚C and -1˚C (Figure 19 and Appendix 2), showed that attributes gradually deteriorated with time as it is assumed in the Quality Index Method that the scores for all quality parameters increase with storage time (Martinsdottir *et al*., 2001). The first sensory changes to occur in stored fish are related to appearance and texture (Huss, 1995). In agreement with this author's findings, the first change observed in 100% air and MA stored tilapia fillets was deterioration in muscle appearance on d2 of storage at both 1˚C and -1˚C.

Colour related quality parameters showed a consistent sample grouping as a function of headspace atmosphere with MA groups recording higher scores. At the beginning of storage time, tilapia fillets were described as dark red and light with trace of blood or bluish on skin and backbone sides accordingly. The colour faded soon after packaging for MA on d2 which was characterised by red brown and light brown on skin and backbone sides, that progressed to light brown and greyish brown respectively towards the end of storage time. Skin discolouration of some MA-treated fish samples have previous been reported (Lauzon *et. al.*, 2002; Stansby and Griffiths, 1935) and is due to denaturation of muscle and pigment protein. The observation was different in 100% air as fillets retained the fresh cherry red colour

characteristics longer especially with storage at -1˚C which was characterised by red brown and light with trace of blood on skin and backbone sides accordingly at the end of storage time d20. This could be owing to the fact that fresh meat cherry-red colour is due to the presence of oxy-myoglobin. Oka (1989) reported that for some fish products oxygen could or should be used, for example high level of oxygen has been used in red fish meat (tuna and yellow tail etc) to maintain red colour, reduce and retard browning caused by formation of metmyoglobin. Kilcast and Subramaniam (2000) correspondingly reported that fresh meats especially beef; oxygen is needed to develop and maintain bright red colour associated with freshness. The preservation of colour characteristics in 100% air stored at -1˚C than counterpart stored at 1˚C can be explained by the fact that superchilling under strict controlled refrigeration, overcome unfavourable changes in fish quality such discoloration (Ando *et al*., 2005 and 2004).

In other quality parameters used in QIM (Figure 20), fresh fillets had a firm texture, no mucus and no off odour for all the groups during early storage time. Off odour and mucus were higher in T1 than in other groups on d20 mainly because strong off odours and mucus are associated with fish spoilage as a result of metabolites released by bacterial action (Olafsdottir *et al*., 1997; Reddy et al., 1995) as reflected by higher aerobic counts in T1 at the end of storage time.

Significant correlation between attributes and storage time was observed for all groups (Appendix 2), this as aforementioned was because the attributes gradually deteriorated with storage time and confirms that the QIM scheme was applicable in all cases. Higher correlation coefficient reported in MA than air packaged groups implies that the QIM scheme reflects the storage time of MA tilapia fillets more accurately. An explanation for the high correlation coefficient in MAP than air at 1˚C based on which the scheme was developed, could be as a result of rapid increase in QI for T1 between d13 and d20 that corresponds well to microbial growth in the group. Besides, after d13 air packaged stored at 1˚C (T1) had developed MAs totally different from 100% air (high level of CO_2) that could have triggered spoilage characteristics especially in colour parameters that reflected rapid deterioration (changes) under higher CO_2. QIM results indicated that on d16, T1 was different from the same group (T1) of preceding sampling day, a trend not observed in other groups. This indicates that T1 was showing spoilage signs on d16. Moreover, T1 on d20 recorded higher QI that could not be attained by MA packaged groups even on successful sampling d23. On the other hand, QI

recorded by T1 on d16 was equitable to QI of T3 on d9 and T4 on d13, whereas T2 did not attained that score on d20.

4.2.3. Sensory evaluation of cooked fillets (QDA)

Sensory quality attributes for cooked tilapia detected at the beginning of storage time (sweet, metallic and arctic charr) were considered positive attributes whereas, attributes (rancid, spoilage, musty and pungent) detected at the end of storage time were considered negative attributes as they are indicators of freshness and spoilage accordingly (Cyprian *et al*., 2008; Sveinsdottir *et al*., 2002). The clear grouping of the attributes evidenced on each side of PC1-axis indicates that fresh samples were easily distinguished from spoiling samples (Sveinsdottir, *et al*., 2003). Positive flavour attributes showed significant difference between air packaged fillets store at 1˚C (T1) and other groups on d16 implying that both storage temperature and atmosphere contributed significantly towards retention of flavour positive characteristics since T1 and T2 were air packaged and T1 and T3 had different headspace atmosphere but both stored at 1˚C.

The negative attributes did not change significantly between the groups with storage time until after d9. End of shelf life is usually determined when spoilage related sensory attributes become evident and most panellists detect them. The average QDA score of above 20 (on the scale 0 to 100) for these attributes (negative) has been applied by various authors as an indication that fish sample is approaching the end of shelf life (Cyprian *et al*., 2009; Bonilla *et al*., 2007; Magnusson *et al*., 2006; Sveinsdottir *et al*., 2002). Air packaged fillets stored at 1˚C (T1) had started showing spoilage characteristics on d13 when negative attributes scores reached 20, besides the group being significantly different from all others. On successful evaluation d16 and d20, air packaged fillets stored at1˚C (T2) and MAP (T3 and T4) did record scores above 20 and were not significantly different ($p>0.05$) whereas T1 scores rose above 20. Similarly on d23 when only MAP fillets were evaluated, both groups (T3 and T4) scored less than 20 for negative attributes. More so on d20 a part of the panel refused to taste T1 samples after smelling. This strongly indicated that the group (air stored at 1˚C) according to sensory evaluation was no longer fit for human consumption on d20. Similar observation was noted by Sveinsdottir *et al.,* (2002) who reported salmon to be unfit for consumption after 20 d when part of the panel refused to taste samples. T2 and T3 recorded scores close to 20 (18 for rancid flavour and off-flavour) on d20 and d23 respectively implying the groups could be sensorial close to end of shelf life. As with negative attributes, colour on top (cooked samples) showed T1 to be different from other groups after d13 of storage.

In the current study, sour flavour was not important in characterising spoilage of sample groups as less change in scores were observed as storage progressed. This could in part be attributed to low microbial growth reported especially in MA packaged fillets and air stored at -1˚C, as sour odour and flavour originates from short-chain fatty acids, alcohols, sulphur compounds, and amines generated by microbial activity (Olafsdottir et al., 1997; Shewan, 1977).

Earthy and musty odour and flavour attribute recorded moderate scores throughout the study. These as earlier explained under section 4.1.1.4 could be characteristic of farmed tilapia, as earthy flavour is commonly observed.

4.2.4. Microbiological analyses

During pre-trial study, pseudomonads were identified as the main specific spoilage organisms in tilapia. This is in agreement with Huss (1994) who reported *Pseudomonas* spp. to be the specific spoilage bacteria of iced stored tropical freshwater fish. TVC maximum limit of log 8 CFU/g has previously been used in tilapia as limit for human consumption (Waliszewski and Avalos, 2001; Reddy *et al.*, 1995). Reddy *et al.* (1995) reported total aerobic, anaerobic and coliform counts to have reached a maximum level of log 8.0 CFU/g on the day of tilapia fillets spoilage. In this study, TVC and *Pseudomonas* spp. reached > log 8 CFU (log 8.6 and log 8.4 respectively) for air packaged during storage at 1˚C (T1) on d16 indicating that they were past consumption limit. However on subsequent evaluation d20, similar or lower counts for TVC and *Pseudomonas* spp. were reported in T1. This observation was mainly attributed to change in headspace gas composition (Figure 17 A) whereby the exponential aerobic growth resulted to high oxygen consumption and an increase in CO_2 a secondary product of metabolism whose accumulation restricts further growth (Adams and Moss, 2008). In air packaged fillets stored at -1˚C, TVC and *Pseudomonas* spp. counts were at consumption limit (log 7 CFU/g) at the end of storage time d20 using log 8 CFU/g as rejection limit for tilapia consumption. The lower counts evidenced in air packaged during storage at -1˚C compared to at 1˚C is due to effective delay of bacterial growth during storage at superchilling conditions (Huss, 1995, Church *et al*., 1998). MA groups (T3 and T4) recorded extended lag phase during early storage days and aerobic counts of < log 4 CFU/g up to d27; this indicates that aerobic counts were not observed to reach human consumption limit for MA packaged fillets. The delayed growth and lower counts of aerobic microorganisms in MA packaged fillets can be attributed mainly to CO_2 inhibition (Sivertsvik *et al*., 2002) and the presence of low level

O_2 (Reddy and Armstrong, 1992) but also to low storage temperature (Huss, 1995) used in the present study.

Real-time PCR method showed a good *Pseudomonas* spp. counts correlation (R^2=0.842) with cultivation on CFC media. This indicates that predictive modelling using PCR (Reynisson *et al.*, 2008) can be used to estimate pseudomonads counts, the main spoilage organisms in air stored tilapia rapidly and eventually predict the remaining shelf life. Cultivations methods need at least 2-5 days before the results can be obtained. Furthermore, on CFC media Enterobacteriaceae can interfere with estimation of pseudomonads counts, unlike real-time PCR that is quite selective.

Low counts (<log 4 CFU/g) were reported for H_2S-producing bacteria throughout the study, with storage at -1˚C for both air and MA (T2 and T4) recording counts below detection limits at the end of time d20 and d27 respectively. This could partly be due to absence of *S. putrefaciens* (the main H_2S-producer) in tropical freshwater fish since the organism is typical of marine waters (Sivertsvik *et al*., 2002). It has also been reported that growth of microbial association during food spoilage depends not only on the imposed environmental conditions, as is well known, but also on microbial interaction. Gram and Melchiorsen (1992) reported *Pseudomonas* spp. to inhibit the growth of *Shewanella putrefaciens* due to the ability of the former to produce siderophores, and this interaction could have been a factor governing the development of spoilage flora since higher counts of *Pseudomonas* spp. were reported in the study.

The facultative anaerobe *Brochothrix thermosphacta* and the strictly fermentative lactobacilli showed unstable growth trends in all groups except T1. As observed with aerobes, moderately high counts were recorded with air packaged fillets than counterparts MA packaged. Growth of these organisms in air packaged fillets in the study might be attributed to the fact that different anaerobic microorganisms show different sensitivity levels to oxygen (Soccol *et al*., 2005). The lag phase extension for *Brochothrix thermosphacta* and lactobacilli was however observed to be a function of storage temperature and atmosphere. Correlating microbial results for various groups, it can be deduced that T2; T3 and T4 were within microbial allowable consumption limits on d20 and d27 accordingly, whereas T1 was passed the limit on d16.

Low *Photobacterium phosphoreum* reported in tilapia fillets air and MA packaged may be because the organism is typical of marine waters. Dalgaard *et al.* (1997) did not detect the

presence of *P. phosphoreum* in fresh water fish, despite finding great growth (> 10^7 CFU/g) of this microorganism in marine species.

4.2.5. Physical and chemical analyses

A gradual decrease in water holding capacity was observed during early storage time in all sample groups (air and MA). The decrease in WHC can be caused by gradually denaturation of muscle proteins with increasing storage time (Oyelese, 2005) that could have been influenced by microbial activities and pH changes. This is the likely explanation for the low moisture content observed in T1 (air stored at1˚C) and T3 (MAP stored at 1˚C) at their respective end of storage time. As time progressed liquid lost (drip) reduced the loosely bound water in flesh muscles yielding to low moisture content and in return an increase in WHC observed on d20. In general, Moisture content and WHC showed no significant different between samples groups. Consequently, the differences in drip loss cannot be explained by differences in water content and WHC. Similar observations were found in Atlantic salmon superchilled at different temperature (Duun and Rustad, 2008). The authors reported fish with a higher water content, to have a higher proportion of loosely bound water.

Storage time and atmosphere interaction did not affect significantly fillets pH values. Similarly Reddy *et al.* (1995) did not find significant differences in pH between packing atmospheres (100% air and 75% CO_2:25% N_2) during storage of tilapia fillets at abused and refrigerated temperature. Moreover, low pH values were observed in MA than air packaged fillets in the current study due to the acidic effect of dissolved CO_2 (Sivertsvik *et. al.*, 2002). This could partly explain the bacteriostatic effects of CO_2 in MAP (Huss, 1995). Besides, the little variations in pH observed between air and MA packaged contributed significantly to drip changes reported between MA and air packaged fillets. The post-mortem pH according to Huss (1988), is the most significant factor influencing the texture of the meat and the degree of 'gaping', i.e. the rapture of the connective tissue. One of the reasons for this is that even minor changes in pH drastically affect the properties of the connective tissue directly affecting WHC and in turn the drip loss.

Higher drip was observed in MA packaged groups with T3 recording the highest while air packaged T2 recorded the least drip. In respect to packaging atmosphere, higher drip was recorded with storage at refrigeration (1˚C) than superchilling (-1˚C). This agrees with Sivertsvik *et al.* (2003) findings that superchilled storage leads to less exudation. According to FAO (1996), drip loss is defined as the nutrients from and value of fish, which is potentially available for human consumption, but fails to be consumed or sold as products. Drip during

transportation of product from source to final consumer or storage, may result to weight below legally allowed tolerance even if it's wholesome and fit for consumption. It's therefore evidenced that air packaged tilapia fillets stored at -1˚C (T2) showed better quality related to analysed physical properties.

4.2.5.1. TVB-N and TMA changes

TVB-N, TMA and other volatile amines are commonly used as indicator for fish deterioration in fresh and lightly preserved seafood (Olafsdottir *et al*., 1997). Volatile bases result from degradation of proteins and non-protein nitrogenous compounds. The study depicted slow TVB-N accumulation in all sample groups during storage at 1˚C and -1˚C. Nonetheless at the end of storage time d20 for air packaged (T1 and T2), T1 was different (21mg N/100g) to other groups but within concentration of 30 mg/100g above which fish is considered unfit for consumption (Oehlenschlager, 1992). This is because changes in some of Volatile nitrogen bases are influenced most importantly by microorganisms which were > log 8 CFU/g in T1 on d20. Ababuocha *et al.* (1996) showed sardines accumulated TVB-N faster when stored at ambient than on ice probably because of increased growth and activity of mesophiles at ambient temperature. Similar sentiments were expressed by Karungi *et al.* (2004) who reported positive correlation of TVB-N and microbes.

The amount of TMA in tilapia fillets during storage time was very low (<1 mg/100 g) and to some extend not detected in most groups. This is probably because TMAO is virtually absent from freshwater species and terrestrial organisms (Hebard *et al*., 1982). Conversely, some studies have reported TMA in tilapia fish (Waliszewski *et al*., 2001; Reddy *et al*., 1995; Gram *et al*., 1989). According to Waliszewski *et al.* (2001) the presence of TMA is attributed to marine fish meal fed on tilapia.

4.2.6. Shelf life

The QDA method is useful in shelf life studies, as can be used to provide information about maximum shelf life (Cyprian *et al*., 2009; Bonilla *et al*., 2007). The results from QDA and microbiological analysis indicated 100% air packaged tilapia fillets stored at 1˚C (T1) had shelf life of 13 - 15 days. This is short shelf life compared to the reported 16 days for tilapia fillets stored in Styrofoam boxes under refrigeration (1˚C) in section 4.1.1.6. The reason for difference could be in the main study, storage time was considered from packaging day unlike in pre-trial study which leads to one day difference. Counterparts 100% air packaged and stored at -1˚C (T2) had approached consumption limits on d20 with average scores of 18 for

negative attributes evaluated according to QDA and similarly in microbiological analysis TVC and pseudomonas counts were log 7 CFU/g that has been considered by Reddy *et al.* (1995) as consumption limit for tilapia air packaged fillets. On the other hand, MA packaged fillets (T3 and T4) on d23 were within consumption limits as evaluated with QDA (< 20 for negative attributes score) and microbiologically suitable for consumption up to d27 on which sensory evaluations (QIM and QDA) were not conducted. Nevertheless, MA packaging affected negatively the colour characteristics of raw fillets as well as its texture. Although Kilcast and Subramaniam (2000) observed sensory characteristics of most foods to deteriorate throughout storage and provided they remain safe, a large degree of change is evidently tolerable to consumers. The present study indicated MA packaged fillets were likely unacceptable during storage at both 1˚C (T3) and -1˚C (T4). This is because colour of the fresh meat is an important indicator of quality and a major factor in influencing retail purchase decisions (Ross, 2000). Reddy *et al.* (1995) reported MA-packaged (75% CO_2:25 N_2) tilapia fillets stored at 4˚C to have shelf life of > 25 days which is in agreement with the microbial and cooked samples findings from the present study.

Tilapia fillets seems to have longer shelf life than fillets from most temperate species; cod fillets 8 days in ice (Bonilla *et al*., 2007), vacuum packed cod fillets 9-10 days at 0°C (Gram *et al*., 1987), and MA and vacuum packaged snapper (*C. auratus*) fillets 9 days at -1°C (Scott, *et al*., 1986). This is because when fish is held under chilled conditions, the fish from warm waters keep longer than fish from cold waters (Gram *et al.,* 1990). This is apparently in relation to the relative proportions of psychrotrophic bacteria on fish from waters of different temperatures. Cold waters tend to favour proliferation of high numbers of psychrotrophs on fish, which in turn enhances spoilage at chilled condition and ultimately shortens the shelf life of fish (Karugi *et al.,* 2004).

5. Conclusions and recommendations

In the main study, the QIM scheme developed during pre-observation and used in shelf life study trials had been revised. The revised scheme consisted of six parameters which gave a total of 13 demerit points. The scores for quality attributes included in the QIM scheme increased differently with storage time at 1˚C and -1˚C, but added all together (QI), they gave a linear relationship between QI and storage time with significant correlations in all sample groups. This implies the developed QIM scheme is applicable to estimate storage time of tilapia fillets during refrigeration as well as superchilling storage. However, during storage the index (QI) was utilised differently in different groups with MA packaged fillets recording higher QI, and storage at 1˚C compared to air packaged and fillets stored -1˚C. This clearly shows that the application of QIM scheme in estimating storage time should be based on regression equation for individual groups as at the end of shelf life; QI was 6.4 and 4.2 for air stored fillets at 1˚C and -1˚C accordingly and 9.7 for MA groups during storage at both 1˚C and -1˚C.

Microbial counts were low (< log 3 CFU/g) in flesh for TVC and *Pseudomonas* spp., whereas for other organisms (SSO) enumerated counts were below the detection limit at the beginning of the storage time. However, at the end of shelf life TVC and pseudomonads counts reached log 7 CFU/g in flesh on day 15 and 20 for air packaged fillets during storage at 1˚C and -1˚C respectively. In MA packaged groups, extended lag phase was observed with counts < log 4 CFU/g up to day 27. Conversely, MA affected negatively on colour, drip loss and texture of fillets which limited it's consideration as a desirable alternative for shelf life extension of tilapia fillets.

Real-time PCR method showed a good correlation with *Pseudomonas* spp. counts, indicating that predictive modelling using PCR can be applied to estimate Pseudomonads counts rapidly and eventually predict the shelf life in air packaged tilapia fillets, since *Pseudomonas* spp. constituted a higher proportion of TVC and it's the main spoilage organism in fillets during air storage.

QDA results showed quality of cooked tilapia did not change much during early storage days, until after d9 for all sample groups. Then scores for positive attributes decreased, while scores for negative attributes increased. Based upon sensory evaluation of cooked tilapia and microbial counts the maximum storage time was 13 -15 days for air packaged during storage

at 1˚C (T1) and 20 days for air superchilled (T2). MA packaged on the other hand was concluded have a shelf life of more than 23 days.

It is observed in the present study that both TVB-N and TMA failed to demonstrate a clear tendency to increase over the storage period and did not provide a useful index of shelf life to reflect spoilage in tilapia fillets during storage. Generally, these analyses may not be considered very reliable for measuring the deterioration of tilapia fillets stored at very low chilling temperature.

Air packaged samples stored at superchilling (T2) retained freshness colour characteristics longer, recorded less drip loss and suppressed microbial growth compared to air refrigerated (T2). In such a condition the quality of the fillets remained good, ensuring fillets safety and sensory parameters up to day 20. Therefore based primarily on sensory evaluation, but also on physical properties and microbiological data it can be concluded that optimal condition for storage of fresh tilapia fillets is 100% air packaged, the suitable temperature being - 1^0C and the storage time 20 days.

In recommendation, the present study was done under well controlled conditions (chilled and superchilled), therefore, the results are more specific than they would be in most commercial situations where conditions can be variable. The data on shelf life reported should be used with discretion and in most cases assumed to represent maximum values.

Low TMA concentrations reported at the end of storage time in the present study could imply that TMAO which is reduced to TMA by some aerobic organisms (especially *Shewanella*) may be virtually absent or in less concentration in tilapia flesh muscles. It's therefore recommended that further study be done using vacuum packaged fillets to reduce the influence of CO_2 on colour characteristics and ascertain quality changes during storage since under anaerobic conditions (vacuum packaged) the main spoilage organisms reduce TMAO to TMA.

ACKNOWLEDGEMENTS

First and far most is to acknowledge the United Nations University Fisheries Training Programme (UNU-FTP) for the scholarship to undertake this training expediently. The academic guidance received from my supervisors Sigurjón Arason and Emilía Martinsdóttir together with study committee members Hannes Magnússon, Hélène L. Lauzon and Kolbrún Sveinsdóttir is highly acknowledged. Additional thanks to Kolbrún Sveinsdóttir for your diligent support; you have been my mentor in data analysis and Hélène L. Lauzon for guidance in project design and packaging.

I gratefully thank the Technological Development and Innovation Fund (Rannis.is) for financing this project. More so, Ragnar Jóhannsson for the excellent ideas to commence tilapia farming in the recirculation aquaculture system (RAS) at Straumfraedihus Keldnaholti in Reykjavik Iceland, it enhanced samples availability that made this project attainable within time frame. My gratitude to the Director Kenya Marine and Fisheries Research Institute, Dr. Johnson Kazungu for giving me study leave to accomplish this laudable work.

Thanks to personnel at Matis for their excellent technical assistance, most importantly to Adalheidur Ólafsdóttir for her guidance during preparation of cooked samples and all the panellists who performed sensory evaluation.

REFERENCES

Ababouch, L.H., Souibri L., Rhaliby, K. and Ouahdi, O. (1996). Quality changes in sardines (*Sardina pilchardus*) stored in ice and at ambient temperature. *International Journal of Food Microbiology*, 13, 123-132.

Abu, H. and E. Okuma (1991). *Rigor mortis* progress of carp acclimated to different water temperatures. In Huss, H.H. (1995). *Quality and Quality changes in fresh fish*. Rome. FAO. Fisheries Technical Paper

Adams, R.M. and Moss, O.M (2008). *Food Microbiology*, 3rd edn. University of Surrey, Guildford (UK): RSC publishers.

Ando M., Takenaga T., Hamase S. and Yamane A. (2005). Effects of superchilling storage on maintainance of quality and freshness of Swordtip squid (Loligo edulis). *Food Science Technology Research.* **11**(3): 355-361.

Ando M., Nakamura H., Haramada R. and Yamane A. (2004). Effects of superchilling storage on maintainance of freshness of Kuruma prawn. *Food Science Technology Research.* **10**(1): 25-31.

Baixas-Nogueras S., Bover-Cid S., Veciana-Nogués T., Nunes M.L. and Vidal-Carou M.C. (2003). Development of a Quality Index Method to Evaluate Freshness in Mediterranean Hake (*Merluccius merluccius*). Journal of Food Science 68 (3): 1067-1071

Bligh, E.G. (1980). Methods of marketing, distribution and quality assurance. In Connel, J.J. and Staff of Torry Research Station eds. *Advances in Fish Science and Technology*, pp.51-54. Oxford: Fishing News Books

Bonilla, C. A., Sveinsdottir, K. and Martinsdottir, E. (2007). Development of Quality Index Method (QIM) scheme for fresh cod (*Gadus morhua*) fillets and application in shelf life study. *International journal of food control* **18**: 352-358

Bolivar, R.B., Mair, G.C. and Fitzsimmons, K. (eds.). 2004. Proceedings of the Sixth International Symposium on Tilapia in Aquaculture, September, 2004. Manila, Philippines.Bureau of Fisheries and Aquatic Resources, Manila, Philippines. 682 pp.

Chow C.J, Hsieh P.P, Tsai M.L. and Chu Y.J. (1998). Quality changes during iced and frozen storage of tuna flesh treated with carbon monoxide gas. J. Food Drug Anal. 10 **6**(3):615-623.

Church, N. (1998). MAP fish and crustaceans- sensory enhancement. *Food Science and Technology Today*. **12**(2): 73-83

Chytiri, S., Chouliara, I., Savvaidis, I.N. and Kontominas, M.G. (2004). Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology*. **21**: 157-165.

Creed P.G. and Pierson B.J. (1999). 'Sous vide – past, present and future', 3rd *European Symposium on Sous Vide*, Leuven, Alma Sous Vide Competence Centre.

Cyprian O., Sveinsdottir K., Magnusson H. and Martinsdottir E. (2008). Application of Quality Index Method (QIM) scheme and effects of short-time temperature abuse in shelf life study of fresh water Arctic charr (*Salvelinus alpinus*). *Journal of Aquatic Food Product Technology*. **17**(13): 303-321

Cyprian, O. (2008). Preliminary shelf life studies of iced tilapia (*Oreochromis niloticus*). MATIS report (Unpublished report).

Dainty, R.H. (1996). Chemical/biochemical detection of spoilage. *International Journal of Food Microbiology*. **33**:19-33.

Dalgaard P., Mejiholm O., Christiansen T.J. and Huss H.H. (1997). Importance of *photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. Letters in Applied Micribiology. **24**: 373-378

Doyle, J.P. (1995). Seafood Shelf Life as a Function of Temperature. [Electronic version]. <http://www.uaf.edu/seagrant/Pubs_Videos/pubs/ASG-30.html>. (04/03/08)

Duun, S.A. and Rustad, T. (2008). Quality of superchilled vacuum packed Atlantic salmon (Salmo salar) fillets stored at -1.4 and -3.6 °C. *Food Chemistry*. **106**: 122-131

Duun, S.A. and Rustad, T. (2007). Quality changes during superchilling storage of cod (Gadus morhua) fillets. *Food Chemistry*. **105**, 1067-1075

Einarsson, H. (1988). Deep chilling –A literature survey. In Duun, S.A. and Rustad, T. (2007). Quality changes during superchilling storage of cod (Gadus morhua) fillets. *Food Chemistry*. **105**, 1067-1075

Engle, C. (1997). Marketing tilapias. Pp. 244-258. In: Costa-Pierce, B.A. and J.E. Rakocy.Tilapia Aquaculture in the Americas. Vol. 1. World Aquaculture Society, Baton Rouge, LA.

FAO (1996). Storage quality of three pelagic species. FAO Fisheries Technical Report Paper 574 pp. 31-33.

FAO (2001). Fisheries technical paper 411: case studies on the allocation of transferable quota rights in fisheries. http://www.fao.org/DOCREP/005/Y2684E/y2684e05.htm

FAO (2002). Fisheries and Aquaculture department. http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus

FAO (2006a). FAO Fisheries Statistics. Capture production, volume 98/1. FAO, Rome, Italy. http://www.fao.org/fishery/statistics/global-production (20/04/2008)

FAO (2006b). FAO Fisheries Statistics. Aquaculture production, volume 98/2. FAO, Rome, Italy. http://www.fao.org/fishery/statistics/global-aquaculture-production (20/04/2008)

FAO (2006c). Cultured Aquaculture species information programme – *Oreochromis niloticus*. Text by Rakocy, J.E. In FAO Fisheries and Aquaculture Department. Rome

FAO (2007). Fisheries and Aquaculture Department. Species Fact Sheets Oreochromis niloticus (Linnaeus, 1758).

FishBase (2007). Oreochromis niloticus Nile tilapia: Summary (20/1/2009)

Fletcher G. C. and Statham J. A. 1988. Deterioration of Sterile Chill-Stored and Frozen Trumpeter Fish (*Latridopsis forsteri*). *Journal of Food Science*. **53** (5): 1336-1339.

Foegeding, A., Lanier, C. and Hultin, O. (1996). Characteristics of edible muscle tissues. In Duun, S.A. and Rustad, T. (2007). Quality changes during superchilling storage of cod (Gadus morhua) fillets. *Food Chemistry*. **105**: 1067-1075

Gram, L. and Dalgaard, P. (2002). Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology*. **13**: 262-266.

Gram, L. and Huss, H. (1996). Microbiological spoilage of fish and fish products. *International journal of Food Microbiology*. **33**: 121-137.

Gram, L. (1992). Spoilage of three Senegalese fish species stored in ice at ambient temperature. In: Gram L. and Huss H. (1996). Microbiological spoilage of fish and fish products. *International journal of Food Microbiology.* **33**: 121-137.

Gram, L., Wedell-neergaard, C. and Huss, H.H. (1990). The bacteriology of fresh and spoiling Lake Victoria Nile perch (*Lates niloticus*). *International Journal of Food Microbiology*. **10**: 303-316.

Gram, L., and Melchiorsen, J. (1992). Interaction between fish spoilage bacteria Pseudomonas sp. And Shewanella putrefaciens in fish extracts and on fish tissue. *International Journal of Food Microbiology*, ***4***(1): 65-72.

Gram, L., Neergaard, C.W., and Huss, H.H. (1987). Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. *Journal of Applied Bacteriology*, ***80***: 65-72.

Hardy, R. (1980). Fish lipids. Part 2. In Connell, J.J. ed. *Advances in Fish science and Technology*, p. 103. Jubilee conference of Torry Research 23-27 July 1979 Oxford: Fishing News

Howgate, P.F. (1982). Quality assessment and Quality control. In Aitken, A., Mackie, I.M., Merrit, J.H. and Windsor Crown, M.L. eds. *Fish Handling and Processing*. Edinburgh, Scotland.

Herrero AM, Huidobro A, Careche M (2003). Development of a Quality Index Method for Frozen Hake (*M. Capensis and M. Paradoxus*). *Journal of Food Science*. **68** (3): 1086-1092

Hovda, M. B., Lunestad, B. T., Sivertsvik, M. and Rosnes, J. T. (2007): Characterisation of the bacterial flora of modified atmosphere packaged farmed Atlantic cod (*Gadus morhua*) by PCR-DGGE of conserved 16S rRNA gene regions. *International Journal of Food Microbiology*. **117**: 68-75.

Huis in't Veld, J.H.J. (1996). Microbial and biochemical spoilage of foods: An overview. *International Journal of Food Microbiology* **33**:1-18.

Huss, H.H., Dalgaard, P. and Gram, L. (1997). Microbiology of fish and fish products. In Luten, J.B., Borresen, T. and Oehlenschlager, J eds. *Seafood from Producer to consumer, intergrated approach to quality*. Amsterdam: Elsevier Science Publishers.

Huss, H.H. (1995). *Quality and Quality changes in fresh fish*. Rome. FAO. Fisheries Technical Paper

Huss, H.H. (1994). *Assurance of seafood quality.* [Electronic version] Rome. Fisheries Technical Paper no.334.

Huss, H.H. (1988). *Fresh fish - Quality and quality Changes* Training Manual. Rome: United Nations, FAO/DANIDA

Huss, H.H. (1981). *Clostridium botulinum Type E and Botulism*. PhD thesis, Technical University Lyngby 2800, Copenhagen Denmark.

ICMSF (International Commission on Microbiological Specifications for foods) 1980. Microbial ecology of foods: Vol I. London: Blackie Academic and Professional.

Jones, N.R. and Disney, J.G. (1996). Technology in Fisheries development in the tropics, Conference proceedings. Handling, processing and marketing of tropical fish. PP 27-31.

Karungi, C., Byaruhanga, B.Y. and Muyonga, H.J. (1994). Effects of pre-chilling duration on quality deterioration of iced Nile perch. *Food Chemistry*. **85**(1)

Kilcast, D. and Subramaniam, P. (edit) (2000). The stability and shelf-life of food. Woodhead Publishing Limited. Cambridge England.

Kinne, O., 1980. Aquaculture, A Critical Assessment of its Potential and Future. *Interdisciplinary Science Reviews*, Vol.5, No. 1, 1980.

Kraft, A.A. (1992). *Psychrotrophic bacteria in Foods: Disease and spoilage*. Boca Raton, Florida: CRC. Press Inc.

Kristinsson, H.G., Mony, S.S. and Petty, H.T. (2005). Properties of tilapia carboxy- and oxyhemoglobin at postmortem pH. *J. Agric. Food Chem*. **53**(9):3643-3649.

Lauzon, H.L. and Martinsdottir, E. (2005). Combined use of CBC chilling, MAP and superchilling to extend the shelf life of cod fillets. In Wang, T., Sveinsdottir, K, Magnusson, H. and Martinsdottir, E. (2008). Combined Application of Modified Atmosphere Packaging and Superchilled Storage to Extend Shelf Life of Fresh Cod (*Cadus morhua*) Loins. *Journal of Food Science*. **73**(1)

Lauzon, H.L., Stefansson, G., Jonsson, G. and Kolbrun, S. (2002). Sensory quality and shelf life of MAP redfish (Sebastes marinus) fillets as affected by previous MA bulk storage of whole fish. Journal of Aquatic Food Product Technology, Vol. 11(3/4) pp 225-273

LeBlanc, R. J. and LeBlanc, E. L. (1992). Effect of superchilling with CO_2 snow on the quality of commercially processed cod (*Gadus morhua*) and winter flounder (*Psedopleuronectes americanus*) fillets. In: Huss. H. H., Jakobsen M. and Liston J. Quality Assurance in the Fish Industry. *Elsevier Science Publishers* B. V. Pp. 115-124.

Lima dos Santos, C.A.M (1978). Bacteriological spoilage of iced Amazonian freshwater catfish. In Gram L. and Huss H. (1996). Microbiological spoilage of fish and fish products. *International journal of Food Microbiology.* **33**, 121-137.

Lima dos Santos, C.A.M. (1981). The storage of tropical fish in ice-a review. *Tropical science*. **23**:97-127

Li L., Hao S., Diao S, Cen J., Yang X., Shi H. and Chen S. (2008). Proposed new color retention method for tilapia fillets (*O. niloticus* and *O. aureus*) by uthanatizing with reduced carbon monoxide. *Journal of Food Processing and Preservation.* **32:** 729–739.

Lioulas, T.S. (1988). Challenges of controlled and modified atmosphere packaging: a food company´s perspective. In Soccol, M.C.H. and Oetterer, M. (2003). Use of Modified Atmosphere in Seafood Preservation. *Brazilian Archives of Biology and Technology*. **46**(4): 569-580

Liston, J. (1980). Microbiology in fishery science. In Connell, J.J. ed. *Advances in Fish Science and Technology*. Jubilee conference of Torry Research 23-27 July. Oxford: Fishing News.

Liston, J. (1992). Bacterial spoilage of seafoods. . In Huss, H.H., Jakobsen, M. And Liston, J. eds. *Quality Assurance in the Fish Industry*, pp. 93-105. Amsterdam: Elsevier Science Publishers.

Luten, J.B. and Martinsdóttir, E. (1997). QIM: a European tool for fish freshness evaluation in the fishery chain. In: Olasfdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E., Heia, K. (1997). *Methods to determine the freshness of fish in research and industry*. Proceedings of the Final Meeting of the Concerted Action "Evaluation of Fish Freshness" FAIR Programme of the EU. International Institute of Refrigeration

Iwamoto, M., Yamanaka, H., Watabe, S. and Hashimoto, K. (1987). Effect of storage temperature on rigor-mortis and ATP degradation in plaice. In Huss, H.H.1995. *Quality and Quality changes in fresh fish*. Rome. FAO. Fisheries Technical Paper

Lund, B. M., Baird-Parker, T. C. and Gould, G. W. 2000. The Microbiological Safety and Quality of Food. *Aspen Publisher Inc., Gaithersburg, Maryland*.

Magnússon, H., Sveinsdóttir, K., Lauzon, H., Thorkelsdóttir, Á., and Martinsdóttir, E. 2006. Keeping quality of desalted cod fillets in consumer packs. J. Food Sci. 71: 70–76.

Martinsdóttir, E. (2002). Quality management of stored fish. In Bremner, H.A. ed. *Safety and Quality Issues in Fish Processing*. pp. 360–378. England: Woodhead Publishing Limited.

Martinsdóttir, E., Sveinsdottir, K., Luten, J.B., Schelvis-Smit, R., Hyldig, G. (2001). Reference manual for the fish sector: sensory evaluation of fish freshness. QIM Eurofish. P.O. Box. 68, 1970 AB IJumiden, The Netherlands.

Malle, P., and Poulmeyrol, M. 1989. A new chemical criterion for the quality of fish: Trimethylamine/Total Volatile Basic Nitrogen (%). *J. of Food protection*. 52: 419-423.

Moller, A. (2007). Opportunities and challenges of fisheries globalization. Paper presented to the International workshop on Sea food processing-local sources, global markets. Organized by Organization for Economic Co-operation and Devolpment (OECD), Paris , 16 April 2007.

Oehlenschlager, J. (1992). Evaluation of some well established and some underrated indices for the determination of freshness and/or spoilage of ice stored wet fish. In Quality Assurance in the Fish Industry, Huss, H.H. (editor). Elsevier Science Publishers B. V., Netherlands, 339-351, (1992).

Oka, H. (1989). Packaging for freshness and preservation of discolouration of fish fillets. In Sivertsvik, M., Jeksrud, W.K. and Rosnes, T.J. (2002). A review of modified atmosphere packaging of fish and fishery products- significance of microbial growth, activities and safety. *International Journal of Food Science.* **37**, 107-127

Ola, J.B. and Oladipo, A.E. (2004). Storage life of Croaker (*Pseudotholitus senegalensis*) in ice and ambient tempereature. African Journal of Biomedical Research. **7**(1): 13-17

Olafsdottir, G., Lauzon, H.L., Martinsdottir, E. Oehlenschlager, J. and Kristbersson, K. (2006). Evaluation of shelf life of superchilled cod (Gadus morhua) fillets and the influence of temperature fluctuations during storage on microbial and chemical quality indicators. *Journal of Food Science*. **71**(2): S97-109

Olafsdottir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E., and Heia, K. (1997). *Methods to determine the freshness of fish in research and industry*. Proceedings of the Final Meeting of the Concerted Action "Evaluation of Fish Freshness" FAIR Programme of the EU. International Institute of Refrigeration.

Otwell, W.S., Balaban, M. and Kristinsson, H. (2003). Use of Carbon monxide for color retention in fish. Proceedings of First Joint Trans-Atlantic Fisheries Technology Conference – TAFT 2003 33rd WEFTA and 48th AFTC meetings, 11-14 June, Reykjavik – Iceland

Oyelese, A.O. (2007). Drip loss measurements, organoleptic assessment and filleting characteristics of the silver cat under low storage temperature. *Journal of food processing and preservation*. **31**: 467-479.

Parry, R.T. (1993). Principles and applications of modified atmosphere packaging of food. In Soccol M.C.H. and Oetterer M. (2003). Use of Modified Atmosphere in Seafood Preservation. *Brazilian Archives of Biology and Technology*. **46**(4): 569-580

Reddy N. R., Villanueva, M., Kautter, D.A (1995). Shelf Life of Modified-Atmosphere-Packaged Fresh Tilapia Fillets Stored under Refrigeration and Temperature-Abuse Conditions. *Journal of Food Protection* . **58** (8): 908 – 914

Reddy N. R., Schreiber, L. C., Buzard, S. K., Skinner G. E. and Armstrong, D.J (1994). Shelf life of fresh tilapia fillets packaged in high barrier film with MAs. In Reddy N. R., Villanueva, M., Kautter, D.A (1995). Shelf Life

of Modified-Atmosphere-Packaged Fresh Tilapia Fillets Stored under Refrigeration and Temperature-Abuse Conditions. *Journal of Food Protection*. **58** (8): 908 – 914

Reddy, N. R. and Armstrong, D.J (1992). Shelf life extension and safety concerns about fresh fish products packed under MA: A review. *Journal of Food safety*. **12**:87-118

Reynisson, E., Lauzon, L.H., Magnusson, H., Hreggvidsson, O.G. and Marteinsson, T.V. (2008). Rapid quantitative monitoring method for the fish spoilage bacteria Pseudomonas. *Journal of Environ. Monit.* **10**: 1357-1362

Rodrigues, O., Losada, V., Aubourg, S. and Barros-Velazquez, J. (2004). Enhanced shelf life of European hake stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. *Food Research International*. **37**, 749-757

Rodrigues, O., Barros-Velazquez, J. Pineiro, C., Gallardo, J. and Aubourg, S. (2006). Effects of stored in slurry ice on the microbial, chemical and sensory quality on shelf life of farmed turbot. *Food Chemistry*. **95**, 270-278

ROSS, P.M. (2000). *The influence of exposure to carbon monoxide on the quality attributes for yellow fin tuna muscle*. In Li L., Hao S., Diao S, Cen J., Yang X., Shi H. and Chen S. (2008). Proposed new color retention method for tilapia fillets (*O. niloticus* and *O. aureus*) by uthanatizing with reduced carbon monoxide. *Journal of Food Processing and Preservation.* **32:** 729–739.

Scott, D. N., Flettcher, G. C. and Summers, G. (1986). MA and vacuum packaging of Snappers. In Sivertsvik, M., Jeksrud, W.K. and Rosnes, T.J. (2002). A review of modified atmosphere packaging of fish and fishery products- significance of microbial growth, activities and safety. *International Journal of Food Science.* **37**: 107-127

Shewan, J.M. (1977). The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In: Gram, L., Neergaard, C.W., and Huss, H.H. 1990. The bacteriology of fresh and spoiling Lake Victoria Nile perch (*Lates niloticus*). *Journal of Food Microbiology*, **10**: 303-316.

Shewan, J.M., Macintosh, R.G., Tucker, C.G., and Ehrenberg, S.C. (1953). The development of numerical scoring assessment of spoilage of white fish. In Lauzon, H.L., Stefansson, G., Jonsson, G. and Kolbrun, S. (2002). Sensory quality and shelf life of MAP redfish (Sebastes marinus) fillets as affected by previous MA bulk storage of whole fish. Journal of Aquatic Food Product Technology. **11**(3/4): 225-273

Sikorski Z. E. and Kolakowski E. 2000. Endogenous Enzyme Activity and Seafood Quality: Influence of Chilling, Freezing, and Other Environmental Factors. In: Haard N. F. and Simpson B. K. Seafood Enzymes: Utilization and Influence on Postharvest Seafood Quality. *Marcel Dekker, Inc*. Pp. 451-487

Silliker, J.H. and Wolfe, S.K. (1980). Microbiological safety comsiderations in controlled-atmosphere storage of meats. *Food Technology*. **34**: 59-63

Sivertsvik M., Rosnes J.T. and Kleiberg G.H. (2003). Effect of Modified Atmosphere Packaging and Superchilled Storage on the Microbial and Sensory Quality of Atlantic Salmon (Salmo salar) fillets. *Journal of Food Science*. **68**(4): 1467-1472

Sivertsvik, M., Jeksrud, W.K. and Rosnes, T.J. (2002). A review of modified atmosphere packaging of fish and fishery products- significance of microbial growth, activities and safety. *International Journal of Food Science.* **37**: 107-127

Simpson, R., Almonacid, S., Acevedo, C. and Cortes, C. (2003). Mathematical model to predict effect of temperature abuse in MAP systems applied to Pacific Hake (Merluccius australis). *Journal of Food Engineering*. **26**: 413-434.

Smith, J.G.M., Hardy, R. and Young, K.W. (1980). A seasonal study of the storage characteristics of mackerel stored at chilled and ambient temperatures. In Connell, J.J. ed. *Advances in Fish Science and Technology*. Jubilee conference of Torry Research 23-27 July 1979. Oxford, England: Fishing News.

Soccol, M.C.H., Oetterer, M., Gallo, C.R., Spoto, M.F. and Biato, D.O. (2005). Effects of modified atmosphere and vacuum on shelf life of tilapia (*Oreochromis niloticus*) fillets. *Brazilian Journal of Food Technology*. **8**(1)

Soccol, M.C.H. and Oetterer, M. (2003). Use of Modified Atmosphere in Seafood Preservation. *Brazilian Archives of Biology and Technology*. **46**(4): 569-580

Stanbridge, L.H. and Board, R,G. (1994). A modification of the Pseudomonas selective medium, CFC, that allows differentiation between meat pseudomonas and Enterobacteriaceae. *Letters in Appl. Microbiology*. **18**(6): 327-328.

Stansby, M.E and Griffiths, F.P. (1935). Handling fresh fish. *Ind. Eng: Chem*. **27**(12): 1452-1458.

Stone, H., and Sidel, J.L. (1985). *Sensory Evaluation Practices*. Florida. Academic Press INC.

Surti, T., Taylor, A. and Ma'ruf, F. (2001). The effect of storage at tropical ambient temperature on the quality and shelf life of grouper (*Plectropomus maculatus*). *Journal of Food Science and Technology*. **36**, 517-522

Sveinsdottir K, Hyldig G, Martinsdottir E, Jorgensen B, Kristbergsson K (2003). Quality Index Method (QIM) scheme developed for farmed Atlandic salmon (*Salmo salar*). *Food Quality and Preference*. **14**: 237-245

Sveinsdottir, K., Hyldig, G., Martinsdottir, E., Jorgensen, B., Kristbergsson, K. (2002). Application of Quality Index Methods (QIM) Scheme in shelf-life study of farmed Atlantic salmon (*Salmo salar*). *Journal and Food Science*. **67**: 1570-1579.

Sorheim, O., Nissen, H. and Nesbakken, T. (1999). The storage of beefm and pork packaged in an atmosphere with low carbon monoxide and high carbon monoxide. *Meat Sci*ence. ***52***: 157–164.

Sykes A.V., Oliveira A.R., Domingues P.M., Cardoso C.M., Andrade J.P, Nunes M.L. (2009). Assessment of European cuttlefish (*Sepia officinalis, L.)* nutritional value and freshness under ice storage using a developed Quality Index Method (QIM) and biochemical methods, LWT. *Food Science and Technology*. **42**:424-432

Waliszewski, K.N. and Avalos, M. (2001). Shelf-life extension of tilapia in crushed ice with additives. *Journal of Food Processing Preservation*. **25**: 299-307

Wang, T., Sveinsdottir, K, Magnusson, H. and Martinsdottir, E. (2008). Combined Application of Modified Atmosphere Packaging and Superchilled Storage to Extend Shelf Life of Fresh Cod (*Cadus morhua*) Loins. *Journal of Food Science*. **73(**1)

Whittle K. J., Hardy R. and Hobbs G. (1990). Chilled Fish and Fishery Products. In: Gormley T. R. Chilled Foods. *Elsevier Science Publishers. Ltd*. Pp. 87-116.

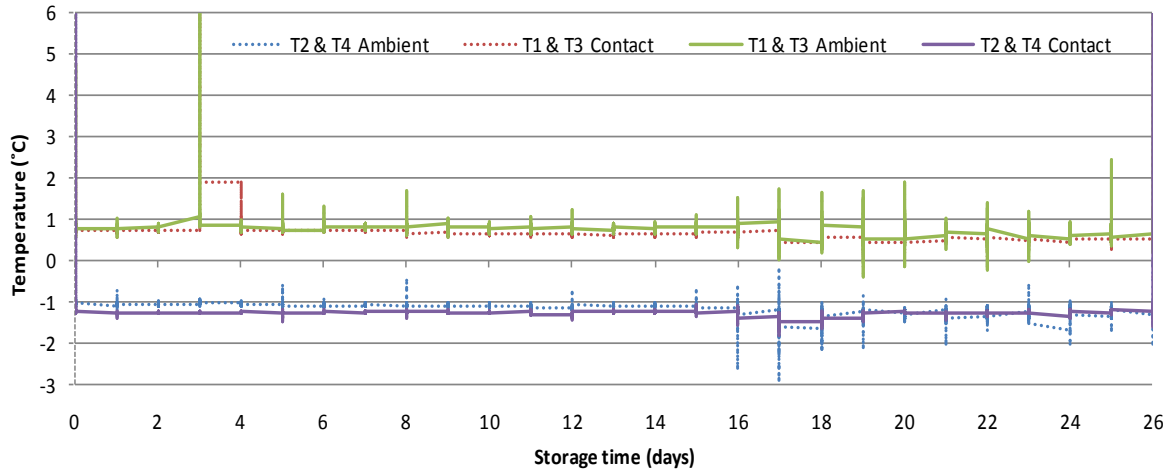
Whittle, K.J., Robertson, I. and MacDonald, I. (1980). Seasonal variability in blue whiting (*Micromesistius poutassou*) and its influence on processing. In Connell, J.J. ed. *Advances in Fish Science and Technology*. Jubilee conference of Torry Research 23-27 July 1979. England: Fishing News.

Wilhelm, K. A. (1982). Extended fresh storage of fishery products with modified atmospheres. In Reddy N.R. , Villanueva, M., Kautter, D.A (1995). Shelf Life of Modified-Atmosphere-Packaged Fresh Tilapia Fillets Stored under Refrigeration and Temperature-Abuse Conditions. *Journal of Food Protection*. **58** (8): 908 – 914

Wolfe, S.K. (1980). Use of CO and CO_2 enrichment atmospheres for meat, fish and produce. *Food Technology*. **34**: 55-58

APPENDICES

Appendix 1: Ambient and contact temperature changes in packaged tilapia fillets during storage. T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C

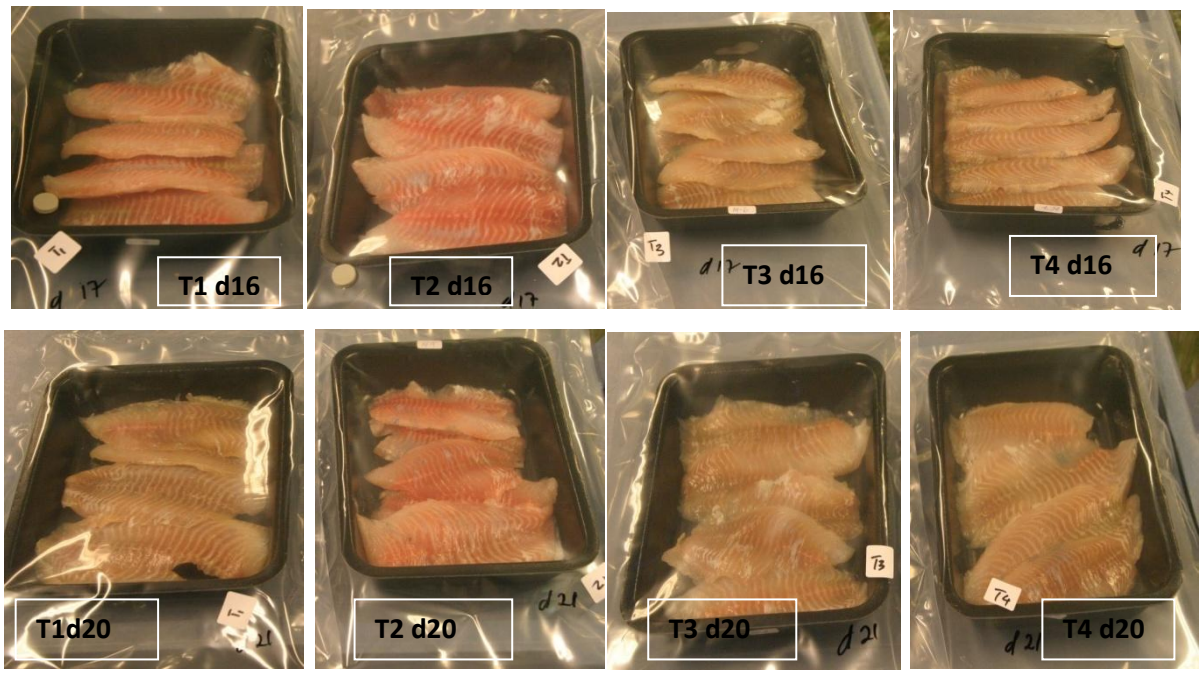


Appendix 2: Quality index score (means ± standard deviation) for packaged tilapia fillets with storage time. Co= control; T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C (N=3).

Storage time (d)	0	2	6	9	13	16	20	23	r
Control	0.5±0.2								
T1		0.7±0.2^{a}	2.2±0.9^{ab}	2.9±0.8^{b}	3.9±0.3^{b}	6.4±0.8^{c}	10.3±0.2^{d}	-	0.942*
T2		0.6±0.1^{a}	1.4±0.2^{ab}	2±0.4^{ac}	2.7±0.5^{bcd}	3.1±1^{cd}	4.2±0.5^{d}	-	0.932*
T3		2.8±0.3^{a}	4.3±1.2^{ab}	6.3±0.6^{bc}	7±0.7^{cd}	8.4±0.5^{ce}	8.8±0.9^{de}	9.6±0.4^{e}	0.948*
T4		2.4±0.4^{a}	4.1±0.7^{ab}	5±0.2^{bc}	6.4±1^{cd}	7.3±0.8^{d}	9.2±0.2^{e}	9.7±0.4^{e}	0.979*

Data within the same row with different letters are significantly ($p<0.05$) different. r= correlation value: *indicate a significant correlation.

Appendix 3: Appearance of packaged deskinned tilapia fillets towards end of storage time d16 and d20. T1= 100% air, stored at 1˚C; T2= 100% air at -1˚C; T3= MA at 1˚C; T4= MA at -1˚C; d=day.



Appendix 4: Appearance of MA packaged deskinned tilapia fillets on d23. T3= MA at 1˚C; T4= MA at -1˚C; d=day.