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THE EFFECT OF DIFFERENT COOLING TECHNIQUES ON THE MICROBIAL QUALITY AND SENSORY SHELF LIFE OF ICELANDIC GOLDEN REDFISH (*Sebastes marinus*) FILLETS

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ABSTRACT

Innovations in the application of different fish cooling techniques have been developed in order to delay spoilage, thus extending shelf life. In the study, effects of different in-process cooling techniques on microbial, chemical and sensory properties of redfish fillets during storage and distribution simulations were evaluated using five treatments. Cooling techniques included the use of slurry ice as a fillet cooling medium (SIC) and/or Combined Blast and Contact (CBC) technique to superchill the fillets. Storage temperature was monitored using I-button loggers. Growth of specific spoilage organisms (SSO), total viable psychrotrophic counts (TVC) and production of microbial metabolites were determined by conventional and molecular microbial and chemical (TVB-N, TMA, pH, salt content) methods. The study showed that superchilling by CBC for skin-on fillets efficiently reached a product temperature of -1°C but resulted in a reduced shelf life of 8-9 days and 10 days for CBC and SIC-CBC treatments, respectively, due to their poorer microbial quality. Skipping the slurry ice cooling step prior to CBC treatment proved undesirable to this fatty product as the sensory results suggested that other deteriorative changes, in addition to microbial changes, contributed to faster quality loss. A shelf life of 11-12 days was obtained for the skinless fillets that were not CBC treated. SIC treatment did not provide sufficient cooling to achieve a measurable freshness and shelf life extension compared to untreated fillets. H_2S-producing bacteria were the dominant SSO and were more resistant to superchilling than *Photobacterium phosphoreum* and pseudomonads. The level of TVB-N in differently treated redfish fillets reached 12-15 mgN/100 g at the end of shelf life, being lower than the EC limit of 25 mgN/100 g. Indeed, low SSO counts (<log 7 CFU/g) and TVB-N levels at sensory rejection indicated that redfish quality deterioration was not only caused by microbial spoilage, but most likely induced by oxidative changes. Finally, the study showed that spoilage was faster in CBC treated skin-on fillets than in non-CBC skinless fillets.

Keywords: *Redfish fillets, superchilling, slurry ice cooling, specific spoilage organisms, sensory shelf life, spoilage indicator.*

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1. INTRODUCTION

1.1 Background

The golden redfish (*Sebastes marinus*) is one of the most commercially important fish in Iceland's fisheries. It is a slow growing species that attains an average total length of 35-40 cm though centennial individuals grow up to 100 cm and 15 kg in weight (Jonsson and Palsson, 2006). Redfish is a typical zooplanktivore that mainly feeds on *Calanus hyperboreus*, euphausiids, capelin *(Mallotus villosus*) and herring (*Clupea harengus*) (Jaworski and Ragnarsson, 2006). Redfish is found all around Iceland in the mid water column but the main fishing grounds are at the edge of the continental shelf at 200 to 400 m depth south and west of Iceland.

Fishing of redfish takes place all year round with peaks of catches in the late winter. Bottom trawling is the exclusive method used in this fishery (Jonsson and Palsson, 2006). Initially the golden redfish had no commercial value for Iceland and was always discarded in the cod catches but with time fish meal and oil production developed and finally redfish gained recognition as a good food fish. About 50% of the redfish catch is now processed and frozen at sea and the rest is iced and exported fresh in containers or by air. The main market for Icelandic redfish is Germany although a considerable percentage is also exported to other western European countries. The market for redfish in eastern Asia is expanding and Japan is now the second largest importer of the golden redfish (Jonsson and Palsson, 2006).

In Uganda, Nile perch (*Lates niloticus*) dominates the international export market in the form of skinless fresh and frozen fillets. Both Nile perch and golden redfish are fatty fish species, 0.6-1.8% lipids for Nile perch (Okeyo *et al*., 2009; E-Covima, 2007) and 1.4-6.8% lipids for redfish (Matis, ISGEM, retrieved 18.02.2011) and contain trimethylamine oxide (TMAO). Reported TMAO levels are 155-201 mgN/100 g for Nile perch (Anthoni *et al*., 1990) and 60-120 mgN/100g for redfish flesh (Mausse, 2000; Etienne, 2005). Despite the biochemical similarities between the two species, taxonomically they are different. Nile perch is a freshwater fish belonging to the family Latidae while golden redfish is a marine fish in the family Sebastidae (Fish base, retrieved 01.03.2011). Ocean perch is a synonymous name for golden redfish that is used for commercial purposes to market the redfish products. The redfish is normally sold either with skin-on or as skinless fillets (HB Grandi, retrieved 18.02.2011) but a longer shelf life is envisaged when the skin and fat layer underneath are removed.

The dominant fishing methods in Uganda for commercial Nile perch fish are gill netting and long line. Lack of fish bleeding in Uganda makes it hard to control the initial quality of fish flown into the European market despite the use of flake ice in plastic chests after hauling the catch and during transport. "The physical damage and stress during capture, the structure and composition of fish and storage temperature greatly influence the spoilage of fish" (Church, 1998). Immediate cooling of fish catches and maintenance of low temperature is a common prerequisite throughout the distribution chain for good quality fish destined for human consumption. This implies that the cooling technique has to be efficient to effectively slow down deteriorative changes in fish.

Innovations in the application of different ice forms to cool down fish have been developed and new techniques are emerging, either intended for on board or in-process cooling. For instance, the use of liquid ice prepared from brine has been documented to cause a rapid initial cooling of fish, but use of brine may create a favourable condition for growth of the

active spoiler *Photobacterium phosphoreum* which becomes dominant under extended storage of cold water marine fish species (Reynisson *et al*., 2010). Therefore, an evaluation of the various new cooling techniques to effectively reduce the effects of spoilage on fish products, hence extending shelf life, is needed as this influences their acceptance on the market.

1.2 Rationale of the study

Redfish fillets are currently being exported from Iceland by sea freight to European markets in expanded polystyrene (EPS) boxes that have drainage holes at the bottom and a layer of conventional ice on top of the fillets. Export by air freight is conducted in closed EPS boxes, generally with cooling mats placed on top of the fillets. Sea freight is more economical and environmentally friendly than air freight, but due to the time factor involved in delivery of products, new methods are being sought to ensure high quality products at delivery.

Skinned redfish fillets are commonly pre-cooled in a slurry ice medium prior to packaging to lower their temperature. Brine absorption by fillets may stimulate microbial growth and catalyse oxidative reactions, hence leading to a lower quality product. An alternative method to effectively cool down the product before packaging and ensure a shelf life extension is therefore needed. Combined Blast and Contact (CBC) cooling, a cooling technology developed by Skaginn hf (Iceland), rapidly cools down fillet temperature to a desired superchilled condition. It currently uses a liquid pre-cooling step based on a salt-containing medium. However, the need for such liquid cooling prior to the CBC treatment should be investigated as avoiding salt uptake in redfish fillets could be favourable. Further, the use of sea freight boxes with drainage holes could be replaced by totally closed, well-insulated EPS boxes to maintain as low and steady product temperature as possible during distribution.

The innovations in the form of ice used for cooling fish largely determine the efficiency of the cooling techniques. The rate of heat transfer (cooling rate) from fish to ice depends on the initial temperature of the fish, size of individual fish, percentage of fat and the amount or form of ice (flake, plate, block or flow/slurry ice) used. The application or testing of such innovative ice media to maintain fish freshness and extend their shelf life has not yet been approached in Uganda. Better knowledge on the effects of such novel cooling techniques on the quality of fatty fish is of great interest. The study therefore aimed at evaluating the effects of different in-process cooling techniques on the microbiological, chemical and sensory properties of redfish fillets during storage and distribution. The cooling techniques implied the use of slurry ice as a fillet pre-cooling medium and/or CBC technique to superchill the fillets before packaging.

To achieve this aim, five groups were investigated; (1) skinned fillets that were not cooled with slurry ice before packaging, stored in closed EPS boxes with a cooling mat, (2) skinned fillets pre-cooled in slurry ice before packaging, stored in closed EPS boxes with a cooling mat, (3 and 4) CBC-treated skin-on fillets with and without a slurry ice pre-cooling step, stored in closed EPS boxes with a cooling mat, (5) skinned fillets, pre-cooled in slurry ice before packaging, stored with a top ice layer in sea freight EPS boxes with drainage holes at the bottom. Group 1 is considered untreated (control), while group 5 represents the current practice of HB Grandi for redfish export by surface.

Specific objectives were set to evaluate the efficacy of the different cooling methods;

1. To evaluate the effect of different cooling treatments on the microbial development in redfish fillets compared to fillets in the control treatment. Enumeration of total culturable psychrotrophic microbiota (total viable counts, TVC) as well as pseudomonads, hydrogen sulphide (H_2S) producing bacteria and *Photobacterium phosphoreum* was carried out.

2. To investigate the effect of the different cooling treatments, storage time and storage temperature on the sensory properties and the formation of microbial metabolites (total volatile basic nitrogen, TVB-N and trimethylamine, TMA, both influencing pH development) in redfish fillets compared to the control treatment.

3. To establish the predominant spoilage bacteria and shelf life of redfish fillets among the different cooling treatments.

2 STATE OF ART

2.1 Fish spoilage

Spoilage of fresh fish is typically initiated by microbial activities (Gram and Huss, 1996) whereas fatty fish spoilage is also characterised by oxidative rancidity of the lipids. Interestingly, it has been shown that blood, more precisely haemoglobin, can induce oxidation (Wang *et al*., 2010). Therefore proper bleeding of fatty fish should be emphasised as one of the prerequisite handling procedures because this not only influences the shelf life of fresh fish products but also greatly affects the texture of the fish (Pacific Sardine Association, 2001).

Quality deterioration of fresh fish can be characterised using a sensory scheme. Initially, newly caught fish loses its fresh fish flavour after a few days of chilled storage (Dalgaard, 2006; Gram and Huss, 1996). The fresh, sweet flavour is attributed to inosine monophosphate (IMP), an ATP degradation product caused by enzymatic autolysis (Huss, 1995). After a period when fish is described as neutral, off-flavours and off-odours are detected. Indeed, further degradation of ATP products results in the loss of freshness characteristics and formation of hypoxanthine (Hx), the cause of bitter off-flavours (Huss, 1995). Ammonia-like, sulphurous and rancid odours become more intense with time and lead to rejection of the fish (Gram and Huss, 1996). It is noteworthy that the rate of formation of hypoxanthine in iced fish differs according to species, being more rapid in redfish than cod (Huss, 1995). This suggests that it may prove more difficult to extend the freshness period of redfish than cod. The time to spoilage has been linked to storage temperature that influences the microbiota growth. Therefore, understanding the spoilage process of fish by specific spoilage organisms (SSO) facilitates the development of methods to determine, predict and extend product shelf life (Dalgaard, 2006; Gram and Huss, 1996). It also allows for the development of proper cooling methods to delay spoilage caused by SSO, like H_2S-producing bacteria, *P. phosphoreum* and pseudomonads under aerobic storage (Olafsdottir *et al*., 2006).

High water temperature generally corresponds to high concentration of micro-organisms on fish but water salinity has little effect on the total concentration though it influences the composition of microbial species on fish. The catching methods have an effect too, for example trawled finfish may have higher concentration of micro-organisms than similar fishes caught by long line (Dalgaard, 2006). Heat labile and sodium-requiring micro-organisms are common in sea and brackish waters as well as seafoods. Thus, isolation of micro-organisms with these characteristics should be performed by spread plating but not pour plating with hot agar. For various fresh and lightly preserved seafoods, Dalgaard (2006) therefore recommended the use of cooled diluent and spread plating on pre-chilled plates of Long and Hammer's agar.

P. phosphoreum is a bioluminescent bacterium responsible for spoilage of different marine fish, and due to its ability to produce biogenic amines, it may have a potential to cause histamine fish poisoning (Dalgaard, 2006). *P. phosphoreum* is a psychrotolerant bacterium growing at 0^oC and typically inactivated above 25-30^oC. This bacterium dominates in the intestinal content of aquatic animals in cold seawater between 0 and 15^oC. Luminous variants of *P. phosphoreum* can grow to high concentrations during normal chilled storage of fish (Dalgaard, 2006). Many marine finfish contain TMAO that stimulates microbial growth. *Aeromonas, Alteromonas*, most Enterobacteriaceae, *Shewanella* and all marine luminous bacteria, particularly *P. phosphoreum*, reduce TMAO to TMA under anaerobic conditions. "TMA contributes to the typical ammonia-like and fishy off-odours and off-flavours in

spoiled seafoods, particularly in products with pH above 6.5" (Dalgaard, 2006). Indeed, Castell and Triggs (1995) demonstrated the variability of the odour threshold of TMA according to pH, as indicated by Table 1. It is also reported that both *Shewanella putrefaciens* and *P. phosphoreum* can reduce TMAO to TMA in marine fish (van Spreekens 1974; Huss, 1995; Gram and Huss, 1996). *Pseudomonas* species are not able to reduce TMAO and their growth is considerably reduced under oxygen limited conditions. Table 2 lists reported SSO as influenced by the fishing areas and atmospheric storage conditions.

Table 1: Variations in pH with odour threshold (Castell and Triggs, 1955)

pH	Odour of 20 ppm TMA solution
< 6.7	no odour
6.7 - 7.4	"fishy" odour
> 7.4	ammonia-like odour

Table 2: The influence of packaging condition and habitat on the dominance of specific spoilage bacteria in chilled (<4°C) fish or stored in ice (modified from Gram and Huss, 1996)

Packaging condition	Specific spoilage organisms of fresh, chilled fish depending on source of fish			
	Temperate waters		Tropical waters	
	Marine	Freshwater	Marine	Freshwater
Aerobic	*Pseudomonas* spp. *S. putrefaciens* *P. phosphoreum**	*Pseudomonas* spp. *S. putrefaciens*	*Pseudomonas* spp. *S. putrefaciens*	*Pseudomonas* spp. *S. putrefaciens* Motile aeromonads**
Vacuum	*S. putrefaciens* *P. phosphoreum*	Lactic acid bacteria	Lactic acid bacteria	Lactic acid bacteria
CO_2	*P. phosphoreum*	Lactic acid bacteria	TMAO reducers	TMAO reducers

*Reynisson *et al*., 2010; and Olafsdottir *et al*., 2006; **Gram *et al*., 1990.

2.2 Shelf life of fresh fish

Shelf life of fish can be considered as the time period from when fish is caught until it is no longer fit for human consumption (Huss, 1995). The shelf life of fresh fish products is influenced by a number of factors, such as the initial microbial load, the fishing method and the post-harvest handling of the catch and varies from species to species. The shelf life of whole redfish is reported to be 16-19 days in ice storage (Rehbein *et al*., 1994; Mausse, 2000) while that of redfish fillets (processed four days post catch) was seven days at 0°C according to Masette (1999).

World market demand for a supply of safe and healthy food is increasing. Hence, food preservation is important to increase shelf life and to maintain nutritional value and quality. Quality fresh fish products are in great demand worldwide. Therefore the ability to predict shelf life of fish products is of interest. However, fish is a perishable product which spoils faster than any other muscle food (Kaale *et al.*, 2010). The high perishability of fish is due to intrinsic factors such as high water activity of about 0.99, high pH, non-nitrogenous

compounds and abundance of nutrients which favour faster microbial growth (Huss, 1995). Storage at superchilled temperature may increase shelf life of food due to slower bacterial growth (Kaale *et al*., 2010) but the temperature has to be well controlled. Below -2°C, three quarters of the water in fish is frozen, slowly causing critical damage to fish tissue structure by large ice crystals formed which enhance enzymatic spoilage (Graham *et al*., 1992; Galart-Jornet *et al*., 2007).

The colour and odour of raw fish flesh are important indices for consumers to evaluate the freshness and quality of fish (Sohn *et al*., 2007), hence these attributes are "shelf life predictors" used by consumers. Shelf life of fish and fishery products is a key factor in the fish industry because it allows processors to plan how to process and transport products to different markets (Huynh *et al*., 2007). Handling practices, processing contamination and storage conditions affect the shelf life of fish and fishery products (Doyle, 1995; Huss, 1995). Further, temperature fluctuation is a key factor that greatly affects quality and shelf life of fish products during processing, transportation and storage in retail shops (Huynh *et al*., 2007). Different cooling techniques and packaging methods have been developed to counteract temperature fluctuations. Some of these cooling techniques have been demonstrated to extend shelf life, for example superchilling in brine at 0 to -4^{o}C and use of slurry ice or liquid ice among others. These cooling techniques basically lower product temperature before packaging, hence retarding the growth of spoilage bacteria and extending shelf life (Huss, 1995). Undesirable effects of superchilling could be the slow formation of ice crystals and increased enzyme activity at temperatures between -1 to -6^{o}C (Robinson, 1985). The achievable extension of shelf life depends on fish species and condition, fat/water content, initial microbial population, atmospheric condition and storage temperature (Huss, 1995). Therefore, the cooling technique to be used will always depend on fish species and characteristics of the product, storage conditions and means of transport from producers to markets.

2.3 On board cooling

Temperature of newly caught fish is generally representative of the water temperature where fishing took place. Temperature may vary due to seasons and fishing areas. For instance, close to Iceland seawater temperature in the south ranges from 2 - 6°C in winter and 8 - 12°C in summer, while lower temperatures are seen in the north, 1 - 4°C in winter and 6 - 8°C in summer months (Matis, retrieved 18.02.2011). This implies that different cooling methods and amounts of ice are needed to suit the cooling requirements.

Flake ice is conventionally used in the fish industry due to its flat shape that gives a large contact surface and a more rapid heat transfer from fish than block and tube ice forms. It is also more easily stored, handled, transported and does moderate physical damage to the fish. However, flake ice has a higher melting rate and requires more storage space than block ice. The air pockets created between flake ice and the top of the fish reduces the flow of heat from fish to ice (Graham *et al*., 1992).

Slurry ice is a mixture of ice particles, water and salt (1.0-2.5% NaCl) which decreases the fish's freezing point and achieves a subzero temperature. However, cooling in slurry ice should not trigger freezing of fish products (Pineiro *et al*., 2004). The fluid nature of slurry ice makes it easily manageable by both pipes and pumps. Slurry ice with a high degree of fluidity has a faster cooling capacity than flake ice and its flexibility offers great efficiency. Slurry ice systems have been installed on board fishing vessels and have demonstrated advantages for on board storage of fish, thus gaining popularity. Use of slurry ice reduces the

physical damage to fish and its products due to its microscopic particles compared to the sharp edges of flake ice particles (Pineiro *et al.*, 2004). Slurry ice melts faster than flake ice but has a higher contact surface area to fish than flake ice, thus covers fish completely during application. However, the initial investment cost is high for use of slurry ice and the quality benefits are species dependent (Pineiro *et al*., 2004). Due to the presence of salt in slurry ice, fish may have a shorter shelf life since salt uptake apparently enhances growth of specific spoilage organisms (Reynisson *et al*., 2010; Cakli *et al*., 2006). This is most likely true for marine TMAO-containing fish species from cold and temperate waters as the presence of the active TMA spoiler *P. phosphoreum* can be expected. The formation of volatile compounds, like TVB-N and TMA, was reported to be higher in haddock after storage in slurry ice than flake ice. Further the predominant spoilage microbiota in haddock stored in slurry ice or flake ice differed (Reynisson *et al*., 2010). In contrast, other reports have indicated the advantage of slurry ice to extend the shelf life of other fish species. Kilinc *et al*. (2007) reported two days extension of shelf life for a 2 hour pre-treatment of sea bream and sea bass with slurry ice while Rodriguez *et al.* (2004) reported seven days of shelf life extension for European hake stored in slurry ice. Although much is documented on the use of slurry ice on whole fish, comparatively little is documented regarding its usage on fish fillets, more precisely redfish fillets.

2.4 In-process cooling

Combined Blast and Contact (CBC) cooling, the new cooling technology developed by Skaginn hf (Iceland) for fish processing, involves superchilling the skin side of the skin-on fillets through a freezing tunnel on a Teflon-coated aluminium conveyor belt at a temperature of about -8oC and simultaneously blasting air over the fillets. Using this technology, rapid lowering of fillet temperature down to between -0.5 to -1oC is achieved before packaging. During this process, 10-15% of the water in lean fish muscle is frozen (Rha, 1975) when packaged, thus extra energy is needed to melt this partly frozen water in the CBC–treated product. Pre-cooling has been found to be important for products subjected to thermal loads during transportation and storage (Magnusson *et al*., 2009; Gao, 2007). Before CBC cooling, the fillets go through liquid cooling (about 1.0-2.5% salt in solution or slurry ice) allowing a slight salt uptake in the fillets to avoid freezing of the flesh in the tunnel. Automated skinning of CBC fillets is easily performed, generally leading to higher product yields than in conventional fillets (Arnthórsdóttir *et al*., 2008). CBC-treated cod fillets have shown a slower quality deterioration rate at early storage compared to traditionally processed fish, hence extending the freshness period and shelf life (Olafsdottir *et al*., 2006). This has been attributed to the cold shock experienced by specific spoilage organisms (SSO) during early storage, slowing down their development in well-controlled thermal conditions. The brining step has been identified as a critical point, contaminating the fillets with bacteria accumulating from the skin-on fillets when the brine medium is not renewed or the fillets properly rinsed before their introduction to the brine bath. In addition the salt and water uptake of fish muscle from a microbially contaminated cooling medium contributes to the rapid growth of fish spoilage bacteria in a conducive environment such as a poorly controlled chill chain. Thus the salt concentration in the cooling medium should be in strict quantities because the salt uptake by fish muscle depends on the concentration and the cooling time in this medium. Salt plays the role of lowering the initial freezing point of the fish muscle. The alternative to this would be to skip the pre-cooling of fillets in the salted medium prior to a well-controlled CBC cooling. Owing to this, there is a need to study further other associated quality defects of fish fillets produced by this technique.

3 MATERIALS AND METHODS

3.1 Experimental design and raw material

Redfish used for this study was obtained from HB Grandi fishing company in Reykjavik (Iceland) in December 2010. The fish was obtained from the deep sea zone of the Atlantic Ocean by trawling and kept on ice in insulated tubs for three days on board the fishing vessel before delivery to the processing plant for filleting and packaging under five different treatments. In total 38 EPS boxes with an average weight of 3 kg of fillets were used and the box size was 35.6 x 21.6 x 6.5 cm.

The five treatments of the experiment were;

- NC, no cooling of fillets during processing. Skinned fillets packed at HB Grandi[1] with a cooling mat in EPS boxes. Fillets considered as untreated (control).
- SIC, slurry ice cooling of skinned fillets performed at HB Grandi followed by packaging with a cooling mat.
- CBC, CBC treated skin-on fillets superchilled in a CBC cooler at Eskja[2] and packed with a cooling mat.
- SIC-CBC, slurry ice cooled for 10 min and CBC treated skin-on fillets at Eskja followed by packaging with a cooling mat.
- SF-SIC, sea freight boxes used for slurry ice cooled skinned fillets at HB Grandi with a crushed plate ice layer at the top of the fillets. SF EPS boxes have holes at their base to drain off water.

The CBC cooling technique was performed at Eskja ehf (Hafnarfjorður, Iceland), a company that owns a CBC cooler. Calibrated temperature loggers were inserted in two EPS boxes for each treatment before storage, three positioned inside to monitor fish temperature at bottom corner, middle/center layer and top center of the box, and one positioned externally on the box. All boxes were stored in a chamber at Matis[3] for almost 6 days at -1^{o}C followed by storage at 2^{o}C for 7 days, simulating sea freight export and storage at wholesaler/retailer. Three fillets (pooled as one sample) from each box were used for microbiological and microbial metabolites analyses, and 11 fillets for sensory evaluation. These fillets were taken below the top layer of fillets. Sampling was carried out on days 1, 6, 10 and 13 of storage, analysing duplicate samples (two boxes per treatment). The mean weight of skin-on and skinned fillets was 149.3 ±18.7 g and 101.4 ± 4.9 g, respectively.

3.2 Sensory evaluation

Four groups of redfish fillets were examined by sensory evaluation (NC, SIC, SIC-CBC and CBC). Group SF-SIC was not evaluated to minimise costs since a maximum of 4 samples per session can be assessed. The aim was to study the effect of the cooling treatments on the redfish quality deterioration and shelf life according to sensory evaluation by a team of trained panellists.

Quantitative Descriptive Analysis (QDA) by Stone and Sidel (2004) and Torry freshness score sheet (Shewan *et al*., 1953) were used to assess cooked samples. A group of 7 to 11

[1] Fishing company in Iceland
[2] Fishing company in Iceland
[3] Food research institute in Iceland

panellists participated in the sensory evaluation. They had refresher training according to International Standards (ISO 8586, 1993), including detection and recognition of tastes and odours, use of scales and in the development and use of descriptors. The members of the panel were experienced in using the QDA method and Torry freshness score for redfish. The sensory attributes were those already described by the sensory panel in earlier projects and these included appearance, flavour, odour and texture attributes. Tables 1 and 2 in Appendix 1 shows the Torry scheme used and the 30 QDA attributes evaluated, respectively.

Portions of about 40 g from the fillets were put in aluminium boxes coded with three digit random numbers. The samples were cooked for 6 min in a pre-warmed oven (Convotherm Electrogerate GmbH, Eglfing, Germany) at 95 - 100^{o}C with air circulation and steam and then served to the panel. Each panellist evaluated duplicates of each sample group in a random order during eight sessions (four samples per session and 2 sessions per sampling day). A computerised system (Fizz, version 2.0, 1994-2000, Biosystèmes) was used for data recording.

3.3 Microbial examination

Portions of each of the three fillets were aseptically obtained and minced together in a mixer. Twenty grams of sample mince was diluted 10-fold, using cooled Maximum Recovery Diluent (MRD, Oxoid[i], UK) and stomached for homogenisation (1 minute). Serial 10-fold dilutions were done as needed in 9 ml cooled MRD. Total viable psychrotrophic counts (TVC) and counts of H_2S-producing bacteria (black colonies) were determined on Iron Agar (IA), modified from Gram *et al.* (1987) with 1% NaCl and no overlay. Plating was performed by spread plating and plates were incubated at 17^{o}C for 5 - 7 days. Enumeration of pseudomonads was performed on modified Cephaloridine Fucidin Centrimide (mCFC) agar as described by Stanbridge and Board (1994). Pseudomonas Agar Base (Oxoid) with CFC selective agar supplement (Oxoid) was used and plates were incubated at 22^{o}C for 3 days. Estimation of *P. phosphoreum* counts was done by a quantitative PCR method developed at Matís (Reynisson, unpublished). Briefly, 10 ml of the 10-fold diluted fish sample in MRD buffer was frozen at -20°C for later DNA extraction. For the DNA extraction, the diluted samples were centrifuged at 11.000 x g for 7 min to form a pellet. The supernatant was discarded and DNA was recovered from the pellet using the promeganesil KF, Genomic system (MD1460) DNA isolation kit (Promega Corporation, Madison, USA) in combination with King Fisher magnetic beads automatic DNA isolation instrument (Thermo Lab systems, Waltham, USA) according to the manufacturers' recommendations. All PCR reactions were done using the MX 3005p instrument. The PCR was done using Brilliant QPCR master mix (Stratagene, La Jolla, CA, USA). Primers were synthesised and purified with HPLC (MWG, Ebersberg, Germany). The DNA standard used for quantification of *P. phosphoreum* was previously calibrated against the PPDM-Malthus conductance method (Dalgaard *et al*., 1996) using fish samples from storage trials.

3.4 Chemical analyses

Total Volatile Basic Nitrogen (TVB-N) and trimethylamine (TMA) were determined in duplicate according to the method described by Malle and Poumeyrol (1989). Briefly, TVB-N and TMA were determined by steam distillation using the Kjeldahl type distillator and part of the minced fish samples prepared for the microbial testing. To 100 g of the mince, 200 ml of 7.5% aqueous trichloroacetic acid (TCA) solution was added and homogenised in a waring blender for one minute, extracting TVB-N and TMA. The distilled TVB-N was collected in a

boric acid solution, then titrated with sulphuric acid. TMA in TCA extract was measured by adding 20 ml of 35% formaldehyde before distillation. TMA was collected in boric acid solution that was titrated with sulphuric acid solution.

The pH was determined in about 5 g of the mince which was mixed with 5 ml of deionised water using the Radiometer PHM 80. The pH meter was calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25^{o}C Radiometer Analytical A/S, Bagsvaerd, Denmark).

The salt content was measured with the Volhard Titrino method according to AOAC ed. 19 from 2000 (no.976.18).

3.5 Data analysis

Graphical presentation and calculation of means and standard deviations were done using Microsoft Excel 2010. Statistical analysis of data was done using NCSS 2000 (Utah, USA) to carry out an analysis of variance, ANOVA for sensory data and one-way ANOVA for other data. Comparison of data with respect to treatments was done using the Duncan's multiple comparison test. The threshold level for significance was 0.05. A Pearson correlation matrix was also obtained to evaluate the relationship between the parameters evaluated, specifically comparing Torry score to chemical and microbial data. Multivariate analysis was conducted in the statistical program Unscrambler (Version 9.7, CAMO software AS, Oslo, Norway) with principal component analysis (PCA), assessing on one hand QDA data determined from 30 attributes in 4 sample groups and on the other hand comparing chemical and microbial data obtained for 5 sample groups. Full cross validation was used. The chemical and microbial data was standardised with 1/SD.

4 RESULTS

The study aimed at evaluating the effects of different in-process cooling techniques on the microbial, chemical and sensory properties of redfish fillets during storage and distribution by simulation trials. The cooling techniques included the use of slurry ice as a fillet pre-cooling medium and/or CBC technique to superchill the fillets before packaging.

4.1 Temperature monitoring

Initial mean product temperature of untreated (NC) and SIC fillets was 1 - 2°C (Figure 1) but slightly lower for SF-SIC (0 - 1°C). SIC-CBC and CBC-treated fillets had the lowest initial temperature (-1°C). It took about 2 - 3 days for the NC and SIC fillets to approach the storage temperature of -1°C as opposed to only 1 day for SF-SIC fillets stored in the boxes with drainage holes. In all treatments, the bottom corner of the EPS boxes recorded the lowest temperature for the first 6 days of storage, after which it slowly surpassed the temperature at other box positions in concordance with higher storage temperature (2°C). Fillet temperature of 0°C was then rapidly reached in NC and SIC treatments (within 9 days), but more slowly in the other treatments (around days 10-11). In fact, the temperature of fillets treated by the CBC technique was the most steady, maintaining -1°C for about 10-11 days. The temperature of the SF-SIC fillets (especially at center and top center) positions was rather stable over the storage period due to the presence of the ice layer.

The mean ambient temperature of the storage chamber recorded by data loggers that were fixed on the external surface of two EPS boxes for each treatment is shown in Figure 1**. Similar fluctuations in temperature were observed for all the treatments. Large fluctuations seen during early storage were due to the sensitivity of the cooling chamber after its opening. Any slight temperature increase triggered a strong cooling in the chamber.

Table 3 compares the mean temperature of the fillets at different positions in the boxes, indicating the bottom corner as being the most sensitive area in an EPS box and responding to its ambient temperature. This is shown by the generally higher product temperature and/or the largest standard deviation. Similar product temperature was observed for the center and top center positions. The lowest mean external temperature of the SIC-CBC treatment could be explained by its lowest product temperature influencing the outer wall of the boxes where loggers were fixed.

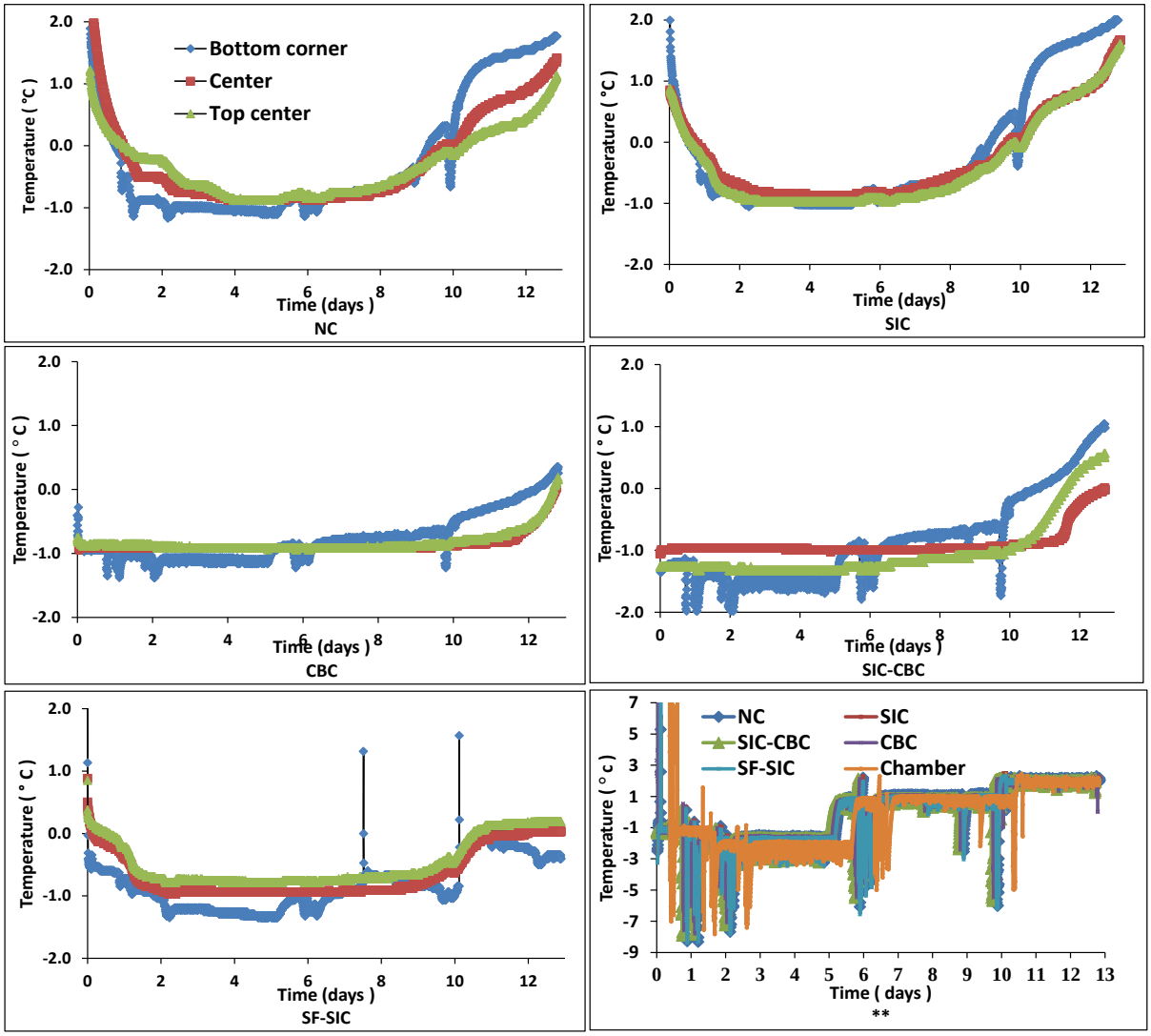

Figure 1: Temperature fluctuation in the fillets for treatments (NC, SIC, SIC-CBC, CBC, SF-SIC) at different positions in EPS boxes over storage time. Data shown is a mean value of two temperature data loggers positioned in two EPS boxes.

** Temperature monitoring on the external surface of EPS boxes ($n_{treatment}$=2) for the different treatments and mean temperature of loggers (n=3) positioned above the boxes in the chamber.

Table 3: Mean temperature (°C ± SD) at different positions of EPS boxes and mean internal product temperature over the storage period

Treatment	Bottom Corner	Center	Top Center	External	Product temperature*
NC	-0.2±1.0	-0.3±0.7	-0.3±0.5	-0.2±2.0	-0.3±0.8
SIC	-0.1±1.0	-0.3±0.7	-0.4±0.7	-0.2±1.9	-0.3±0.8
SIC-CBC	-0.8±0.9	-0.9±0.5	-1.0±0.6	-0.6±1.1	-0.9±0.7
CBC	-0.8±0.4	-0.9±0.2	-0.8±0.2	-0.3±1.9	-0.8±0.3
SF-SIC	-0.8±1.0	-0.5±1.8	-0.3±1.8	-0.2±2.7	-0.5±1.6

*Mean value of six loggers at three positions (bottom corner, center and top center) in two boxes. All treatments had a cooling mat on top of fillets stored in closed EPS boxes while SF-SIC had a top ice layer and drainage holes at the bottom of the boxes.

4.2 Shelf life of redfish fillets

Assessment of quality deterioration of cooked redfish fillets was done by two methods, using the Torry freshness and QDA schemes. The skinned fish fillets that received no extra cooling during process (NC) or were subjected to the slurry ice cooling (SIC) prior to packaging had a similar shelf life of 11-12 days, as determined by the Torry scheme (Figure 2). The shelf life of CBC and SIC-CBC fillets was 8-9 and 10 days, respectively. Despite this difference, significant difference between CBC treatments and NC or SIC treatment was only observed on day 13 ($p<0.05$). It is noteworthy that the characteristic freshness was already lost for all treatments on day 6 post-processing, as a score of 7 indicates a neutral flavour.

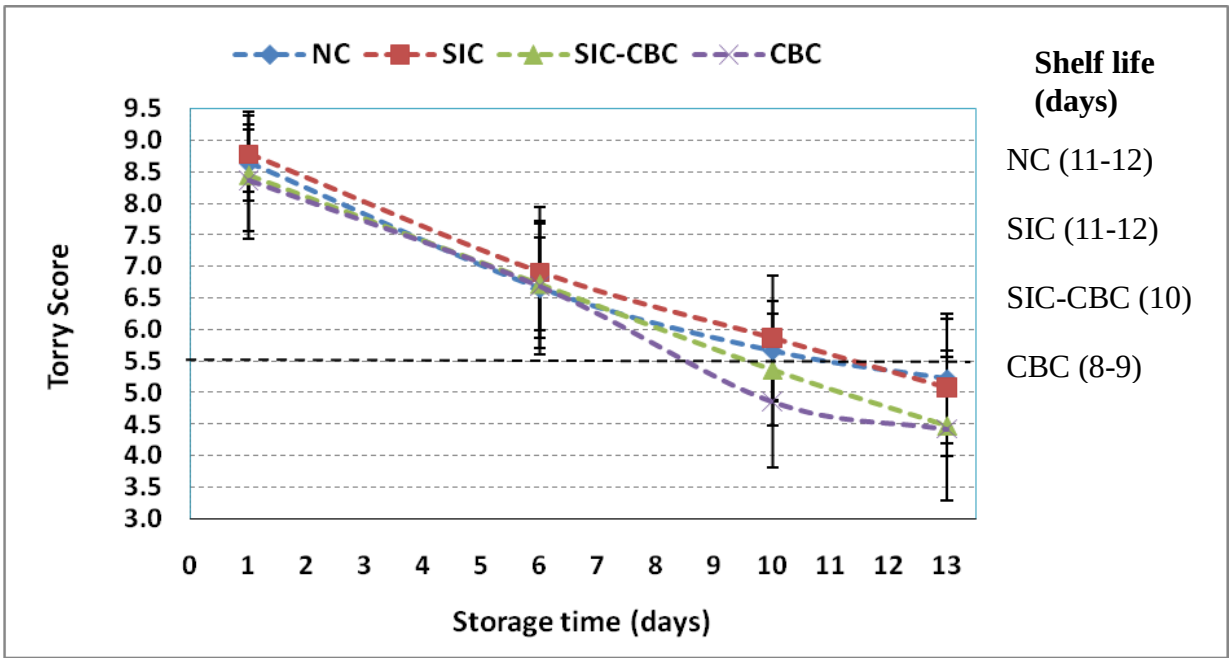

Figure 2: Torry freshness score for differently treated redfish fillets as a function of storage time.

Vertical bars indicate the standard deviation of mean score (n=7-11 panellists) for each sampling day and for each treatment analysed in duplicate. Fish is considered unfit for human consumption at a score of 5.5.

The QDA scheme counted 30 attributes of appearance, texture, odour and flavour, some representing freshness and spoilage characteristics (see Table B in Appendix 1). Figures 3a-f show the negative sensory attributes (flavour and odour) that were used to relate fish spoilage and shelf life evaluation for each of the treatments (NC, SIC, SIC-CBC, CBC) assessed using the mean QDA score. At the mean QDA score of 20 and above, most of the panellists detect the sensory attributes (Bonilla *et al.*, 2007; Magnusson *et al.*, 2006). Most of the spoilage attributes (sour odour, TMA odour, sour flavour, TMA flavour and off-flavour) were hardly detected during the first 6 days of storage for all the treatments, with the exception of table cloth odour. The intensity of table cloth odour increased with time up to the last day of storage when it reached a mean score of 20 or more in all treatments. Similarly, TMA odour for SIC-CBC treatment increased as time progressed to the mean score of 20 at the end of storage time but CBC-treated fillets were just reaching this threshold at the end of the storage period. Generally, skin-on fillets (SIC-CBC and CBC) attained spoilage characteristics faster than skinned fillets (NC and SIC). This agrees with the Torry results. Tables including sensory statistical data are presented in Appendix 2.

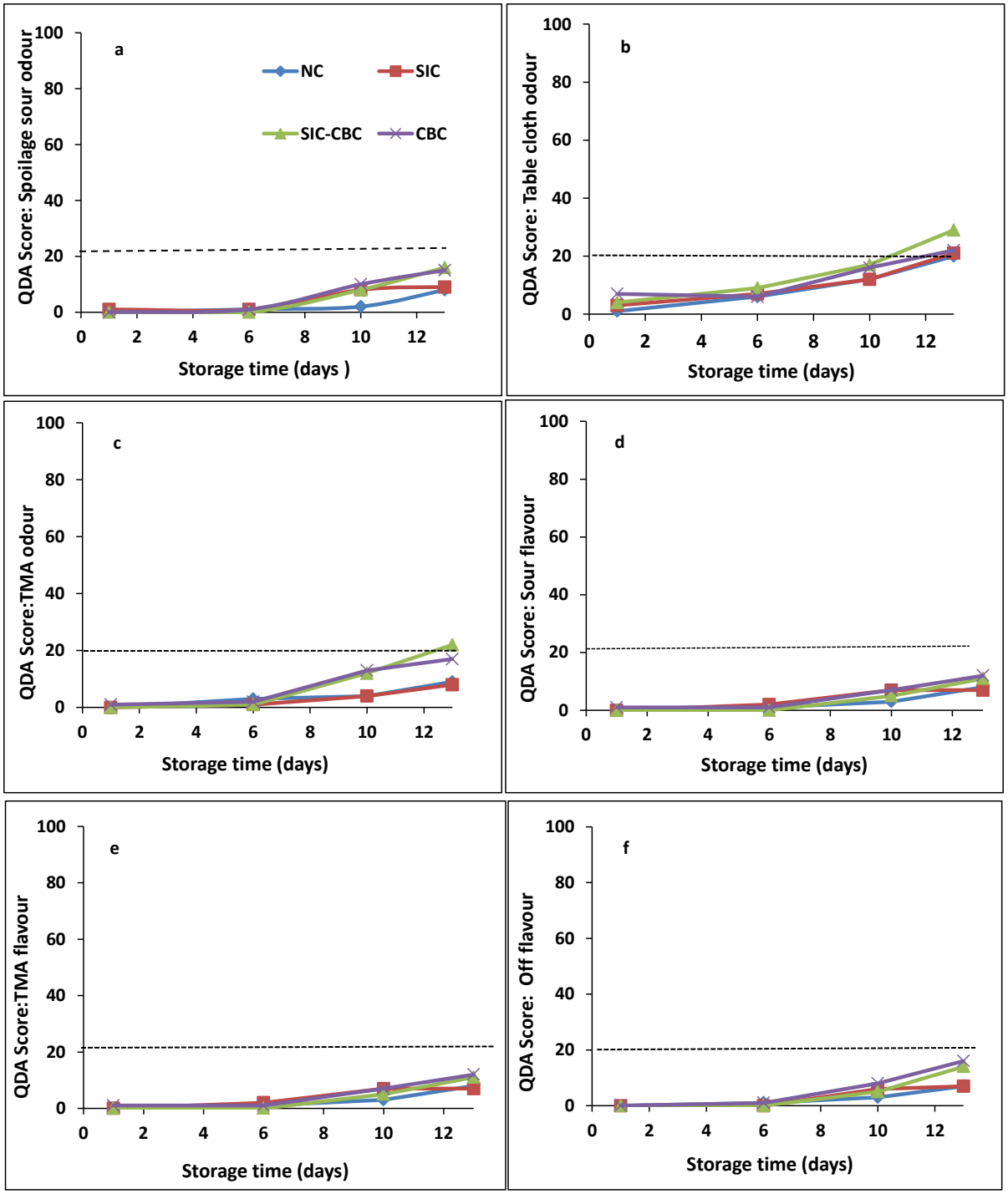

Figure 3: Mean QDA score of sour odour (a), table cloth odour (b), TMA odour (c), sour flavour (d), TMA flavour (e) and off-flavour (f) for the differently treated redfish fillets (n=7-11 panellists receiving duplicate samples).

In order to analyse all sensory attributes with time a multivariate analysis by PCA was done (Figure 4). The two principal components (PC1 and PC2) explain 91% of the sensory variation with the main variation being due to the difference caused by the storage time in all the treatments (PC1, 86%), going from right to left for fresh to spoiled fish based on the loadings (sensory attributes) shown. The position of sample CBC-d10 in the area of d13-samples indicates its faster deterioration compared to the other treatments. PC2 accounts for

5% of the sensory variation among the samples, with TMA and table cloth odours being the most influential variables in the downward direction and appearance characteristics (colour, heterogeneity and white precipitation) in the upward direction. It is therefore observed that CBC samples developed a negative appearance with storage time, while spoilage odours were mostly apparent in SIC-CBC samples. Sensory attributes characteristic for redfish during early stages of storage like sweet, metallic and cod liver flavour, shellfish, vanilla/warm milk, cod liver and sweet odour are positioned on the right in the upper part of the figure. After a storage period of 6 days, these attributes became less intense. Towards the end of storage, a grouping of treatments is seen on the bi-plot, differentiating between SIC-CBC and other treatments. The position of NC-d13 and SIC-d13 suggests a similar spoilage pattern, while that of CBC is positioned further upwards.

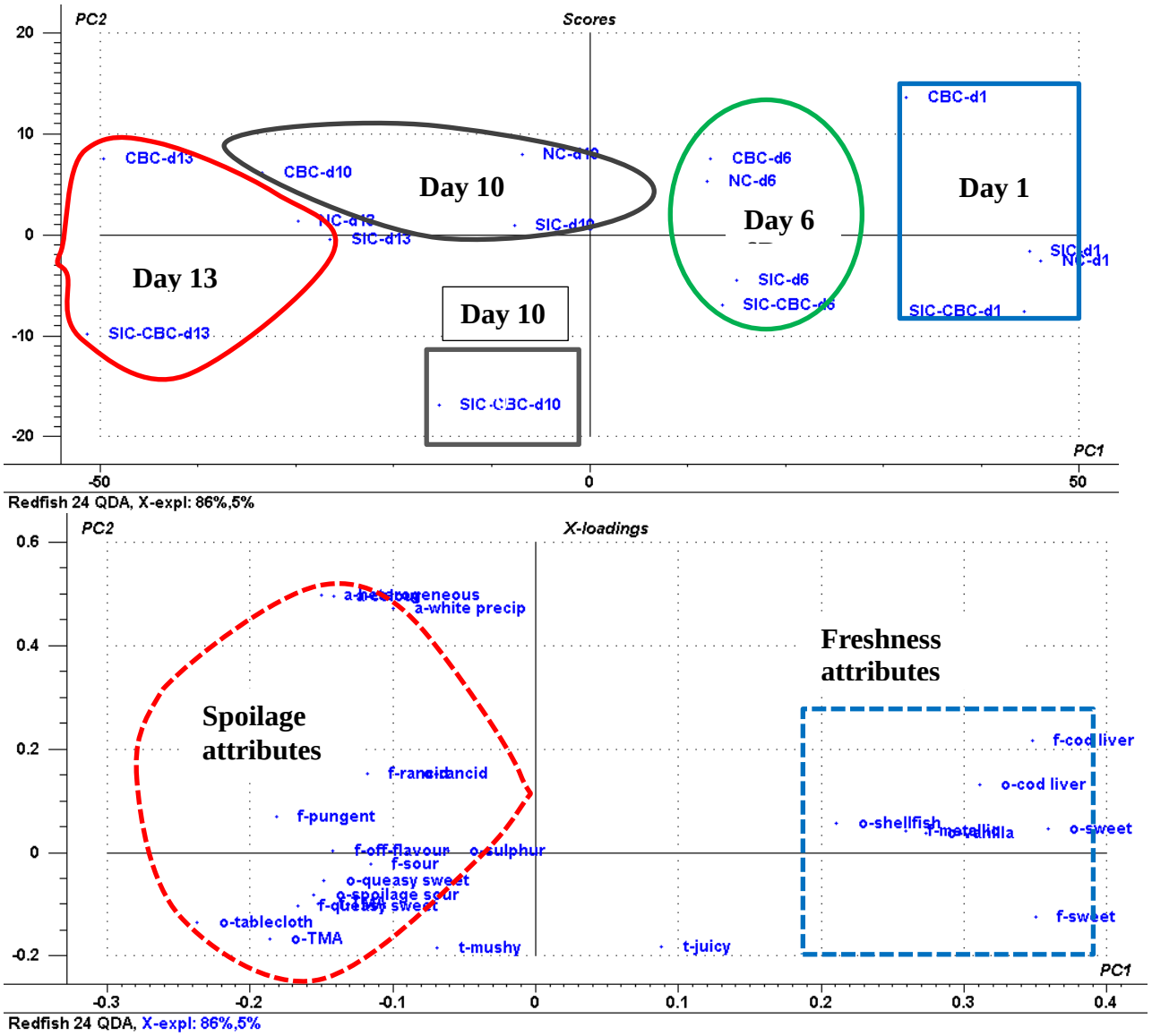

Figure 4: Principle component analysis (PCA) describing sensory quality, odour (o-), appearance (a-), flavour (f-) and texture (t-) of the sample treatments (NC, SIC, SIC-CBC, CBC) with storage time (d1 to d13). PC1 vs PC2 (X-expl.: 86% and 5%). Bi-plot of scores (samples, above) and loadings (sensory attributes, below).

4.3 Microbial analyses

To evaluate the effect of different cooling treatments on the microbial development with time in redfish fillets, enumeration of total culturable psychrotrophic microbiota (total viable counts, TVC) as well as pseudomonads, hydrogen sulphide (H_2S) producing bacteria and *Photobacterium phosphoreum* was done.

4.3.1 Total viable counts (TVC)

The microbiological analysis conducted 1 day post-packaging indicated that skin-on fillets (SIC-CBC and CBC) had a slightly higher microbial load (about 0.5 log CFU/g) than skinless fillets (NC, SIC, SF-SIC) (Figure 5a) ($p>0.05$). Microbial development progressed similarly for all groups but significantly higher counts were observed on skin-on fillets compared to skinless fillets on days 6 and 10 ($p<0.05$). The slurry ice used to pre-cool the SIC-CBC treatment had TVC of log 4.5 CFU ml^{-1} as measured early during processing. Microbial analysis of the slurry ice medium used for SIC treatment applied at HB Grandi was not performed. The results show though that the pre-cooling slurry ice treatment applied to fillets in-process (SIC and SIC-CBC) did not influence the microbial load observed on day 1 compared to corresponding groups (NC and CBC, respectively) ($p>0.05$). However untreated (NC) and SF-SIC fillets generally had the lowest counts compared to other treatments. After 13 days of storage, slightly higher counts were obtained for both CBC treatments compared to the 3 other ones, but the difference was insignificant ($p>0.05$). Correlation of Torry score to TVC gave an R value of -0.906.

4.3.2 H_2S-producing bacteria

The slurry ice used to pre-cool the SIC-CBC treatment had a H_2S-producing bacteria count of log 4.0 CFU ml^{-1}. After day 1 of storage, the H_2S-producing bacteria counts of the different treatments ranged from log 1.3 to 3.0 CFU/g, being significantly lower for NC skinless fillets than skin-on CBC-treated groups ($p<0.05$) (Figure 5b). A significant increase in H_2S-producing bacteria counts was observed as storage time progressed for all treatments ($p<0.05$). After 10 days of storage, counts of NC and SF-SIC fillets were significantly lower than in the other treatments ($p<0.05$) while no significant difference was seen three days later. Counts of H_2S-producing bacteria gave the highest correlation value to Torry score (R=-0.940) among all other microbial and chemical parameters evaluated (see Appendix 2).

4.3.3 Pseudomonads

The slurry ice used to pre-cool the SIC-CBC treatment had a pseudomonad count of log 2.5 CFU ml^{-1}. The growth of pseudomonads in the differently treated redfish fillets with storage time is shown in Figure 5c. A significant difference in the counts of pseudomonads was seen from day 6 of storage between CBC treatments and other treatments ($p<0.05$). After 6 days of storage, significantly lower counts were obtained for SIC and SF-SIC fillets compared to the skin-on CBC-treated groups, while 4 days later the three groups, NC/SIC/SF-SIC, had significantly lower counts than CBC treatments. On the last sampling day, counts of CBC fillets were significantly higher than in all other treatments. Generally, the pseudomonads on skin-on fillets (SIC-CBC and CBC) had higher counts than the skinless fillets throughout the storage time. Correlation of Torry score to pseudomonad counts gave an R value of -0.832.

4.3.4 Photobacterium phosphoreum

There was no *P. phosphoreum* to be detected in the slurry ice used to pre-cool the SIC-CBC treatment. The number of *P. phosphoreum* increased in the fillets with storage time for all the cooling treatments (Figure 5d). SF-SIC treatment had similar low counts as the control (NC) on day 6. Significant difference among groups was first observed on day 10, with SIC fillets having higher counts than their NC and SIC-CBC counterparts ($p<0.05$). On day 13, NC fillets had significantly lower counts than SF-SIC fillets, which in turn were significantly less contaminated than those from both CBC treatments. No counts could be obtained for SIC fillets on that day. The slowest overall growth rate of *P. phosphoreum* was observed in the NC treatment. Correlation of Torry score to *P. phosphoreum* counts gave an R value of -0.852.

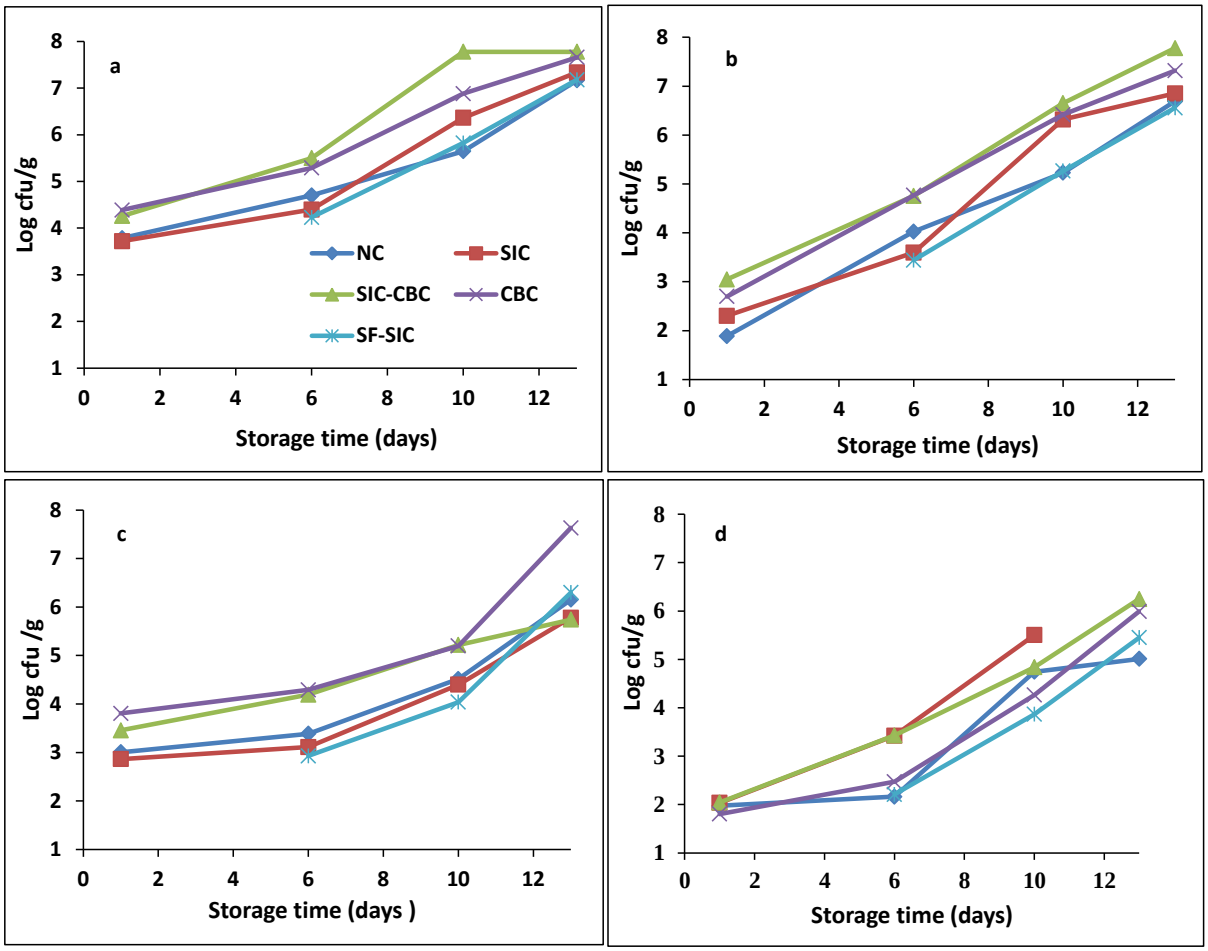

Figure 5: Growth of total culturable psychrotrophic microbiota (TVC, a), H_2S-producing bacteria (b), pseudomonads (c) and *P. phosphoreum* (d) in differently treated redfish fillets.

The values indicate mean of 2 samples obtained from two EPS boxes for each treatment.

4.4 Total Volatile Basic Nitrogen (TVB-N) and Trimethylamine (TMA)

TVB-N content and formation of TMA in the flesh of the fillets was measured during storage. No significant change was observed in the TMA and TVB-N content for the first 10 days of storage in all treatments (Figures 6a and b), but a significant increase was noticed on day 13 ($p<0.05$) for both CBC-treated groups. TMA content in SIC fillets also significantly increased between days 10 and 13. Otherwise, a low TMA content had formed in NC and SF-SIC fillets on the last sampling day. A table including chemical statistical data is presented in Appendix 2.

The proportion of TMA to TVB-N was determined to evaluate the importance of TMA among other basic products as storage time progressed. Table 4 provides this P-ratio (TMA/TVB-N). In agreement with TVB-N and TMA values presented, little difference was observed during the first 10 days. After 13 days of storage, SIC-CBC, SIC and CBC treatments had important levels of TMA (>50%) over other basic compounds.

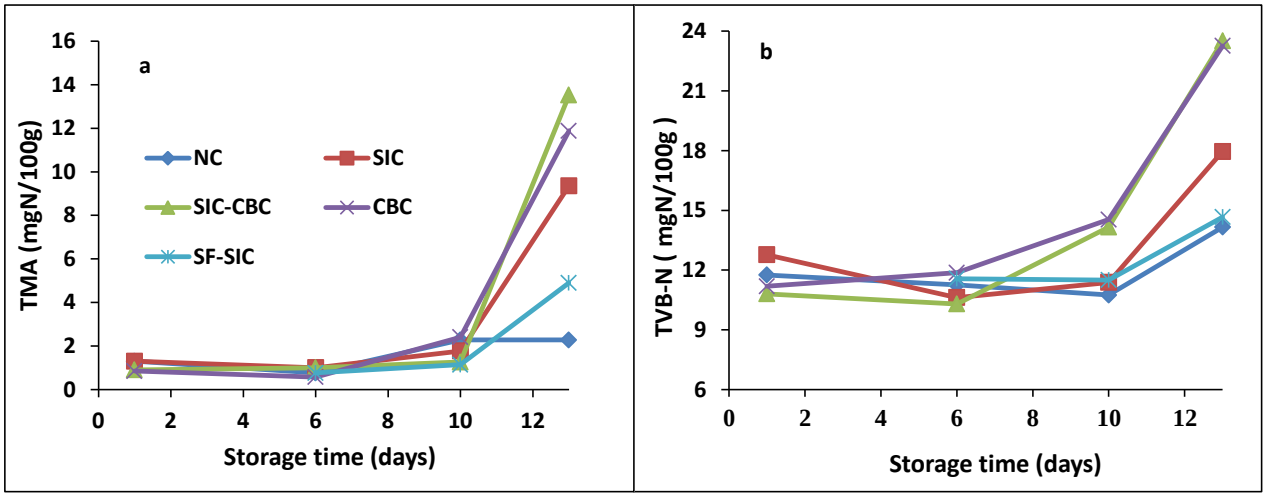

Figure 6: Trimethylamine (a) and Total Volatile Basic Nitrogen (b) as a function of storage time for differently treated redfish fillets

The values indicate mean of 2 samples obtained from two EPS boxes for each treatment.

Table 4: P-ratio of TMA to TVB-N (mgN/100g) for the differently treated redfish fillets at different storage time

Time (days)	NC	SIC	SIC-CBC	CBC	SF-SIC
1	0.109	0.101	0.079	0.0876	NA
6	0.068	0.089	0.099	0.048	0.069
10	0.211	0.156	0.089	0.165	0.096
13	0.161	0.521	0.575	0.511	0.333

4.5 pH and salt content

Variation in pH with storage time for the differently treated redfish fillets is shown in Figure 7a. Generally the pH in all treatments gradually decreased as the storage time increased until day 10 when it increased in SIC, SIC-CBC and CBC treatments but did fall slightly for NC and SF-SIC. On the last sampling day, the pH value for SIC-CBC fillets was significantly higher than that obtained for NC, SIC and SF-SIC fillets ($p<0.05$).

Figure7b shows changes in salt content of redfish fillets as storage time progressed. There was no noticeable change in salt concentration of redfish fillets for each treatment from day 1 to 10 after which the salt content increased for each treatment, most likely due to water loss in the fish muscle. SIC-CBC treatment had the highest salt content throughout the storage time, followed by SF-SIC and SIC treatments during the first 10 days of storage. CBC and NC treatments had a similar salt content throughout the storage period.

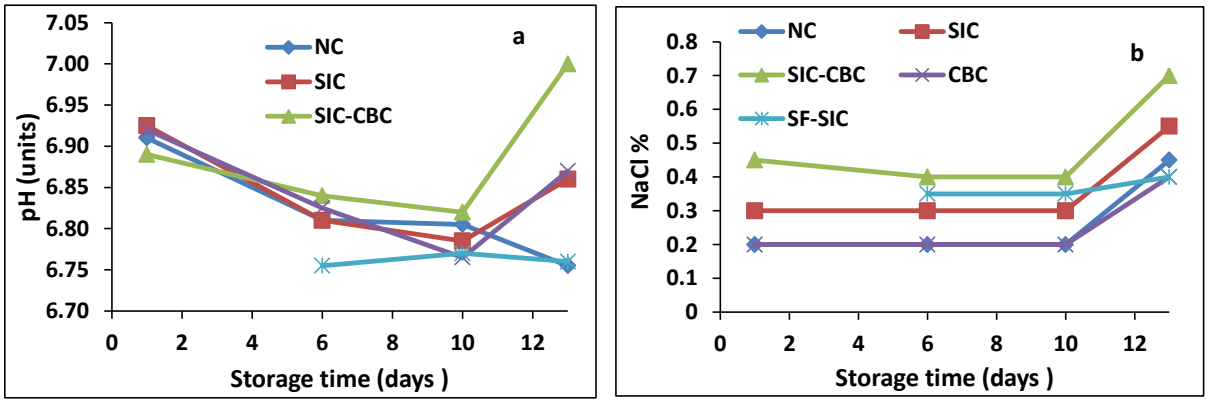

Figure 7: Variations in pH (a) and salt content (b) for differently treated redfish fillets.

The values indicate mean of 2 samples obtained from two EPS boxes for each treatment.

4.6 Overall analysis of microbial and chemical data

Figure 8 is a bi-plot relating microbial (TVC, H_2S-producing bacteria, pseudomonads, *P. phosphoreum)* and chemical variables (TVB-N, TMA and pH) measured in the five treatments during storage time. PC1 describes the different treatments with their respective time of storage from left to right and PC2 accounts for the influence of the microbial and chemical parameters on the samples. Both PC1 and PC2 explain 93% of the variation observed among the samples, pH being the most influential variable in the above quadrants and H_2S-producing bacteria as well as other microbial parameters being the most influential variables downwards. This explains the diagonal trend seen in the samples from left to right as time progressed, in concordance with the pH changes by initial lowering of the values followed by an increase caused by the production of basic compounds (TVB-N and TMA), especially in the sample SIC-CBC-d13. Lower pH values as well as TVB-N and TMA content were observed in the following samples on day 13; SIC, SF-SIC and NC, explaining their position on the plot in comparison to SIC-CBC. Further, this bi-plot shows that CBC and SIC-CBC treatments underwent a faster spoilage process than NC, SIC and SF-SIC treatments, based on the position of the samples.

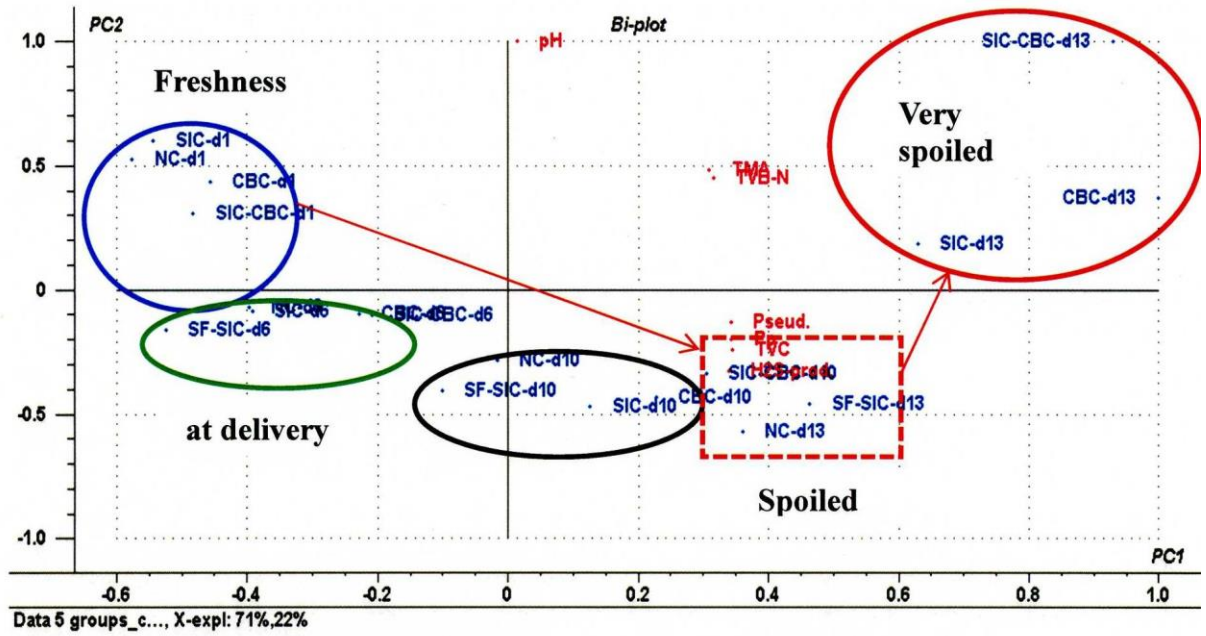

Figure 8: Principle component analysis (PCA) relating microbial and chemical data of the sample treatments (NC, SIC, SIC-CBC, CBC, SF-SIC) with storage time (d1 to d13). PC1 vs PC2 (X-expl: 71% and 22%). Bi-plot of scores (samples in blue) and loadings (chemical and microbial parameters in red).

5 DISCUSSION

The study describes the microbial quality, chemical and sensory characteristics of redfish skinless or skin-on fillets processed three days post catch as influenced by different in-process cooling techniques. It further shows the behaviour of specific spoilage organisms (SSO) during slow and fast superchilling of the skinless and skin-on fillets, respectively, over the storage period simulating distribution and storage. The cooling techniques implied the use of slurry ice as a fillet pre-cooling medium and/or CBC technique to superchill the fillets before packaging. However, mechanical problems with the deskinning machine at the CBC processing site did not allow for production of superchilled skinless fillets as intended. Instead, two groups of superchilled skin-on fillets (CBC+/-SIC treatments) were compared to skinless fillets prepared as three groups; untreated (no in-process cooling) or cooled by slurry ice and then stored in closed EPS boxes or sea freight boxes with drainage holes. Table 5 summarises the main findings for the differently treated redfish fillets at their sensory rejection point.

Table 5: Microbial and chemical indicators of spoilage for differently treated redfish fillets at the end of shelf life according to the Torry scheme

Treatment	NC	SIC	SIC-CBC	CBC
Sensory shelf life (days)	11-12	11-12	10	8-9
Product Temp (°C)*	-0.4±0.7	-0.4±0.7	-1.1±0.3	-0.9±0.2
TVC (log CFU/g)	6.4	6.9	7.8	6.3
H_2S-prod. bacteria	6.0	6.6	6.7	5.8
Pseudomonads	5.4	5.1	5.2	4.9
P. phosphoreum	4.9	>5.5	4.8	3.6
TVB-N (mgN/100g)	12.3	14.7	14.2	13.5
TMA (mgN/100g)	2.3	5.6	1.3	1.7
P-ratio	0.187	0.381	0.091	0.126
pH (units)	6.8	6.8	6.8	6.8

***Mean product temperature of redfish fillets over the maximum shelf life period indicated

The results indicate that H_2S-producing bacteria were the most dominant spoilage organisms in all treatments. *P. phosphoreum* and H_2S-producing bacteria counts were observed to be higher in slurry ice cooled fillets (SIC, SIC-CBC) than in NC and CBC treated fillets, respectively at the end of sensory shelf life. This further demonstrates the effect salt uptake had on SSO growth. By lowering the muscle freezing point, salt uptake in SIC and SIC-CBC treatments may have provided some protection for SSO in superchilled fillets and allowed them to grow faster than at a similar temperature with a lower salt level as in NC and CBC products. Similar findings were reported by Reynisson *et al.*, (2010) and Lauzon *et al.*, (2009). In addition to that, salt uptake may have shielded the SIC-CBC fillets from deteriorative changes caused by freezing damages to some extent, in comparison to CBC fillets as demonstrated by the shorter shelf life of CBC fillets. H_2S-producing bacteria were observed to be more tolerant to superchilling conditions than *P. phosphoreum*. This agrees with what Olafsdottir *et al.*, (2006) reported during aerobic storage of cod fillets. Pseudomonads were apparently the least affected by the treatments applied among the SSO evaluated.

Overall, SIC-CBC and CBC treatments maintained the lowest fillet temperature over the storage period, followed by NC and SIC (Table 5). Despite the lowest product temperature maintained, shelf life extension was not observed in the SIC-CBC and CBC-treated fillets compared to NC and SIC treated fillets. Higher SSO counts, especially H_2S-producing bacteria for the skin-on CBC-treated fillets may have caused the faster deterioration rate observed, as demonstrated by the more rapid formation of bacterial metabolites (TVB-N and TMA) and a poorer sensory quality. It is therefore recommended to remove the skin to enhance the quality of the product or at least package the fillets in EPS boxes with flesh facing flesh side of fillets and vice versa for the skin side. This was not done in this experiment because the skinning machine had a mechanical failure during the experiment.

Despite the similar microbial development of CBC and SIC-CBC fillets, sensory defects relating to the colour and heterogeneous appearance of the cooked flesh, as well as stronger rancidity odour and flavour, were attributed to CBC-treated (superchilled) fillets, especially those not previously cooled in slurry ice. These characteristics suggest that oxidative changes contributed to the shorter shelf life of CBC fillets compared to non CBC treatments. Going beyond the initial freezing point of fish muscle, slow freezing and temperature fluctuations can damage fish tissues, causing water loss, protein denaturation and enzymatic changes (Huss, 1995). Other quality changes at subzero conditions include non-enzymatic reactions like oxidation and protein insolubilisation (Fennema, 1985). The reason for the shorter shelf life seen in CBC than SIC-CBC fillets could be explained by the freezing damages occurring in lower salt-containing CBC fillets than in SIC-CBC treated ones. It is therefore concluded that the SIC treatment before CBC processing may be a preferable step to reduce freezing damage, especially for fatty fish species, or the CBC tunnel conditions should be adjusted to suit better the small fillet size of redfish compared to cod for which it was already adjusted.

The purpose of in-process slurry ice cooling is to reduce the product temperature before packaging. This is a critical processing step since improper cooling and fillet microbial contamination may speed up spoilage during storage and distribution of the product. The NC and SIC treated redfish fillets had a similar initial temperature which explains why SIC treatment did not enhance the sensory shelf life of the fillets. However, salt uptake by the fillets during slurry ice application led to a faster growth of SSO in SIC fillets compared to NC fillets. Despite this microbial relationship with salt, SIC treatment did not shorten the shelf life of the fillets in comparison to NC treatment. The observed higher levels of basic volatile compounds (TMA and TVB-N) in SIC than NC fillets at a later stage of storage are due to increased microbial growth, particularly TMA producers (H_2S-producing bacteria and *P. phosphoreum*). This agrees with what Malle and Poumeyrol (1989) reported that these are indicators of spoilage at an advanced stage. Therefore, no evident efficacy of SIC treatment at HB Grandi with respect to cooling was observed compared to NC treatment.

TMA formation in fresh fish is an indicator of microbial spoilage. Its formation is temperature and oxygen level dependent. It is known to be a proper indicator for iced/chilled fish stored in air. A P-ratio of 50% would indicate that all produced TVB-N from the initial value would be TMA. The percentage itself does not really indicate whether spoilage is reached. It depends on the initial TVB-N value of the product. The study gave a TVB-N value of 12-15 mgN/100g of redfish fillets at the sensory rejection. This is lower than the TVB-N value of 25 mgN/100 g reported by Masette (1999), and corresponds to the European Commission regulation limit for unprocessed redfish suitable for human consumption (EC No.2074/2005). However this lower value is explained by the low microbial loads, generally < log 7 CFU/g, detected at sensory rejection (Table 5). The initial low and fluctuating environmental temperature may have as well favoured other deteriorative changes than those

of microbial origin. Indeed, the initial low storage temperature may have affected the activity of spoilage microbiota. Further it should be envisaged that the spoilage pattern of whole redfish may differ to that of fillets where more complex deteriorative changes may take place under aerobic storage. In addition, the different in-process treatments led to different microbial loads which in turn are expressed in the metabolite profile observed. According to Malle and Poumeyrol (1989) a P value above 40%, representing the ratio of TMA to TVB-N, is an indicator of advanced stage of fish tissue degradation. This study showed that P-ratio varies with the cooling method applied to redfish fillets, with SIC treatment leading to the highest value. This is because of the difference in SSO loads accumulating in the differently treated fillets as storage progressed. This suggests that spoilage of redfish fillets was not entirely microbial hence the lower levels of TVB-N than the EC recommended limit.

The fall in pH during the first 10 days of storage is due to breakdown of residual glycogen via glycolysis to pyruvic acid and then lactic acid production as reported by Huss (1995). Despite this drop, it was still above 6.7, implying that the fillet texture was still reasonably firm. This agrees with the sensory results of the study according to texture attribute. The rise in pH after 10 days of storage can be explained by the increased production of basic volatile compounds in the deteriorating fish due to increased microbial activity.

The study clearly showed that bottom corners of EPS boxes are weak points since they respond faster to the ambient temperature. This implies that redfish fillets stored in the same EPS box may be of varying quality depending on the position they are, especially under extreme thermal load. This creates a need to develop EPS boxes with better insulating properties. The sea freight EPS boxes with drainage holes were observed to respond fast and directly to the fluctuations in ambient temperature during storage. This makes redfish fillets greatly susceptible to fast microbial and quality deterioration in case the ambient temperature of the storage chamber or distribution vessel is abused. However, in well monitored temperatures, sea freight EPS boxes should perform as well as closed EPS boxes.

6 CONCLUSION

The study showed that skinning of redfish fillets contributed to a longer shelf life and that in-process cooling of skinless fillets did not enhance freshness nor shelf life of the product. CBC treatment did not delay spoilage of redfish fillets despite the fact that this cooling technique led to the lowest product temperature. The shorter shelf life was caused by the highest microbial contamination due to the presence of the skin on the fillets. CBC treatment of fatty skin-on fillets apparently requires a slurry ice pre-cooling step prior to CBC to reduce freezing damages and oxidative flesh deterioration in the superchilled state as well as deskinning of fillets before packaging.

Despite the insignificant salt uptake observed in fillets pre-cooled in slurry ice, it contributed to a faster growth of specific spoilage organisms, particularly the TMA producers, H_2S-producing bacteria and *P. phosphoreum*. The low counts (<log 7 CFU/g) of specific spoilage organisms and TVB-N levels at sensory rejection indicated that spoilage of redfish fillets was not entirely microbial. Other deteriorative changes contributed to appearance and off-odour/off-flavour defects, especially in CBC-treated fillets. Oxidation of lipids, protein insolubilisation and enzymatic action in superchilled fillets could be responsible for this observation. H_2S-producing bacteria contributed though to an important part of the dominant spoilage microbiota of redfish fillets and were described as a good microbial spoilage indicator, reflecting best the quality deterioration observed in cooked products. The shelf life of SF-SIC treatment was apparently similar to NC treatment based on chemical and microbial data. Finally, the benefit of CBC treatment to extend shelf life of redfish skinless fillets remains to be demonstrated.

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APPENDIX 1 – Torry scheme and QDA attributes

Table A – Torry freshness scheme

Score	Odour	Flavour
10	Initially weak odour of boiled cod liver, fresh oil, starchy	Boiled cod liver, watery, metallic.
9	Shellfish, seaweed, boiled meat, oil, cod liver	Oily, boiled cod liver, sweet, meaty characteristic.
8	Loss of odour, neutral odour	Sweet/ characteristic flavours but reduced in intensity.
7	Woodshavings, woodsap, vanillin	Neutral
6	Condensed milk, boiled potato	Insipid
5	Milk jug odours, boiled clothes- like	Slight sourness, trace of "off"-flavours, rancid
4	Lactic acid, sour milk TMA	Slight bitterness, sour, "off"-flavours, TMA, rancid
3	Lower fatty acids (eg acetic or butyric acid) composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide, rancid

Table B – List of QDA attributes evaluated

Sensory attribute	scale		definition
ODOUR			
sweet odour	none	much	Sweet odour of fresh redfish
cod liver	none	much	Boiled cod liver
shellfish, algae	none	much	Characteristic, fresh odour
vanilla/warm milk	none	much	Vanilla, sweet warm milk
boiled potatoes	none	much	Whole, hot, boiled potatoes in a saucepan
rancid	none	much	Rancid odour
table cloth	none	much	Dirty damp dish cloth from the kitchen (left for 36 hrs.)
TMA	none	much	TMA odour, reminds of dried salted fish, amine
queasy sweet	none	much	Spoilage odour, queasy sweet, overripe fruits
spoilage sour	none	much	Spoilage sour, sour odour, sour milk, acetic acid
sulphur	none	much	Sulphur, matchstick, boiled cabbage
APPEARANCE			
colour	light	dark	Light: white colour. Dark: yellowish, brownish, grey
heterogeneous	homogeneous	heterogeneous	Homogeneous: even colour. Heterogeneous: stains, uneven colour
white precipitation	none	much	White precipitation on the sample surface
flakiness	none	much	The fish sample slides into flakes when pressed with a fork
FLAVOUR			
cod liver	none	much	Boiled cod liver
metallic	none	much	Characteristic metallic flavour of fresh redfish
sweet	none	much	Characteristic sweet flavour of fresh redfish
rancid	none	much	Rancid flavour
pungent	none	much	Pungent flavour
queasy sweet	none	much	Spoilage flavour queasy sweet, overripe fruits
sour	none	much	Spoilage sour, sour taste
TMA	none	much	TMA flavour, reminds of dried salted fish, amine
off-flavour	none	much	Intensity of off-flavour (spoilage flavour)
TEXTURE			
soft	firm	soft	Softness in first bite
juicy	dry	juicy	Dry: draws liquid from mouth. Juicy: releases liquid when chewn
tender	tough	tender	Tenderness when chewn
mushy	none	much	Mushy, porridge like texture
meaty mouthfeel	none	much	Reminds of meat texture, rough fibers
sticky	none	much	Glues together teeth when biting the fish

APPENDIX 2 – Mean data with statistical analysis
Table C - One –way ANOVA by NCSS, Duncan's Multiple–Comparison Test

Sample	Torry		TVC		H2S-prod.		Pp		Pseud.		pH*		TVB-N		TMA		Salt	
			log cfu/g		log cfu/g		log cfu/g		log cfu/g				mgN/100g		mgN/100g		%	
P value	*0.0000*		*0.0000*		*0.0000*		*0.0000*		*0.0000*		*0.0355*		*0.0013*		*0.0007*		*0.0005*	
NC-d1	8.7	a	3.8	a	1.9	a	2.0	a	3.0	ab	6.91	ce	11.8	a	1.3	a	0.4	ab
NC-d6	6.6	b	4.7	b	4.0	de	2.2	a	3.4	ac	6.81	ad	11.3	a	0.8	a	0.2	a
NC-d10	5.7	bc	5.6	c	5.2	f	4.7	c	4.5	df	6.81	ad	10.7	a	2.3	a	0.2	a
NC-d13	5.2	c	7.2	ef	6.7	g	5.0	c	6.2	h	6.76	a	14.2	a	2.3	a	0.5	ac
SIC-d1	8.7	a	3.7	a	2.3	ab	2.0	a	2.9	a	6.93	de	12.8	a	1.3	a	0.5	ac
SIC-d6	6.8	b	4.4	ab	3.6	cd	3.4	ac	3.1	ab	6.81	ad	10.6	a	1.0	a	0.3	ab
SIC-d10	5.9	bc	6.4	d	6.3	g	5.5	d	4.4	de	6.79	ac	11.4	a	1.8	a	0.3	ab
SIC-d13	5.1	c	7.3	ef	6.9	g	NA		5.8	gh	6.86	ad	18.0	ab	9.4	c	0.6	bc
SF-SIC-d6	NA		4.2	ab	2.7	ab	2.2	a	2.9	a	6.76	a	11.6	a	0.8	a	NA	
SF-SIC-d10	NA		5.8	c	5.3	f	3.9	ad	4.0	ce	6.77	ab	11.5	a	1.1	a	NA	
SF-SIC-d13	NA		7.2	ef	6.6	g	5.5	d	6.3	h	6.76	ab	14.7	a	4.9	ac	NA	
SIC-CBC-d1	8.4	a	4.3	ab	3.0	bc	2.0	a	3.5	ac	6.89	b	10.8	a	0.9	a	0.6	bc
SIC-CBC-d6	6.7	b	5.5	c	4.8	ef	3.1	ac	4.2	ce	6.84	ad	10.3	a	1.0	a	0.4	ab
SIC-CBC-d10	5.4	cd	7.8	f	6.7	g	4.8	c	5.2	fg	6.82	ad	14.2	a	1.3	a	0.4	ab
SIC-CBC-d13	4.5	d	7.8	f	7.1	g	6.2	e	5.7	gh	7.00	e	23.5	b	13.5	d	0.7	c
CBC-d1	8.3	a	4.4	ab	3.0	bc	1.8	a	3.8	bd	6.92	de	11.2	a	0.9	a	0.2	a
CBC-d6	6.7	b	5.3	c	4.8	ef	2.5	ab	4.3	de	6.83	ad	11.9	a	0.6	a	0.2	a
CBC-d10	4.9	cd	6.9	e	6.4	g	4.3	b	5.2	g	6.77	ab	14.5	a	2.4	ab	0.2	a
CBC-d13	4.4	d	7.7	f	7.3	g	6.0	e	7.6	i	6.87	ae	23.3	b	11.9	d	0.4	ab

* Fisher's LSD Multiple-Comparison Test used for pH comparison; NA, not available

Table D – QDA mean data with statistical significance from days 1, 6, 10 and 13

ANOVA by NCSS, Duncan's Multiple–Comparison Test

	Day 1						Day 6					
Sensory attribute	ms	NC	SIC	SIC-CBC	CBC	*p value*	ms	NC	SIC	SIC-CBC	CBC	*p value*
ODOUR												
sweet odour		48	46	46	43	0.523		39	39	39	36	0.705
cod liver		39	38	40	36	0.595		27	26	24	30	0.284
shellfish, algae		28	29	29	24	0.259		26	27	23	26	0.677
vanilla/warm milk		35	33	32	30	0.501		27	25	27	22	0.479
boiled potatoes		17	22	21	22	0.096		29	25 **b**	30	33 **a**	0.058
rancid		0	1	1	0	0.234		1	1	1	3	0.305
table cloth	*	1 **b**	3	4	7 **a**	0.032		6	7	9	6	0.695
TMA		0	0	0	1	0.499		3	1	1	2	0.478
queasy sweet		3	2	2	3	0.898		7	10	11	7	0.164
spoilage sour	*	0 **b**	1 **a**	0	0	0.015		1	1	0	1	0.657
sulphur		0	0	0	0	0.404		0	0	1	2	0.549
APPEARANCE												
colour	**	26 **b**	27 **b**	27 **b**	40 **a**	0.009		32	28	27	35	0.130
heterogeneous	*	30 **b**	33 **b**	31 **b**	43 **a**	0.018		40	38	35	42	0.679
white precipitation	***	32 **b**	32 **b**	25 **b**	44 **a**	0.001	**	43 **a**	33 **c**	33 **bc**	40 **ab**	0.002
flakiness		46	47	42	44	0.481		48	46	44	45	0.735
FLAVOUR												
cod liver		46	45	42	44	0.424		29	29	27	31	0.720
metallic	*	38 **a**	35	33	32 **b**	0.017		27	28	23	29	0.188
sweet		40	44	46	40	0.226		23	27	30	28	0.323
rancid	**	1 **b**	0 **b**	0 **b**	2 **a**	0.002		2	1	1	4	0.188
pungent		3	2	1	2	0.512		11	6	9	11	0.221
queasy sweet		2	2	2	3	0.977		10	12	9	9	0.598
sour		0	0	0	1	0.658		1	2	0	1	0.577
TMA		0	0	0	1	0.480		2	3	1	3	0.272
off-flavour		0	0	0	0	0.206		1	0	0	1	0.309
TEXTURE												
soft		42	42	45	45	0.634		38	35	39	34	0.190
juicy		49	49	51	47	0.513		44	47	44	42	0.292
tender		51	48	51	48	0.445		47	48	49	44	0.201
mushy		19	18	21	19	0.826		22	21	25	20	0.333
meaty mouthfeel		27	28	26	27	0.888		21	21	16	21	0.064
sticky		34	33	30	32	0.212		27	26	26	27	0.908

ms (marginal significance, p = 0.05 - 0.0); * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$)

	Day 10						Day 13					
Sensory attribute	**ms**	**NC**	**SIC**	**SIC-CBC**	**CBC**	*p value*	**ms**	**NC**	**SIC**	**SIC-CBC**	**CBC**	*p value*
ODOUR												
sweet odour		28	24	24	20	0.281	*	21	22 **a**	13 **b**	15	0.027
cod liver		20	22	16	15	0.190		13	15	10	11	0.129
shellfish, algae		17	19	13	14	0.188	*	13 **a**	13	8 **b**	10	0.026
vanilla/warm milk	*	17 **a**	18 **a**	13	9 **b**	0.013		13	13	9	10	0.143
boiled potatoes		28	26	25	29	0.733		29	27	24	22	0.165
rancid	*	6	3	1 **b**	10 **a**	0.034		7	5	7	11	0.393
table cloth		12	12	17	16	0.260	*	20 **b**	21 **b**	29 **a**	22 **b**	0.019
TMA	*	4 **b**	4 **b**	12 **a**	13 **a**	0.001	***	9 **b**	8 **b**	22 **a**	17 **a**	0.001
queasy sweet		11	8	9	11	0.620		16	16	19	16	0.771
spoilage sour	*	2 **b**	8 **a**	8 **a**	10 **a**	0.013	*	8	9 **b**	16	15 **a**	0.026
sulphur		4	2	3	5	0.306		3	4	7	6	0.222
APPEARANCE												
colour	*	39 **a**	35	28 **b**	40 **a**	0.028	***	36 **b**	34 **b**	40 **b**	48 **a**	0.001
heterogeneous	**	49 **a**	41 **bc**	34 **c**	50 **ab**	0.001		42	45	43	51	0.212
white precipitation		39	39	34	42	0.214	*	46 **a**	43	36 **b**	43 **a**	0.017
flakiness		41	44	44	39	0.437	*	49 **a**	46	45	42 **b**	0.041
FLAVOUR												
cod liver	*	28	28 **a**	19	17 **b**	0.024		17	16	10	14	0.147
metallic	*	26 **a**	20	22	16 **b**	0.038		15	14	11	10	0.110
sweet		21	21	22	14	0.298		13	16	12	9	0.133
rancid		5	8	2	9	0.121		11	8	9	14	0.486
pungent		12	11	10	20	0.083	**	13 **b**	12 **b**	21 **a**	19	0.009
queasy sweet		9	11	14	13	0.591	*	14	12	20	20	0.036
sour		3	7	5	7	0.526		8	7	11	12	0.155
TMA	*	3 **b**	6	10 **a**	10 **a**	0.020	**	7 **b**	6 **b**	18 **a**	15 **a**	0.001
off-flavour		3	6	5	8	0.351	*	7 **b**	7	14	16 **a**	0.025
TEXTURE												
soft		43	45	47	42	0.814		41	42	44	40	0.487
juicy	**	43 **a**	45 **a**	46 **a**	35 **b**	0.007		42	42	43	42	0.969
tender		48	53	51	44	0.061		52	54	50	49	0.216
mushy		28	26	32	27	0.600		22	24	25	22	0.780
meaty mouthfeel		25	25	29	22	0.354		28	26	25	27	0.566
sticky		37	35	39	35	0.162		27	28	28	25	0.497

ms (marginal significance, p = 0.05 - 0.0); * (p < 0.05); ** (p < 0.01); *** (p < 0.001)

Table E – Pearson correlation matrix with R values for Torry, microbial and chemical data

Pearson Correlations Section (Row-Wise Deletion)

	TVC C2	H2S C3	Pseud. C4	Pp C5	pH C6	TVB-N C7	salt C9	TMA C11	Torry C12
C2	1.000	0.970	0.893	0.879	-0.154	0.633	0.262	0.558	-0.906
C3	0.970	1.000	0.878	0.877	-0.255	0.557	0.166	0.513	-0.940
C4	0.893	0.878	1.000	0.782	-0.102	0.687	0.239	0.629	-0.832
C5	0.879	0.877	0.782	1.000	-0.133	0.622	0.290	0.628	-0.852
C6	-0.154	-0.255	-0.102	-0.133	1.000	0.424	0.401	0.474	0.232
C7	0.633	0.557	0.687	0.622	0.424	1.000	0.516	0.956	-0.582
C9	0.262	0.166	0.239	0.290	0.401	0.516	1.000	0.525	-0.125
C11	0.558	0.513	0.629	0.628	0.474	0.956	0.525	1.000	-0.558
C12	-0.906	-0.940	-0.832	-0.852	0.232	-0.582	-0.125	-0.558	1.000

Pp- *Photobactecterium phosphoreum*