Nýsköpun & neytendur
Consumers & Products

Líftækni & lífefni
Biotechnology & Biomolecules

Vinnsla & virðisaukning
Value Chain & Processing

Mælingar & miðlun
Analysis & Consulting

Erfðir & eldi
Genetics & Aquaculture

Öryggi & umhverfi
Food Safety & Environment

The effect of cooling methods at processing and use of gel packs on storage life of cod (*Gadus morhua*) loins. Effect of transport via air and sea on temperature control and retail-packaging on cod deterioration

Emilía Martinsdóttir
Hélène L. Lauzon
Björn Margeirsson
Kolbrún Sveinsdóttir
Lárus Þorvaldsson
Hannes Magnússon
Eyjólfur Reynisson
Arna Vigdís Jónsdóttir
Sigurjón Arason
Maria Eden

Nýsköpun og neytendur

Skýrsla Matís 18-10
Maí 2010

ISSN 1670-7192

The effect of cooling methods at processing and use of gel packs on storage life of cod (*Gadus morhua*) loins. Effect of transport via air and sea on temperature control and retail-packaging on cod deterioration

Emilía Martinsdóttir[1)]
Hélène L. Lauzon[1)]
Björn Margeirsson[1)]
Kolbrún Sveinsdóttir[1)]
Lárus Þorvaldsson[1)]
Hannes Magnússon[1)]
Eyjólfur Reynisson[1)]
Arna Vigdís Jónsdóttir[1,2)]
Sigurjón Arason[1,2)]
Maria Eden[3)]

[1)]Matís ohf
[2)]Háskóli Íslands
[3)]ttz Bremerhaven

Skýrsluágrip Matís ohf

Icelandic Food and Biotech R&D

Report summary

ISSN: 1670-7192

Titill / Title	**The effect of cooling methods at processing and use of gel-packs on storage life of cod (*Gadus morhua*) loins – Effect of transport via air and sea on temperature control and retail-packaging on cod deterioration**		
Höfundar / Authors	*Emilía Martinsdóttir[1)], Hélène L. Lauzon[1)], Björn Margeirsson[1)], Kolbrún Sveinsdóttir[1)], Lárus Þorvaldsson[1)], Hannes Magnússon[1)], Eyjólfur Reynisson[1)], Arna Vigdís Jónsdóttir[1,2], Sigurjón Arason[1,2)], Maria Eden[3)] [1)]Matís ohf [2)]Háskóli Íslands [3)] ttz Bremerhaven*		
Skýrsla / Report no.	18-10	*Útgáfudagur / Date:*	Maí 2010
Verknr. / project no.	1682/1704		
Styrktaraðilar / funding:	*EU (contract FP6-016333-2) Chill-on, AVS, R&D Fund of Ministry of Fisheries in Iceland, the Technology Development Fund at the Icelandic Centre for Research*		
Ágrip á íslensku:	Tilgangur tilraunanna var að kanna áhrif mismunandi kælingar við vinnslu og hitasveiflna í flutningi með og án kælimottu á geymsluþol þorskhnakka. Í vinnslu var borið saman að nota enga forkælingu fyrir flökun, vökvakælingu og roðkælingu (CBC) sem alltaf er með vökvakælingu. Áhrif hitasveiflna þar sem líkt var eftir í hitabreytingum í flutningi (RTS) voru borin saman við geymslu við stöðugt hitastig (-1 °C). Einnig voru metin áhrif þess að nota kælimottu í geymslu og flutningi. Sýni voru gæðametin með skynmati, örveru- og efnamælingum. Fylgst var með hitastigi með hitasíritum. Roðkældir þorskhnakkar í frauðplastkössum voru fluttir til Bremerhaven með flugi og skipi þar sem þeim var endurpakkað í loft og loftskiptar pakkningar (MAP) og þær geymdar við 1 °C. Gerðar voru efna- og örverumælingar til að fylgjast með gæðabreytingum. Hitastig roðkældra hnakka var lægra en hinna fyrstu 2 daga tilraunarinnar. Kælimotturnar höfðu ákveðin áhrif til lækkunar hitastigs þegar hitasveiflur voru í ferlinu og lægri hiti hélst gegnum allan geymslutímann. Samt sem áður hafði notkunin ekki áhrif á lengd ferskleikatíma eða geymsluþols samkvæmt skynmati. Örverufjöldi var heldur lægri ef hitasveiflur urðu í ferlinum en lítill munur var við stöðugt hitastig. Geymsla við stöðugt, lágt hitastig (-1 °C) lengdi geymsluþol um ca. 3 daga samkvæmt skynmati og var það í samræmi við örverutalningar og mælingar á TVB-N og TMA. Í tilraunum í Bremerhaven kom fram að örverufjöldi var yfirleitt lægri þegar notaðar voru loftskiptar umbúðir í samanburði við fisk í lofti. Þetta var sérstaklega áberandi í flugfiskinum. Fiskurinn sem fluttur var með skipi geymdist samt jafn lengi og fiskurinn sem fluttur var með flugi. Þetta orsakast af því að flugfiskurinn varð fyrir meiri hitasveiflum í flutningi og yfirborðshiti hans mældist 4 °C við komuna til Bremerhaven. Flutningstími með skipi var miklu lengri (+48 klst) en yfirborðshiti mældist undir 2 °C við móttöku. Notkun kælimotta hafði lítil áhrif á hitastigið í flutningi en samt sem áður var yfirborðshiti aðeins lægri í fiski með kælimottum við komuna til Bremerhaven bæði með flugi og skipi.		
Lykilorð á íslensku:	*Kælitækni, hitasveiflur, þorskhnakkar, ferskleiki, geymsluþol, skemmdarbakteríur*		

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D

Report summary

ISSN: 1670-7192

Summary in English:	The main aim of the experiment was to investigate the effects of different cooling techniques during processing and temperature fluctuations during transport on the storage life of cod loins with and without gel packs. The following cooling techniques were studied: combined blast and contact (CBC) cooling (with liquid cooling prior to the CBC cooling), only liquid cooling and where no special cooling was used prior to deskinning and trimming. The effect of real temperature simulation (RTS) during storage was compared to a steady storage temperature of -1 °C. The samples were analysed with sensory, microbial and chemical methods. The temperature was monitored from packaging using temperature loggers. CBC cooled loins were transported to Bremerhaven via air and ship freight after packaging in EPS boxes. The fish was repacked in air and modified atmosphere and stored at 1 °C. Deteriorative changes were evaluated by microbial and chemical indicators. CBC cooling resulted in a lower temperature profile the first two days of the experiment. The use of gel packs lowered somewhat the temperature increase in the products when RTS was applied and lower temperature was maintained during the entire storage period. According to sensory evaluation, the use of gel packs did not result in prolonged freshness period or shelf life. According to microbial and chemical analysis no marked difference was seen whether gel packs were used or not in groups stored at a steady temperature. However, microbial counts were somewhat lower and slower formation of TVB-N and TMA occurred in RTS groups where gel packs were used compared to no gel packs. Storage at a steady -1 °C resulted in prolonged shelf life of three days according to sensory evaluation. This was confirmed by microbial and chemical analysis as lower microbial counts, TVB-N and TMA values were generally obtained in the steady temperature group than in the group receiving the RTS treatment. The storage studies carried out at Bremerhaven on modified atmosphere vs. air packed loins showed generally lower microbial counts, especially in the air transported fish. Deterioration process of air and sea freight fish was however similar. Re-packaging of sea freight fish at a later stage did not significantly affect its deteriorative process compared to re-packed air freight fish. This might be due to the fact, that the air freight fish was subject to high temperatures during transport and surface temperature reached over 4 °C. The sea freight fish had a much longer transport phase, but arrived with surface temperatures below 2° C. This shows that not only the time of re-packaging but also the temperature profile during transport are important factors influencing the deteriorative process and shelf life. Gel packs did not have significant cooling effect in this experiment. However the surface temperature in boxes with a gel pack was slightly lower than in boxes without a gel pack independently of transport mode used.
English keywords:	*Cooling techniques, real temperature simulation, cod loins, freshness, shelf life, spoilage bacteria*

CONTENTS

LIST OF FIGURES

1 INTRODUCTION

Maintaining a low and steady temperature through the entire cold chain is vital for the shelf life of valuable, fresh cod loins. In recent years many new technologies have been introduced for that purpose. One of the companies which has been designing and developing such technologies is Skaginn hf. a food processing equipment manufacturer located in Akranes, Iceland. Skaginn hf. has been developing and designing a new cooling technology for fish processing for the past few years called Combined Blast and Contact cooling (CBC). The technique involves superchilling the skin side of fillets by moving them through a freezer tunnel on a Teflon coated aluminium conveyor belt at a temperature of approximately -8 °C and simultaneously blasting cold air over the fillets. This rapid cooling process freezes the skin without freezing the flesh. Before the CBC cooling, the fish goes through a pre-cooler/fluid-ice which contains approximately 2.5% salt which makes it possible for the fillets to go through the CBC process without freezing the flesh. This superchilling process facilitates further handling of the fillets, in particular deskinning and effective cooling with a resulting loin temperature around -1 °C when packed.

The study is divided into two parts, studying the effects of different cooling techniques and temperature control during transport using the same raw material. The main aim of the first part of the experiment (Experiment I) was to investigate the effects of different cooling techniques and environmental temperature fluctuations on the storage life of cod loins, with and without gel pack. The following cooling techniques were studied: liquid cooling of fillets with or without CBC cooling compared to no cooling at all. The effect of real air freight temperature simulation (RTS) during storage was compared to a steady storage temperature (-1 °C). Additionally, the influence of using a gel pack in the boxes during storage was studied. The samples were analysed with sensory evaluation, microbial and chemical methods. The aim of the second part of the experiment (Experiment II) was to study temperature control during transport via air- and sea freight. For this purpose, CBC cooled cod loins packed in EPS boxes were transported via air-and sea freight to Bremerhaven, Germany. After the transport, the samples were retail-packed in air and modified atmosphere (MA), and analysed with microbial and chemical methods. The temperature history of the groups was studied using temperature loggings.

2 MATERIAL AND METHODS

2.1 Experimental design

Cod used in the experiments (I and II) was trawler caught east of Iceland on February 22nd, 2009 (average ambient air temperature of 5 °C). After bleeding, gutting and washing, the cod was stored in crushed plate ice (fish to ice ratio approx. 3:1) in 460 L tubs on board vessels to main processing in the northern part of Iceland. At the processing plant, the cod was processed in different ways (see below) on February 24th (11am-2pm). The average temperature of the fish during the processing step was 1 °C. The products (cod loins) were packed in 5 kg expanded polystyrene (EPS) boxes, the boxes were palletized and the pallets containerized. The procedure for the experiments I and II was then as follows:

<u>Experiment I.</u> The pallets were transported to Reykjavik on the same day (5pm to midnight) in a refrigerated container and arrived at Matís, Reykjavík, around 9.30am on the next day. At Matís the loins were stored either at -1 °C or under real air freight temperature simulation (RTS) for up to 13 days from processing.

The experimental groups were as follows:

A. Liquid cooling (LC) and CBC cooling, with a cooling gel pack (GP) and stored under RTS conditions at Matís.
B. Liquid cooling and CBC cooling, stored under RTS conditions at Matís.
C. Liquid cooling, with a cooling gel pack and stored under RTS conditions at Matís.
D. No cooling (NC) during processing, with a cooling gel pack and stored under RTS conditions at Matís.
E. Liquid cooling and CBC cooling, with a cooling gel pack and stored at -1 °C (S) at Matís.

F. Liquid cooling and CBC cooling, stored at -1 °C at Matís.

Following abbreviations of experimental groups will be used hereafter:

A. CBC-RTS-GP
B. CBC-RTS
C. LC-RTS-GP
D. NC-RTS-GP
E. CBC-S-GP
F. CBC-S

Sampling was done regularly from processing up to 13 days as shown in Table 1.

Table 1. Sampling days for Experiment I. CBC = combined blast and contact cooling, RTS = real temperature simulation during storage, GP = gel pack in boxes, LC = liquid cooling only, NC = no cooling during processing, S = superchilled during storage

Group	Treatment	Storage	Sampling days*
CBC-RTS-GP	CBC cooling	RTS, gel pack	0, 1, 3, 6, 8, 10
CBC-RTS	CBC cooling	RTS, no gel pack	0, 1, 3, 6, 8, 10
LC-RTS-GP	Liquid cooling	RTS, gel pack	0, 1, 3, 6, 8, 10
NC-RTS-GP	No cooling	RTS, gel pack	0, 1, 3, 6, 8, 10
CBC-S-GP	CBC cooling	-1 °C, gel pack	0, 2, 7, 9, 13
CBC-S	CBC cooling	-1 °C, no gel pack	0, 2, 7, 9, 13

***Sampling on day 0 was done for microbial and chemical analysis only.**

Experiment II. A part of the cod loins' lot described above (CBC cooled loins with and without gel pack) was transported to Bremerhaven, Germany via air and sea freight after packaging in EPS boxes.

The cod loins were transported from the pick-up location in Bremerhaven/Cuxhaven to TTZ for temperature check, collection of data loggers and repackaging as retail portions (2 loins per package; average loin weight 312 g). A part of the fish was stored under modified atmosphere (MA) at 1 °C for up to 21 days from processing in Iceland. During storage, samples were analysed by microbial and chemical methods at TTZ, Bremerhaven, Germany.

Fish loins were divided into 8 groups (see Table 2) differing in transport mode (sea or air), packaging conditions (with or without gel pack) and packaging method (MAP or air). Sampling was done at arrival (two (air freight) and six (sea freight) days from processing and then 3, 6, 11, 14 and 18 days from repackaging for air freight fish but 4, 8, 10 and 15 days from repackaging for sea freight fish. On the last sampling day for air freight fish only MAP samples were examined. The last sampling day was in fact 20 and 21 days from processing for air and sea freight fish, respectively.

Table 2. Sampling groups in Experiment II

		MA packaged	**Air packaged**
Air freight	**With gel pack**	FG MAP	FG AIR
	Without gel pack	GG MAP	GG AIR
Sea freight	**With gel pack**	FS MAP	FS AIR
	Without gel pack	GS MAP	GS AIR

Figure 1a. Packaging of fish loins

Figure 1b. Gas composition of modified atmosphere packages.

Fish was repackaged (Figure 1a) upon arrival under MA (45% N_2, 5% O_2, 50% CO_2) (Figure 1b) and air packaging. Each bag contained 2 cod loins stored in a 1 °C cold chamber. The data logger stored together with the fish revealed an average temperature of 1.1 ± 0.6 °C . The standard deviation is quite high due to large doors of the cold chamber, which had to be opened during sampling.

2.2 Temperature and humidity measurements

<u>Experiment I and II</u>. Two types of loggers and one type of relative humidity and temperature logger were used for temperature and humidity recording:

a. iButton temperature loggers (Figure 2), type DS1922L. (http://www.maxim-ic.com/quick_view2.cfm/qv_pk/4088). This logger has an accuracy of ±0.5 °C, a resolution of 0.0625 °C and an operating range of -40 to 85 °C. The diameter is 17 mm and the thickness is 5 mm. The iButton loggers were used in the experiments for measuring the product temperature. They were placed in plastic bags, in order to avoid microbial contamination.

b. Onset temperature loggers type UTBI-001 (http://www.onsetcomp.com/products/data-loggers/utbi-001). This logger has an accuracy of ±0.2 °C, a resolution of 0.02 °C and an operating range of -20 to 70 °C. The diameter is 30 mm and the thickness is 17 mm. Those loggers were used for measuring ambient temperature in climate chambers.

a)

Figure 2.a. iButton temperature logger

b)

Figure 2.b. Onset Tidbit temperature logger

c. UB12 HOBO® Relative humidity and temperature data logger

Temperature range: - 20 °C to + 70 °C
RH: 5 – 95 %
Accuracy: ± 2.5 %
Capacity: 43.000 measurements
Resolution: 0.03 %
Time accuracy: ± 1 minute per week.
Size: 74 x 22 x 58 m.
Weight: 46 g.

Four iButton temperature loggers were put in each EPS box and two on the outside of each box. In Experiment 1, five Onset temperature loggers were used in each climate chamber for measuring ambient temperature. The configuration of temperature loggers in Experiment II is described in Section 4.1.
Figure 3 shows the location of the temperature loggers inside the boxes. The numbering scheme used is consistent with Table 3. The names used for each logger hereafter are also displayed in Table 3.

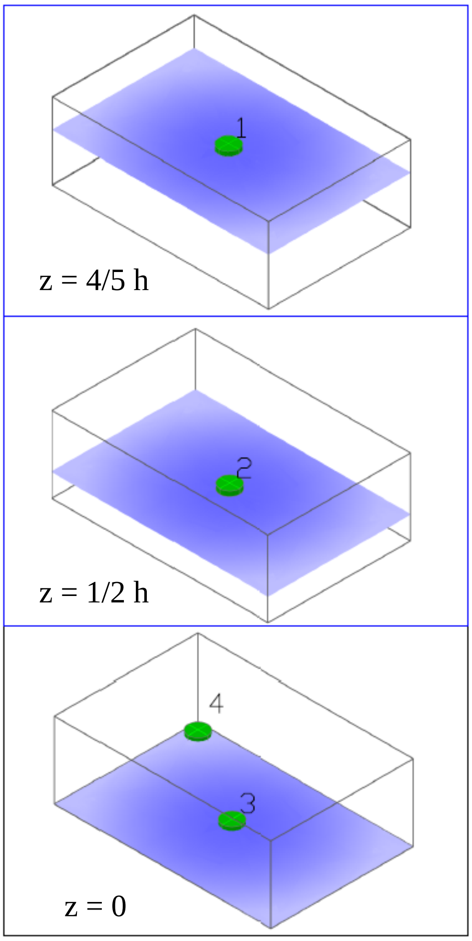

Figure 3. Location of temperature loggers inside each box. The fish pile thickness in the box is represented by h and the vertical position of the loggers by z.

Table 3. Location and numbering scheme of temperature loggers

#	Horizontal location	Vertical location	Name
1	Middle	Top	Top
2	Middle	Middle	Middle
3	Middle	Bottom	Bottom

4	Corner	Bottom	Corner

2.3 Sensory evaluation

Experiment I. Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (2004), and the Torry freshness score sheet (Shewan et al. 1953) were used to assess cooked samples (MA09sky027-040) of cod (Table 1). Eleven panellists all trained according to international standards (ISO 8586, 1993); including detection and recognition of tastes and odours, use of scales and development and use of descriptors, participated in the sensory evaluation. The members of the panel were familiar with the QDA method and experienced in sensory analysis of cod. One session was used for training prior to the sensory evaluation. The panel was trained in recognition of sensory characteristics of the samples and describing the intensity of each attribute for a given sample using an unstructured scale (from 0 to 100%). Most of the attributes were defined and described by the sensory panel during other projects (Sveinsdottir et al. 2009; Magnússon et al. 2006). The sensory attributes were 30 and are described in Table 4.

Samples weighing ca 40 g were taken from the loins and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 6 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95-100 °C with air circulation and steam, and then served to the panel. Each panellist evaluated duplicates of each sample in a random order in 14 sessions (maximum four samples per session). A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording.

Data analysis. Principal Component Analysis (PCA) on significant mean values of QDA sensory attributes was performed, using full cross validation. Analysis of variance (ANOVA) was carried out on QDA data in the statistical program NCSS 2000 (NCSS, Utah, USA). The program calculates multiple comparisons using Duncan's multiple comparison test. The significance level was set at 5%, if not stated elsewhere.

Table 4. Sensory vocabulary for cooked samples of cod (*Gadus morhua*)

Sensory attribute	Short name	Description of attribute
Odour		
sweet	o-sweet	Sweet odour
shellfish, algae	o-shellfish	Shellfish, algae, characteristic fresh odour
meaty	o-meat	Reminds of boiled meat or halibut
vanilla/warm milk	o-vanilla	Vanilla, sweet heated milk
boiled potatoes	o-potatoes	Reminds of boiled potatoes
frozen storage	o-frozen	Refrigerator, freezer storage odour
table cloth	o-cloth	Reminds of a table cloth (damp cloth to clean kitchen table, left for 36 h)
TMA	o-TMA	TMA odour, reminds of dried salted fish, amine
sour	o-sour	Sour odour, sour milk, spoilage sour, acetic acid
sulphur	o-sulphur	Sulphur, matchstick
Appearance		
colour	a-dark	Left end: light, white colour. Right end: dark, yellowish, brownish, grey
appearance	a-disc	Left end: homogeneous, even colour. Right end: discoloured, heterogeneous, stains
white precipitation	a-prec	White precipitation on the fish surface
Flavour		
salt	f-salt	Salty taste
metallic	f-metallic	Characteristic metallic flavour of fresh cod
sweet	f-sweet	Characteristic sweet flavour of fresh boiled cod
meaty	f-meaty	Reminds of boiled meat
frozen storage	f-frozen	Reminds of wood which has soaked in refrigerator/freezing flavour
pungent	f-pungent	Pungent flavour, bitter
sour	f-sour	Sour taste, spoilage sour
TMA	f-TMA	TMA flavour, reminds of dried salted fish, amine
off-flavour	f-off	Strength of off-flavour (spoilage flavour/off-flavour)
Texture		
flakiness	t-flakes	The fish portion slides into flakes when pressed with the fork
soft	t-soft	Left end: firm. Right end: soft. Evaluate how firm or soft the fish is during the first bite
juicy	t-juicy	Left end: dry. Right end: Juicy. Evaluated after chewing several times: dry - draws juice from the mouth
tender	t-tender	Left end: tough. Right end: tender. Evaluated after chewing several times
mushy	t-mushy	Mushy texture
meaty mouthfeel	t-meaty	Meaty texture, meaty mouthfeel, crude muscle fibers
clammy	t-clammy	Clammy texture, tannin (dry red wine)
rubbery	t-rubbery	Rubbery texture, springy

2.4 Microbial measurements

Experiment I. Total viable psychrotrophic counts (TVC) and counts of H_2S-producing bacteria were evaluated 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. Plates were incubated at 17 °C for 5 d. Bacteria forming black colonies on IA produce H_2S from sodium thiosulphate and/or cysteine. Cephaloridine Fucidin Cetrimide (CFC) agar was modified according to Stanbridge and Board (1994) and used for enumeration of presumptive pseudomonads. Pseudomonas Agar Base (Oxoid) with CFC Selective Agar Supplement (Oxoid) was used. Plates were incubated at 22 °C for 3 d. *Pseudomonas* spp. form pink colonies on this medium. Counts of *Photobacterium phosphoreum* were estimated by using the PPDM-Malthus conductance method (Dalgaard and others 1996), as described by Lauzon (2003).

In all experiments, cooled Maximum Recovery Diluent (MRD, Oxoid) was used for dilutions and agar media were surface-plated. All samples were analysed in triplicate and results presented as an average.

Experiment II. The following analysis was carried out at TTZ, Bremerhaven, Germany: TVC and H_2S-producing bacteria on Triple Sugar Iron Agar (Merck) incubated at 15 °C for 5 d, and pseudomonads on CFC medium (Oxoid) incubated at 25 °C for 3 d. In all experiments, cooled MRD was used for dilutions and agar media were surface-plated. All samples were analysed in duplicate and results presented as an average. Counts of *Photobacterium phosphoreum* were obtained by a quantitative PCR method. Briefly, one ml of the tenfold diluted fish samples 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 Promega Magnesil KF, Genomic system (MD1460) DNA isolation kit (Promega Corporation, Madison, USA) in combination with KingFisher magnetic beads automatic DNA isolation instrument (Thermo Labsystems, Waltham, USA) according to the manufacturers´ recommendations.

All PCR reactions were done using the Mx3005p instrument. The PCR was done using Brilliant QPCR mastermix (Stratagene, La Jolla, CA, USA). Primers were synthesized and purified with HPLC (MWG, Ebersberg, Germany). The DNA standard used for quantification was previously calibrated against the PPDM-Malthus conductance method.

2.5 Chemical analysis

Total Volatile Base Nitrogen (TVB-N) and Trimethylamine (TMA)

Experiment I. The method of Malle and Tao (1987) was used for total volatile bases (TVB-N) and trimethylamine (TMA) measurements. TVB-N was measured by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen) and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic acid (TCA) solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulphuric acid solution. TMA was measured in TCA extract by adding 20 ml of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine. All chemical analyses were done in triplicate.

Experiment II. TVB-N was measured at two to three sampling points and in duplicate by the direct steam distillation into boric acid using a Kjeldahl-type distillatory according to German Food Law (LMBG).

pH- measurements

Experiment I. The pH was measured in 5 grams of minced loins 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 °C) (Radiometer Analytical A/S, Bagsvaerd, Denmark).

Experiment II. pH measured accordingly as above.

Salt and water content

Experiment I. The water content of each loin was measured by accurately weighing out 5 grams of the minced sample in a ceramic bowl with sand. The sample was then mixed to the sand and dried in an oven at 103 ± 2 °C for 4 h. The water content was based on weight differences before and after the drying of three replicates for each sample (ISO 6496, 1999). Salt content was measured with the Volhard Titrino method according to AOAC (2000).

2.6 Drip measurements and water holding capacity

Experiment I. Drip was evaluated through the storage by measuring the weight of the fish before and after packaging. The drip (%) was then calculated as the ratio of the weight of the water lost during storage to the original weight of the fish.

The water holding capacity (WHC) was determined by a centrifugation method (Eide et al., 1982). Approximately 2 g of the minced fish was weighed accurately and centrifuged (Heraeus Biofuge Stratos, Kendro Laboratory products, USA) at 210 x *g*, for 5 min at 0-5

°C. The weight lost during centrifugation ($\Delta m_{centrifuged}$) was evaluated as water loss and no corrections were made for other components as may be necessary for fish with high fat content. WHC was calculated as the ratio of the water retained in the sample, compared to mass of water before centrifugation ($m_t * x_t^w$):

$$WHC = \left(\frac{m_t * x_t^w - \Delta m_{centrifuged}}{m_t * x_t^w} \right) * 100$$

3 RESULTS AND DISCUSSION: EXPERIMENT I

3.1 Temperature measurements

Ambient temperature

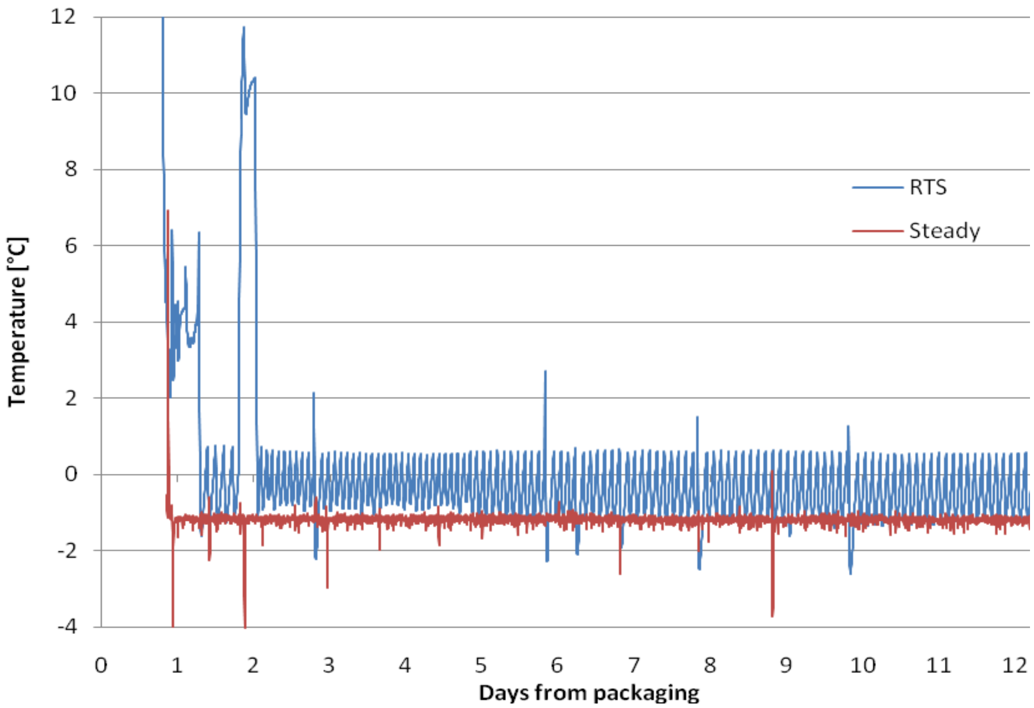

Figure 4. Temperature in the climate chamber of the real temperature simulation (RTS)

Figure 4 shows how the real (air freight) temperature simulation was performed as well as the temperature in the steady climate chamber. The boxes arrived at Matís facilities at 8:45 on the morning of 25th of February 2009. The boxes which belonged to the RTS groups were then inserted into a climate chamber with air temperature of 4.6±3.0 °C. After 12 h the temperature was lowered down to -0.6±0.7 °C for 12 h and then raised again to 9.8±2.2 °C for 6 h. At last, the temperature was kept at constant at -0.5±0.5 °C for the remaining storage period. The groups subjected to steady temperature were put in a climate chamber where a temperature of 1.2±0.2 °C was maintained during the entire storage period.

Real temperature simulation

From Figures 5-8, it can be seen that the location most sensitive to temperature load is the bottom corner of the EPS box, i.e. the fastest to respond to any temperature load in all four cases.

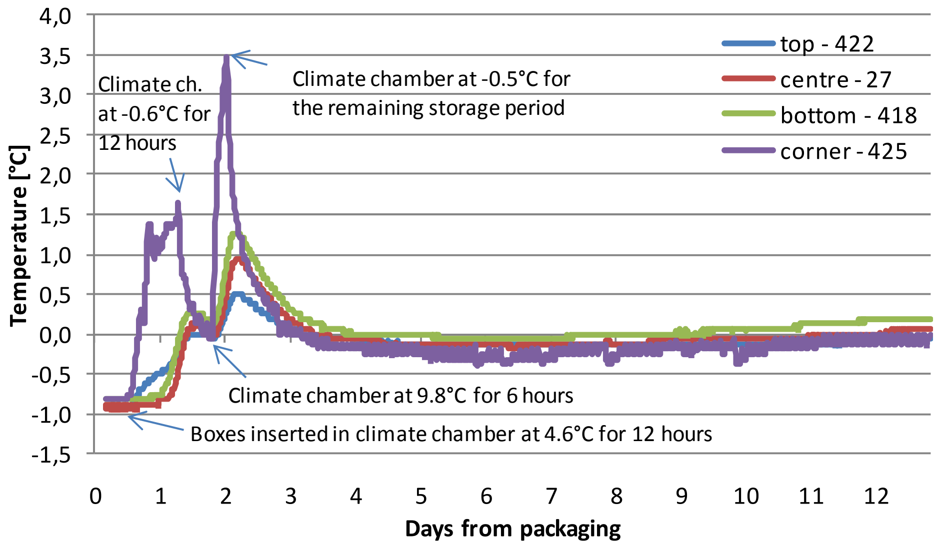

Figure 5. Group A (CBC-RTS-GP) – CBC cooled and packed with a gel pack.

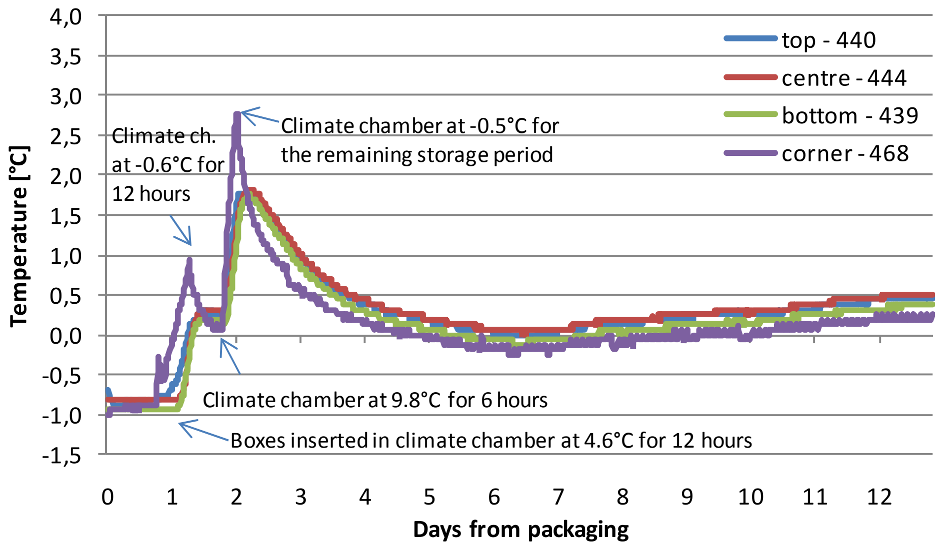

Figure 6. Group B (CBC-RTS) – CBC cooled and packed without gel pack.

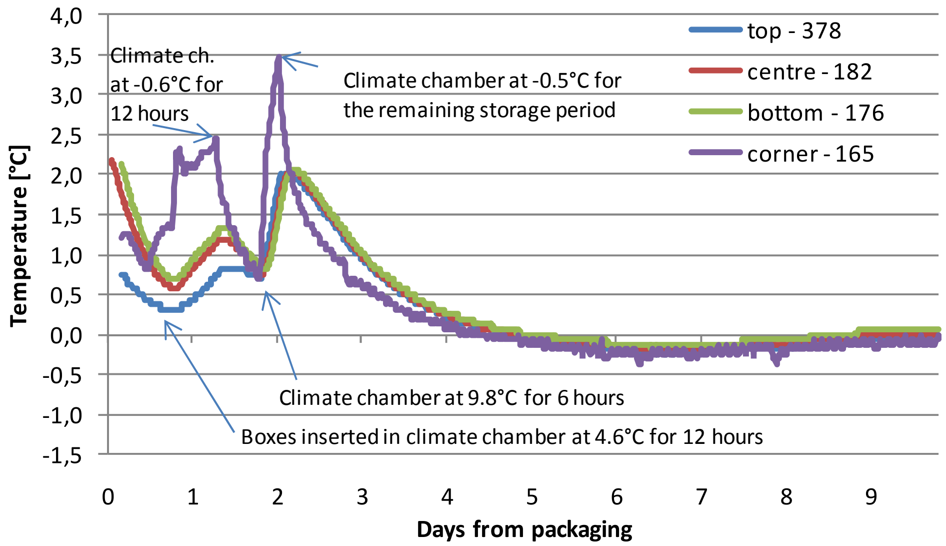

Figure 7. Group C (LC-RTS-GP) – liquid cooled and then packed with a gel pack.

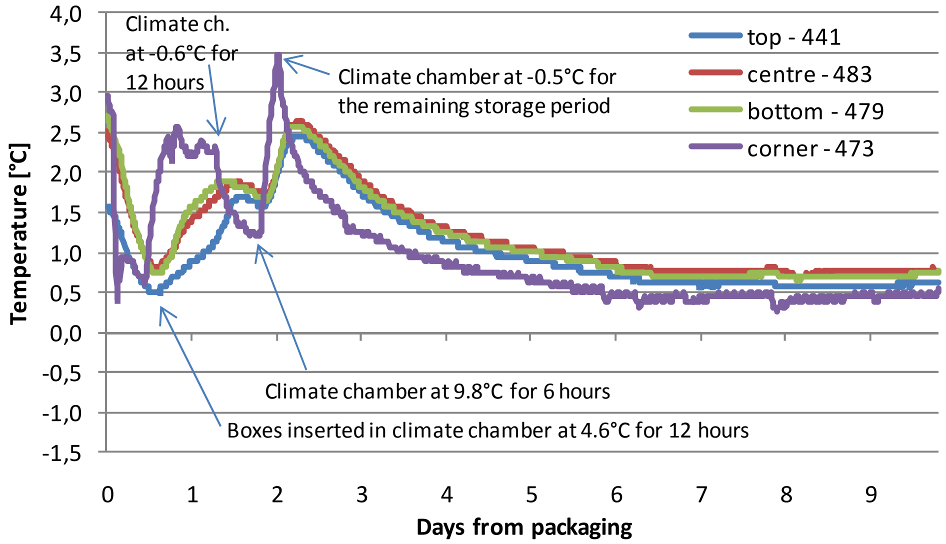

Figure 8. Group D (NC-RTS-GP) – No cooling during processing and packed with a gel pack.

The average product temperatures are shown in Table 5. It can be seen that the group cooled with liquid cooling before packaging (C) maintained a lower temperature during the storage period than the group which received no cooling (D). The average product temperature of the group treated by liquid cooling was 0.4±0.6 °C compared to 1.1±0.5 °C for the untreated group. The average product temperature of the groups which had received CBC cooling was even lower, -0.1±0.3 °C with a gel pack and 0.2±0.5 °C without gel pack.

It is also evident from Figures 5-8 that not only is the initial temperature of the products which received CBC cooling lower, but they are also more resistant and slower to respond to ambient temperature fluctuations.

The gel pack seems to have a slight effect on the average product temperature during storage as slightly lower temperature was measured in the CBC cooled group with a gel pack than without. The gel pack might have had a larger effect in groups C and D as the product temperature at packaging is higher than the groups treated with CBC cooling.

The difference in initial product temperature is evident between the groups which received CBC cooling and those which received only liquid cooling or no cooling. The temperature at packaging in groups C and D was approximately 2-3 °C. The temperature in the top layer was also lower both at packaging and during storage than at other locations, probably due to the gel pack located on top of the product inside the boxes.

Interestingly the temperature in all the groups above reached a minimum after approximately 6-7 d and then began to rise, even though the ambient temperature was lower than the product temperature. This is probably due to exothermic reactions during the spoilage process.

Table 5. Average product temperature and standard deviation during storage

	group	mean (°C)	stdev (°C)
A	CBC-RTS-GP	-0.1	0.3
B	CBC-RTS	0.2	0.5
C	LC-RTS-GP	0.4	0.6
D	NC-RTS-GP	1.1	0.5
E	CBC-S-GP	-0.3	0.3
F	CBC-S	-0.2	0.2

Steady temperature conditions

Figures 9-10 show the product temperature of the groups which were kept at a steady ambient temperature of -1.2±0.2 °C during the entire storage period at Matís facilities.

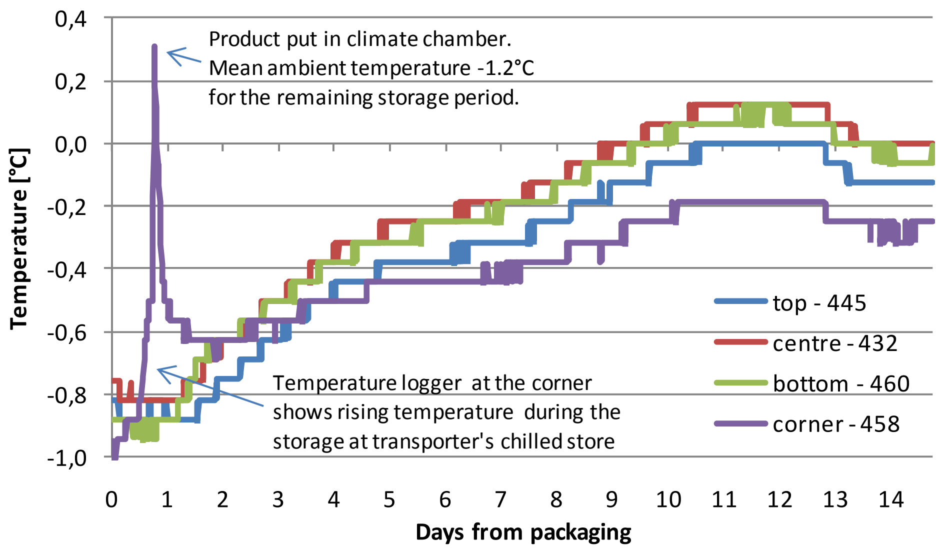

Figure 9. Group E (CBC-S-GP) - CBC cooled and packed with a gel pack.

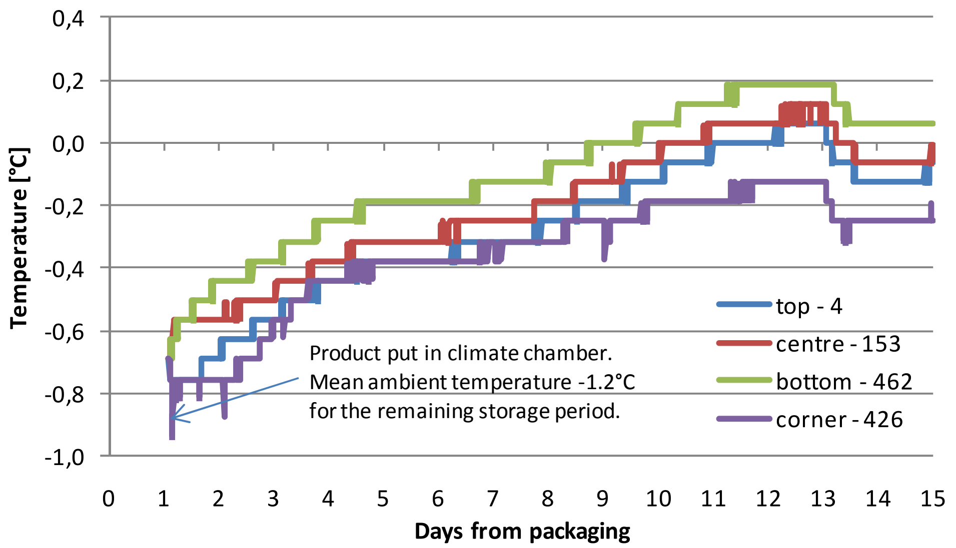

Figure 10. Group F (CBC-S) – CBC cooled and packed without gel pack.

The same phenomenon can be seen in the groups kept at a steady temperature as was seen after day 7 in the RTS groups, i.e. rising of the product temperature despite a low and steady surrounding temperature which again could be explained by exothermic reactions during the spoilage process.

It can also be seen from Figures 9-10 that the location which has the lowest temperature is the bottom corner location, since it is the most vulnerable to the surrounding temperature. The temperature at the top of the products is also lower than at the centre and the bottom of the box. This is probably due to the gel pack located on top of the product.

Comparison of temperature profiles among groups

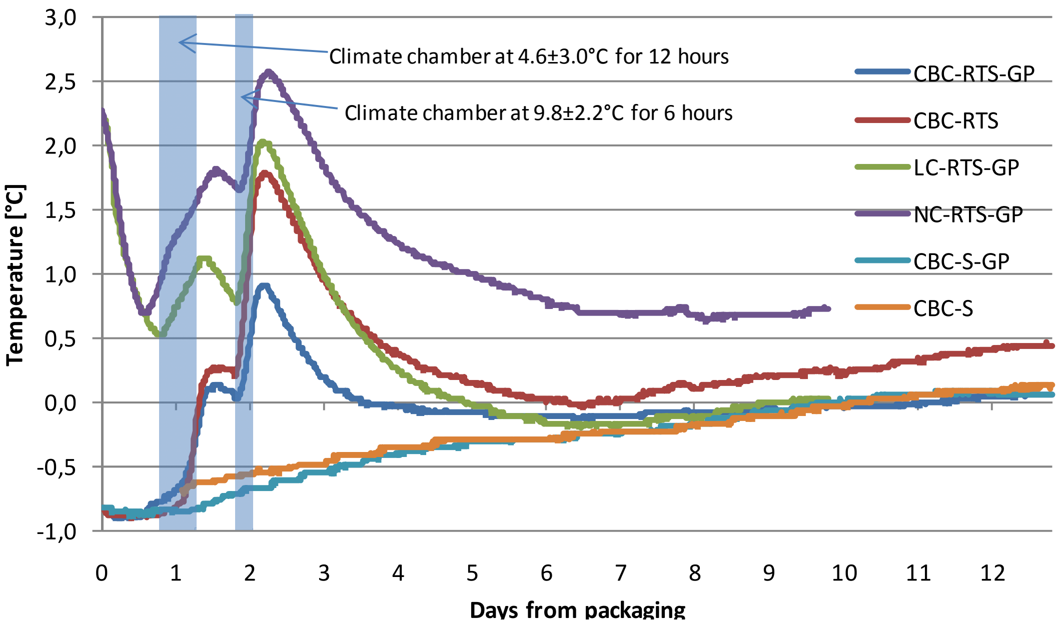

Figure 11. Average product temperature history of all the groups during transportation and storage at Matís facilities.

Figure 11 shows the product temperature history of the groups which were subjected to real temperature simulation (RTS) as well as those which were kept at a steady, subzero temperature (-1.2±0.2 °C) during the storage at Matís facilities.

The influence of CBC cooling can clearly be seen on Figure 11 as the group CBC-RTS-GP shows significantly lower temperature profile during the temperature load applied for the first two days of the experiment.

The gel pack also seems to have some effect in dampening the temperature response of the products when the temperature load is applied as can be seen from comparing groups CBC-RTS-GP and CBC-RTS. By comparing the same groups it is also evident that lower temperature can be maintained during the entire storage period with a gel pack, as at the end of the storage period CBC-RTS is at 0.44 °C while CBC-RTS-GP is at 0.06 °C.

Liquid cooling also influenced the temperature development of the product during the storage period as a lower temperature was maintained during the entire storage period with liquid cooling than no cooling, even though the temperature at packaging was similar to that where no cooling was applied.

3.2 Sensory evaluation

Figure 12 shows show how the samples were characterized by the sensory attributes. Altogether 95% of the sensory variation was explained in the first two principal components. The main variation between the samples was due to differences explained by storage time. Sensory attributes characteristic for cod at the beginning of storage, such as sweet and metallic flavour, sweet and shellfish odours are located to the right in the upper part of Figure 1b describing samples after 1-2 days of storage (Figure 12a). As storage time progressed, these sensory attributes become less evident but the vanilla odour and juicy texture become more characteristic, and then potato odour and white precipitation (lower part of Figure 12b). The sensory attributes characteristic for cod at the end of storage, such as TMA and sour flavours and odours, located to the left in the upper region are used to describe the samples at the end of the storage period. The sample group NC-RTS-GP appeared to retain the freshness characteristics longer than the other sample groups as after six days of storage, it was comparable to other groups after three days of storage (Figure 12a). LC-RTS-GP however, lost the freshness characteristics faster and was more described be spoilage attributes after eight days of storage than other groups (results from LC-RTS-GP after 10 days were removed from multivariate data analysis due to extreme values of spoilage related attributes).

Tables A-D in appendix show in more detail how the sample groups were characterized by sensory attributes.

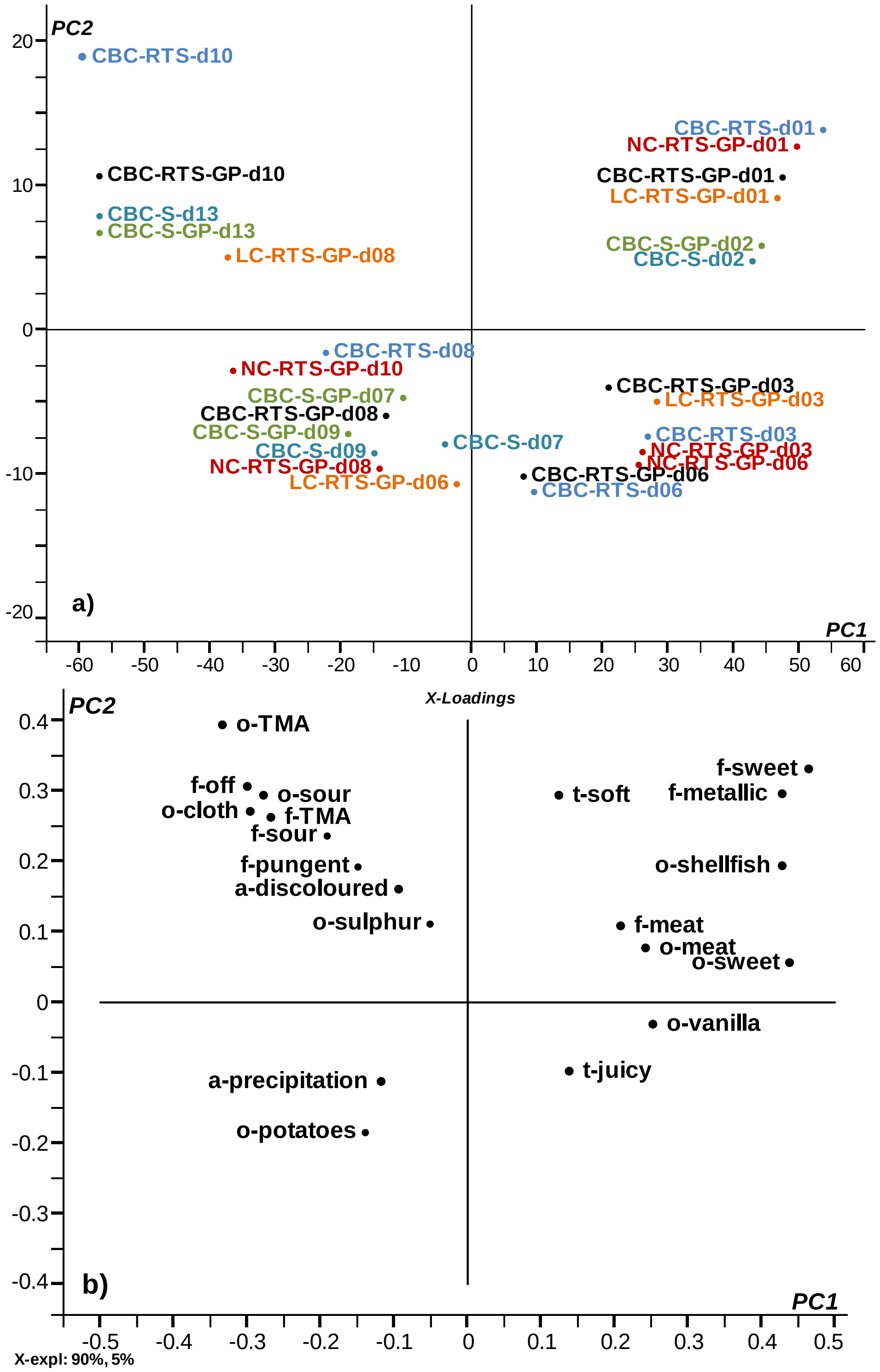

Figure 12. PCA describing sensory quality, odour (o-), appearance(a-), flavour (f-) and texture (t-) of the sample groups with storage time (d). PC1 VS PC2 (X-expl.: 90% and 5%). a) scores, b) X-loadings. (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

Figure 13 shows how the Torry freshness score changes with storage time. A Torry score around 7 indicates the fish has lost most of its freshness odour and flavour characteristics, and has a rather neutral odour and flavour (Shewan et al. 1953). These limits were obtained after six days for LC-RTS-GP, after eight days for CBC-S, but after around seven days for other sample groups. When the average Torry score is around 5.5 most of the sensory panellists detect spoilage attributes, and these limits have been used as the limits for consumption at Matis (see e.g. Olafsdottir et al. 2006). According to this, the maximum shelf life of LC-RTS-GP was 8-9 days, CBC-RTS-GP and CBC-RTS was 9-10 days, but CBC-S-GP and CBC-S was 12 days. NC-RTS-GP was approaching these limits on day 10.

Figure 14 shows how the sweet flavour changed with storage time. When the score for this attribute is around 25-30, the fish has lost most of its characteristic sweet flavour. LC-RTS-GP has reached these limits after around seven days, after nine days for CBC-S, and after around 7-9 days for other groups, which was approximately one day longer than what was observed from the Torry score.

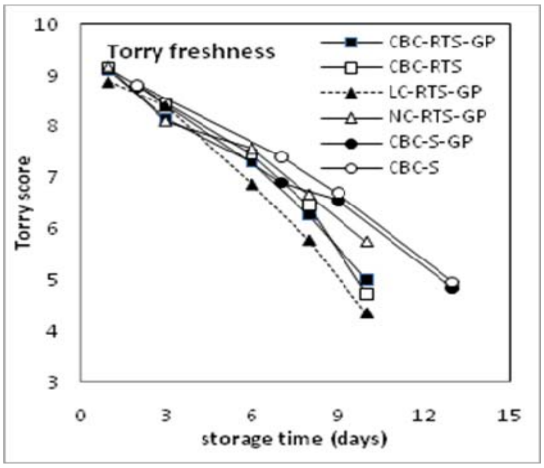

Figure 13. Average Torry freshness scores (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

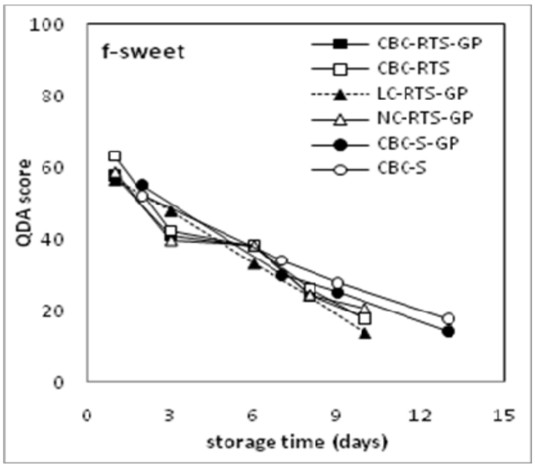

Figure 14. Average QDA scores of sweet flavour (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

Figures 15-20 show how odour and flavour attributes related to spoilage change with storage time. End of shelf life is usually determined when sensory attributes related to spoilage become evident. When the average QDA score for those attributes is above the value 20 (on the scale 0 to 100) most panellists detect them (Bonilla et al. 2005; Magnússon et al. 2006). According to this criterion, LC-RTS-GP had a maximum shelf life of 8-9 days, CBC-RTS nine days, CBC-RTS-GP 10 days, CBC-S-GP and CBC-S 12-13 days, but NC-RTS-GP was approaching end of shelf life after 10 days. These results are in agreement with the results from the Torry scores.

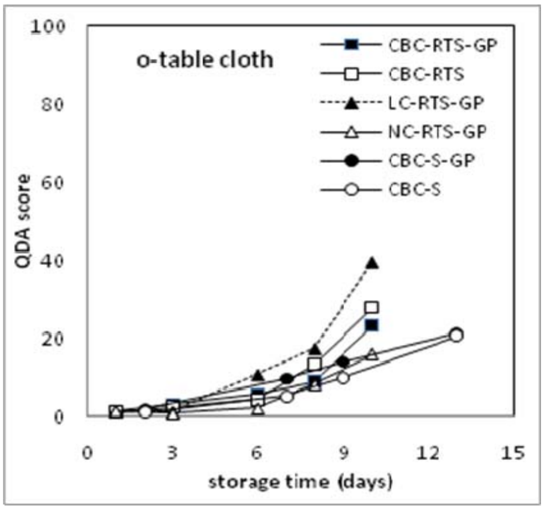

Figure 15. Average QDA scores of table cloth odour (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

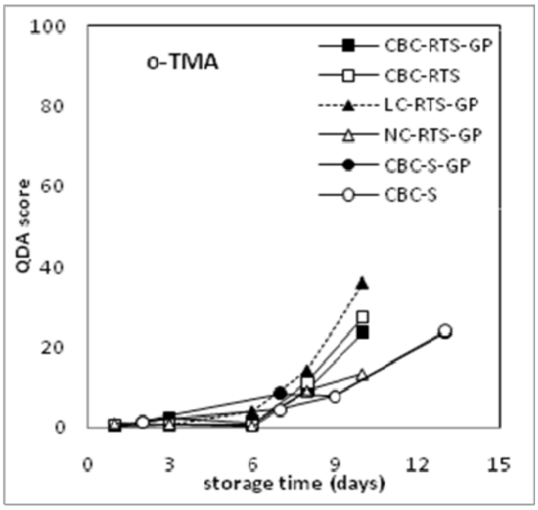

Figure 16. Average QDA scores of TMA odour (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

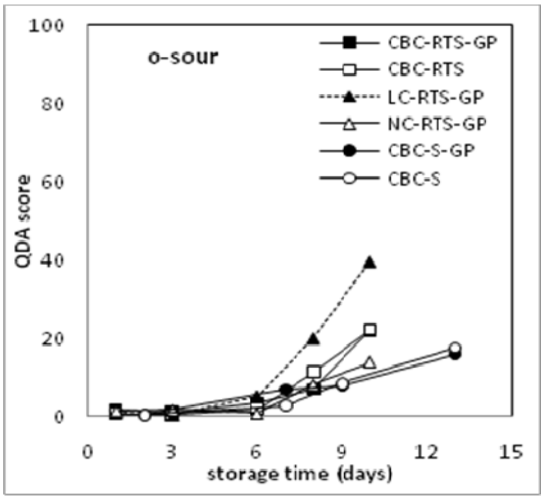

Figure 17. Average QDA scores of sour odour (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

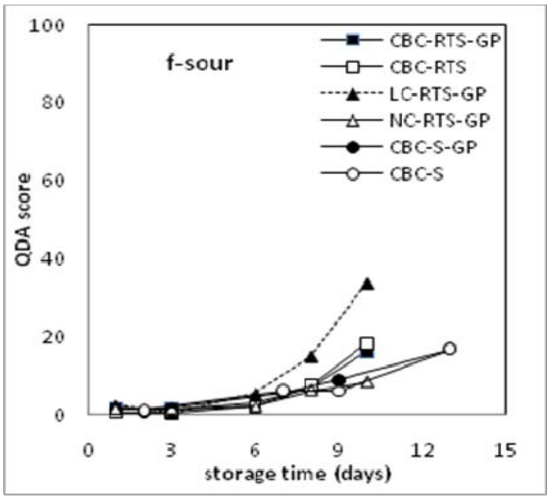

Figure 18. Average QDA scores of sour flavor (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

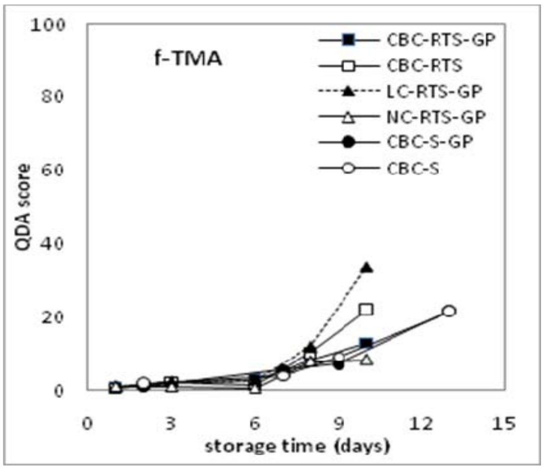

Figure 19. Average QDA scores of TMA flavor (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

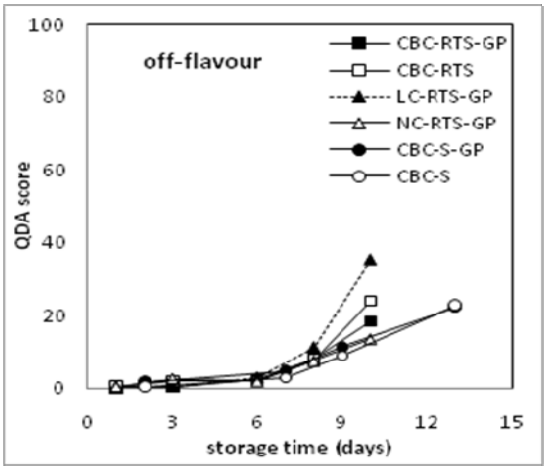

Figure 20. Average QDA scores of off- flavor (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

A comparison of the freshness period (the end of this period is when the fish has lost the freshness characteristics and reached the neutral phase) and the maximum shelf life (the end of this period is when odour and flavour attributes related to spoilage have become evident) is shown in Table 6.

Table 6. Freshness period (days) and maximum shelf life (days) according to sensory evaluation (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

Group	Freshness period (days)	Shelf life (days)
CBC-RTS-GP	7-9	9-10
CBC-RTS	7-9	9-10
LC-RTS-GP	6-7	8-9
NC-RTS-GP	7-9	10+
CBC-S-GP	7-9	12-13
CBC-S	8-9	12-13

The different treatments of the groups did not influence the sensory characteristics of the samples in other ways than resulting in different lengths of freshness period and maximum shelf life. The estimation of these periods was based on freshness and spoilage related odour and flavour attributes. Thus, immersing the fillets in brine resulted in a two day shorter freshness period and maximum shelf life as compared to untreated loins (LC-RTS-GP compared to NC-RTS-GP), probably due to brine contaminated by spoilage bacteria. CBC cooling resulted in a two day longer freshness period and a one day longer shelf life as compared to loins receiving the same treatment except CBC cooling (CBC-RTS-GP compared to LC-RTS-GP). However, due to the brine immersion, CBC cooling did not result in a prolonged shelf life as compared to loins not receiving this treatment. Further, the results indicated that the shelf life of untreated loins was longer than of the CBC groups. The use of gel pack was insignificant as it did not result in prolonged freshness period or shelf life (CBC-RTS-GP vs. CBC-RTS and CBC-S-GP vs. CBC-S). Storage at -1 °C (CBC-S-GP and CBC-S) resulted in prolonged shelf life of ca. 3 days compared to storage under the simulated air freight temperature conditions. Only a tendency of prolonged freshness period was observed.

3.3 Microbial measurements

Results from microbial counts are shown in Figures 21 – 25. The cod was processed 2 days from catch. Microbial counts of the control group were low (Figure 21). These samples were taken of newly filleted cod fillets on the day of processing before any further treatment (d0). Treatment with liquid cooling and CBC led to increase in microbial counts compared to the control group. The brine was quite contaminated with bacteria, especially *Photobacterium phosphoreum*. However, numbers of bacteria did not increase in the brine during the working day apart from slight increase in counts of *P. phosphoreum.*

Microbial counts at the beginning of storage were in all cases lower in the group where no cooling was applied (NC-RTS-GP) compared to the group were liquid cooling was used (LC-RTS-GP). Not much difference was noticed thereafter regarding total viable counts (TVC), H_2S-producing bacteria and *P. phosphoreum*. Counts of pseudomonads were lowest up to day 6 of storage in the groups with no cooling and liquid cooling. During that time counts were higher in the liquid cooling group than in the no cooling one. These results indicate strongly that the fillets got contaminated from the brine used for liquid cooling which contained high microbial loads (Figure 21).

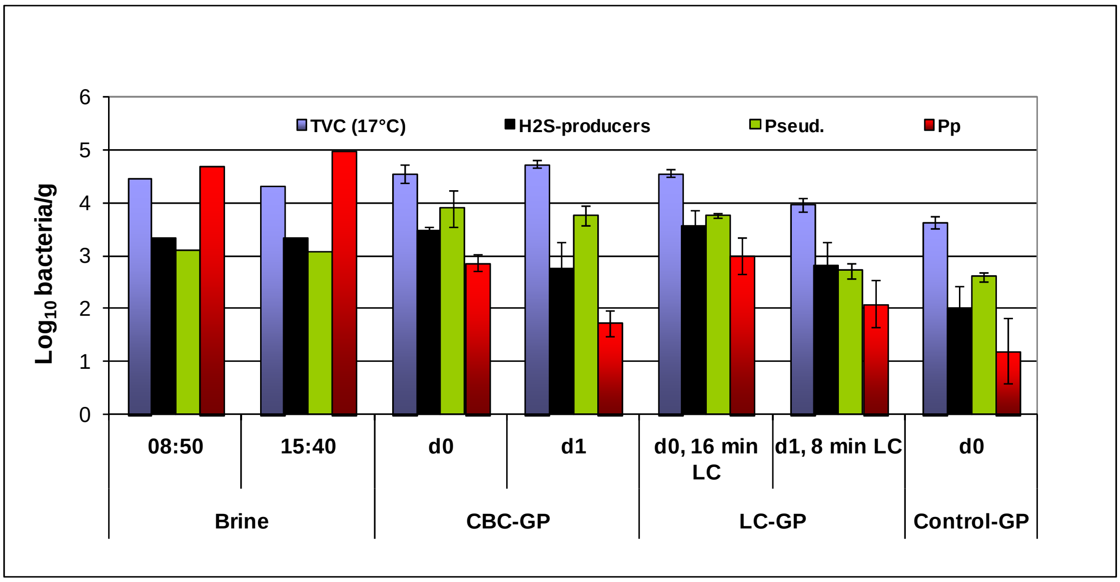

Figure 21. Microbial counts of cod loins and brine. (CBC: Combined blast and contact cooling, LC: Liquid cooling, GP: Gel pack).

Microbial counts were usually somewhat lower in the CBC-RTS groups where a gel pack was used compared to no gel pack. This was especially noticable for numbers of H_2S-producing bacteria and *P. phosphoreum* during part of the storage. In the CBC-S groups however, no marked difference was seen whether a gel pack was used or not.

Lower microbial counts were generally obtained in the CBC-S group than in the the CBC-RTS group. This was especially noticable for counts of *P. phosphoreum*. Lower storage temperature was applied in the former group, with initial temperature being close to -1 °C (see Figure 11).

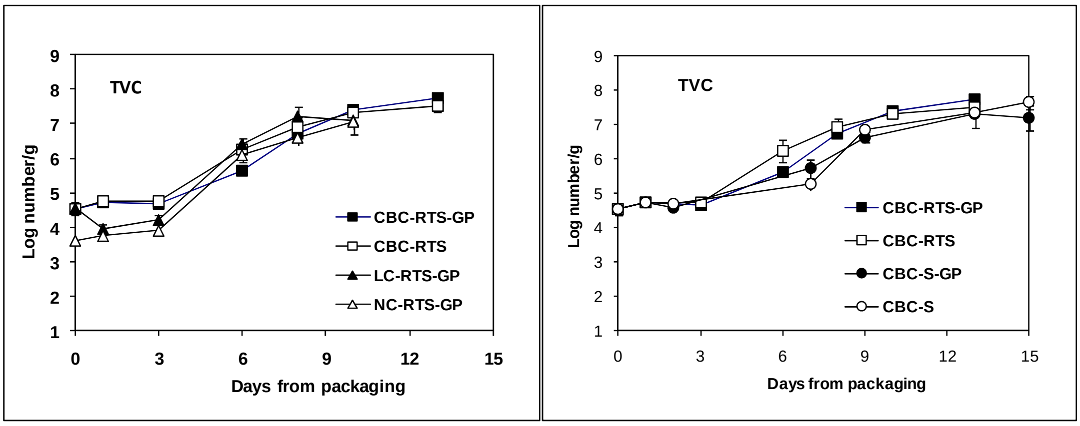

Figure 22. Total viable counts (TVC) in cod loins. Average values of triplicate samples are shown. Error bars show SD. (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

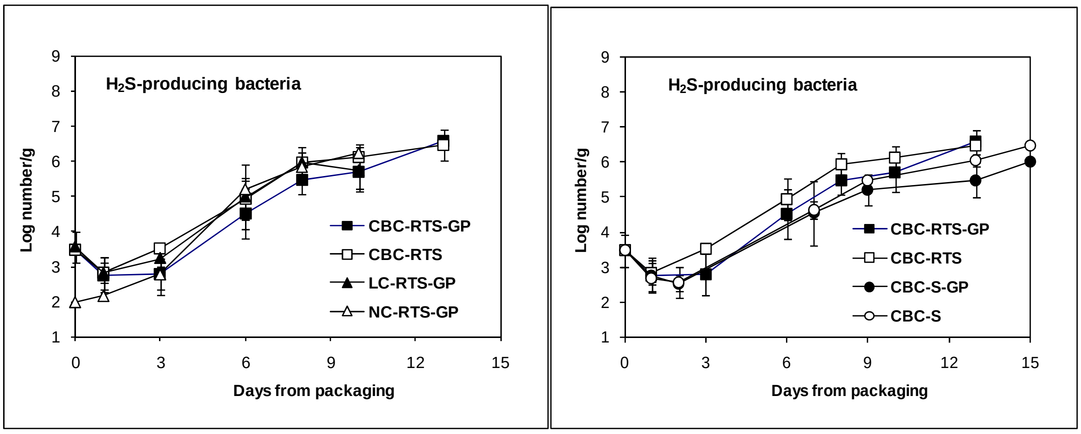

Figure 23. Growth of H_2S-producing bacteria in cod loins. Average values of triplicate samples are shown. Error bars show SD. (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

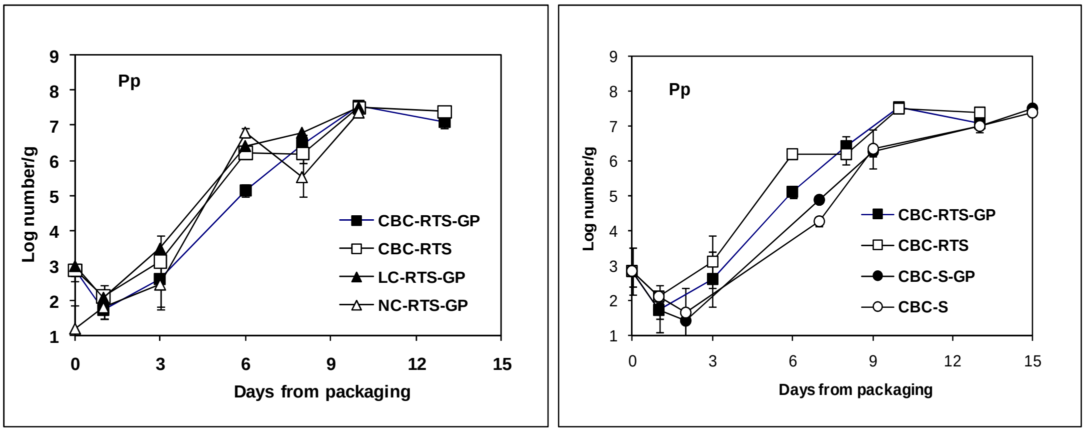

Figure 24. Growth of *Photobacterium phosphoreum* in cod loins. Average values of triplicate samples are shown. Error bars show SD. (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

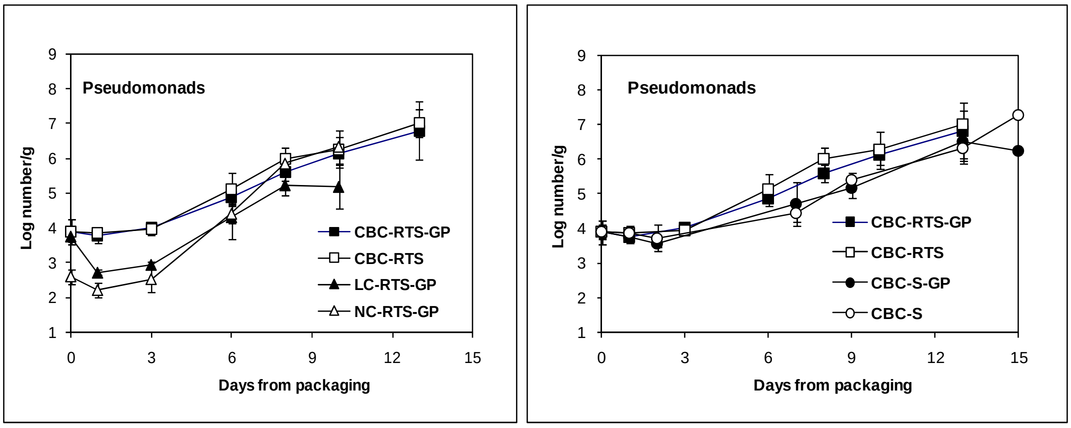

Figure 25. Growth of presumptive pseudomonads in cod loins. Average values of triplicate samples are shown. Error bars show SD. (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

3.4 Chemical measurements

Total Volatile Base Nitrogen (TVB-N) and Trimethylamine (TMA)

Results from TVB-N and TMA measurements are shown in Figures 26-27. The results agree well with the results from microbial counts. Lowest TVB-N and TMA values were obtained in the group NC-RTS-GP where no cooling was applied. Considerable higher values were found in the group LC-RTS-GP. Use of gel packs led to slower formation of TVB-N and TMA as can be seen by comparing groups CBC-RTS-GP and CBC-RTS.

Lower values were obtained in the CBC-S group than in the the CBC-RTS group. Lower storage temperature was applied in the former group, with initial temperature being close to -1 °C (see Figure 11).

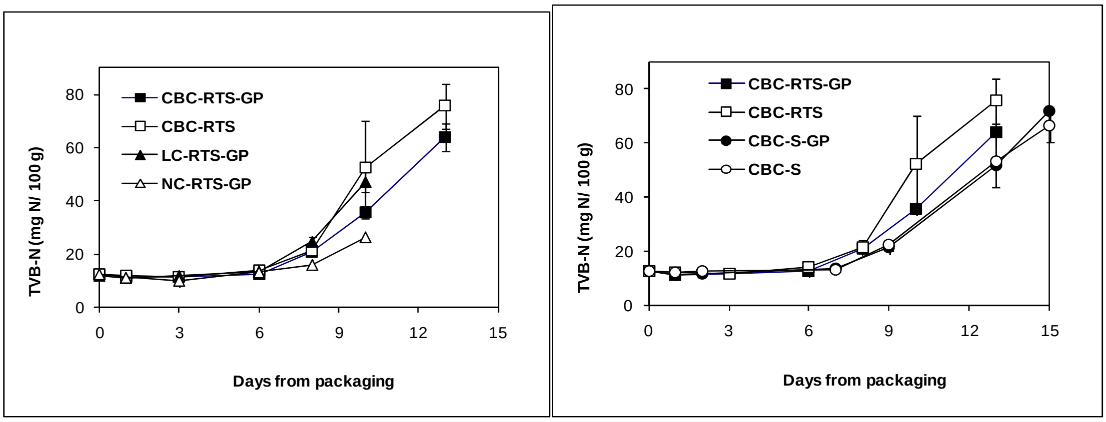

Figure 26. Total Volatile Base Nitrogen (TVB-N) in cod loins. Average values of triplicate samples are shown. Error bars show SD. (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

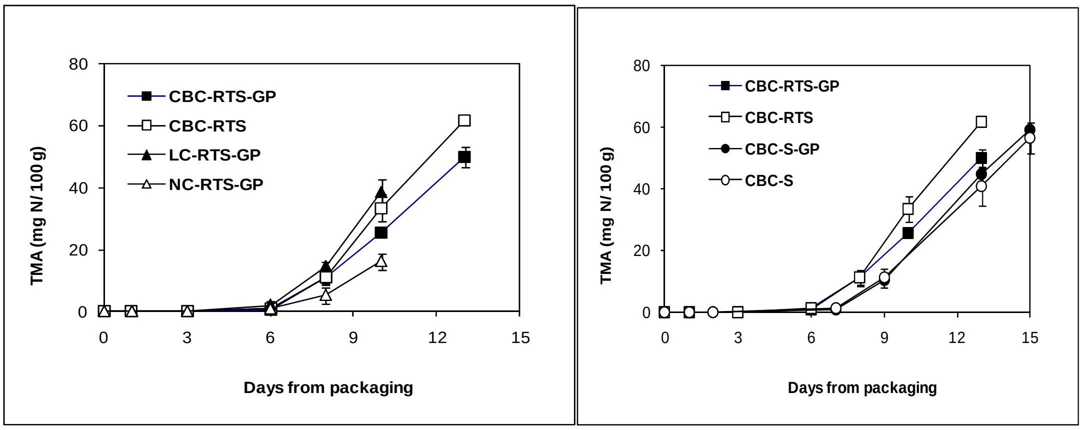

Figure 27. Trimethylamine (TMA) in cod loins. Average values of triplicate samples are shown. Error bars show SD. (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

pH – measurements

In general, the pH increased with storage time (Figure 28). Effects of cooling methods and storage conditions were not significant, except on day 13. Then the pH was higher in CBC-cooled loins that were stored at temperature fluctuations without gel pack.

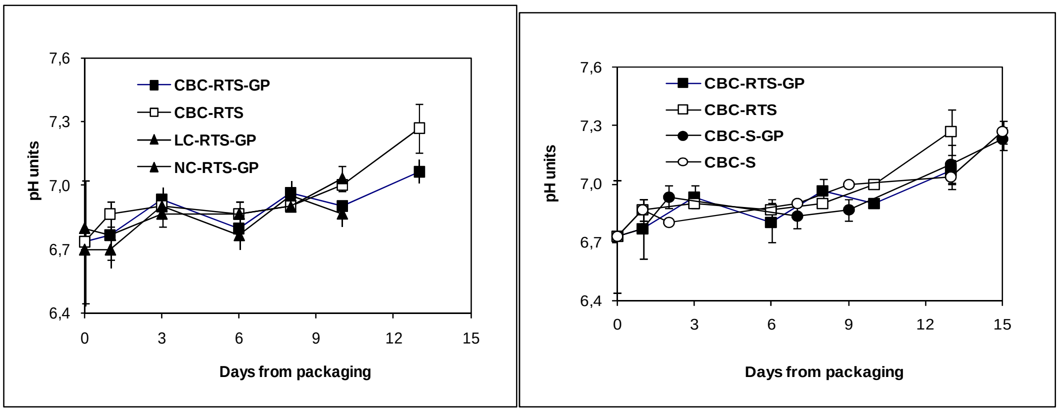

Figure 28. Acidity (pH) in cod loins. Average values of triplicate samples are shown. Error bars show SD. (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1°C).

Salt and water content

The salt content of the loins was slightly increased by a immersion in the liquid which contained 2.2% NaCl. It was in the range of 0.3% to 0.4% in the immersed loins whereas it was 0.2% in the reference group (NC-RTS-GP) (Figure 29). The water content was similar in all groups over the storage period or in the range of 81% to 82% (Figure 30).

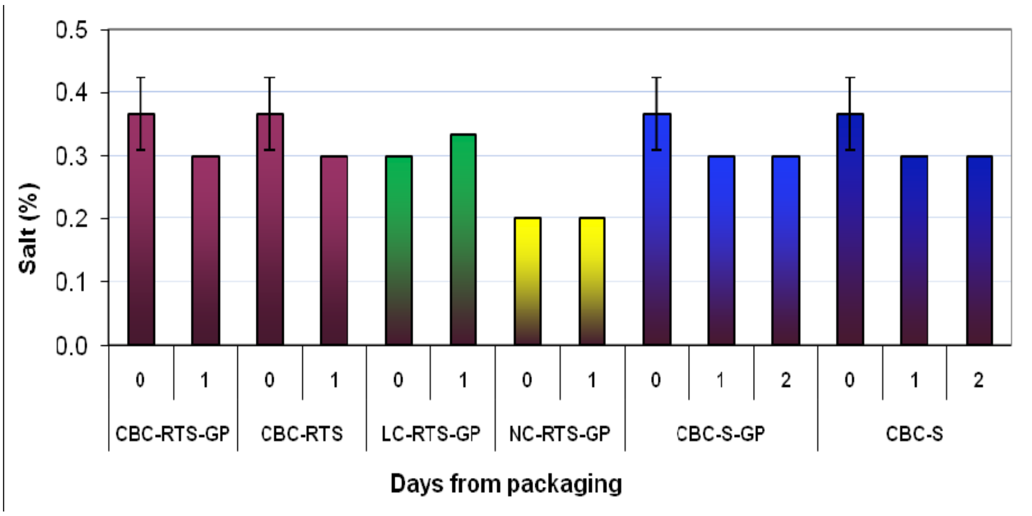

Figure 29. Salt content of cod loins that were immersed in liquid containing salt during processing. Reference loins were packed without prior cooling. (C: No cooling, LC: Liquid cooling, CBC: Combined blast and contact cooling, GP: Gel pack, RTS: Real temperature simulation, S: Steady temperature).

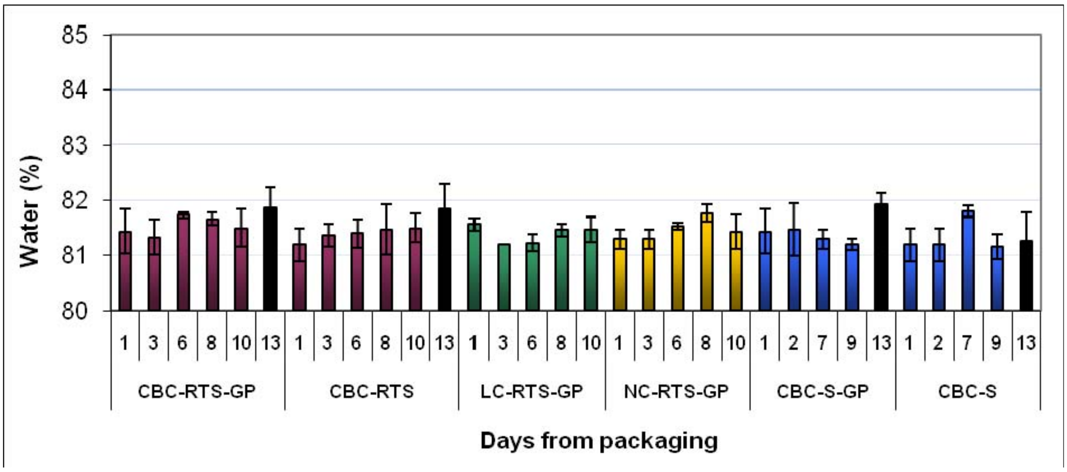

Figure 30. Changes in water content of cod loins that treated by different cooling and storage conditions. Reference loins were packed without prior cooling. (C: No cooling, LC: Liquid cooling, CBC: Combined blast and contact cooling, GP: Gel pack, RTS: Real temperature simulation, S: Steady temperature).

3.5 Drip and water holding capacity (WHC)

The drip of the cod loins increased with storage time in all groups from approximately 1% to 3-4%. However, the drip in loins that were only immersed before packaging (LC-GP-RTS) was nearly 3% right at day 1 and increased to approximately 4% during the storage period (Figure 31).

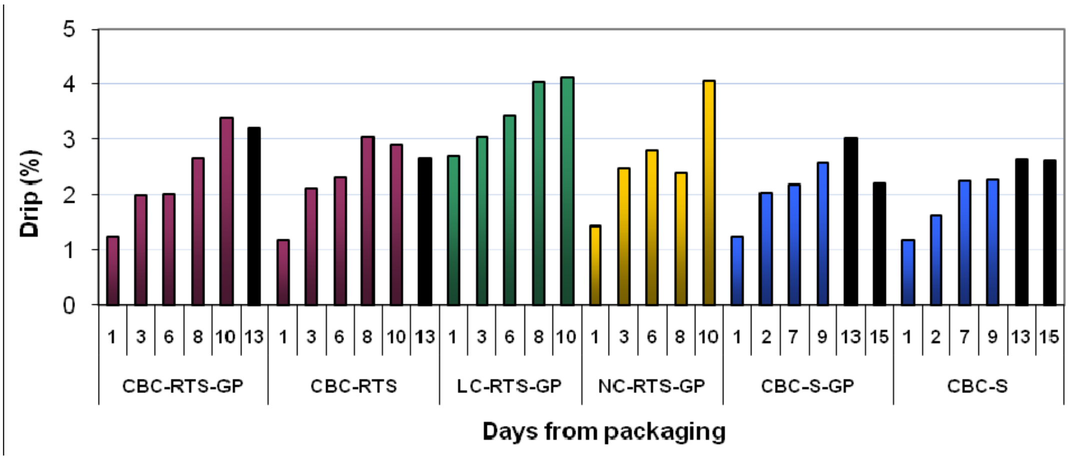

Figure 31. Changes in drip of cod loins that treated by different cooling and storage conditions. Reference loins were packed without prior cooling. (C: No cooling, LC: Liquid cooling, CBC: Combined blast and contact cooling, GP: Gel pack, RTS: Real temperature simulation, S: Steady temperature). Black columns indicate the drip may be underestimated as the loins were apparently slightly frozen during sampling.

The WHC (Figure 32) tended to decrease during storage as degradation of the muscle increased. At the end of the storage period slight rises in the WHC were observed. These changes were believed to result from the increased drip, i.e. as more of the loosely bound water leaked out of the muscle, the proportion of water that was tightly bound grew.

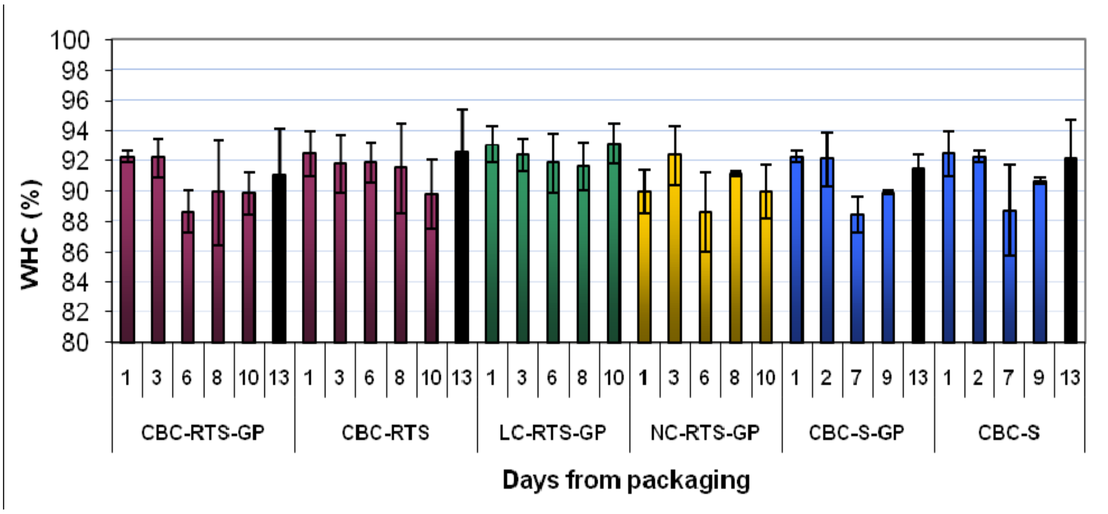

Figure 32. Changes in WHC of cod loins that treated by different cooling and storage conditions. Reference loins were packed without prior cooling. (C: No cooling, LC: Liquid cooling, CBC: Combined blast and contact cooling, GP: Gel pack, RTS: Real temperature simulation, S: Steady temperature). Black columns indicate the drip may be underestimated as the loins were apparently slightly frozen during sampling.

4 RESULTS AND DISCUSSION: EXPERIMENT II

4.1 Temperature control during air and sea transport from Iceland to Germany

Temperature mapping

During processing, fish fillets were chilled in a CBC equipment down to -0.9 to -0.5 °C. Temperature was mapped in eight EPS boxes and four of them contained a cooling gel pack on top of the loins. Half of the boxes were exported by air freight and the other half by sea. Fluctuations in temperature during transportation were analyzed, evaluating which parts of the transportation process are critical for the quality loss of the product. The product and ambient temperatures were monitored, resulting in the temperature mapping of two pallets, one transferred by sea and the other with air freight.

Four Onset TidBit logger temperature loggers were placed on the air freight-pallet surface, top centre, top corner, bottom corner and between boxes (approx height of 0.5 m). Four DS1922L ibutton temperature data loggers were placed inside each of the eight EPS boxes (Figure 33). In addition, two ibutton temperature data loggers were placed on the outer surface of the EPS boxes.

Figure 33. DS1922L ibutton logger at the bottom centre of an EPS box.

The ibutton temperature data loggers placed inside the EPS boxes were located at the following locations: bottom corner, bottom centre, mid centre and upper centre (below the top loin). The three loggers on outer surface were located as Figure 34 shows. The EPS boxes are designed for 5 kg of fresh fish products.

Figure 34. EPS boxes, Onset Tidbit and DS1922L ibutton temperature data loggers on outer surface.

Transport by air

Results of the temperature mapping from packaging in Iceland to arrival in Bremerhaven by air freight transportation are shown in Figures 35 - 38.

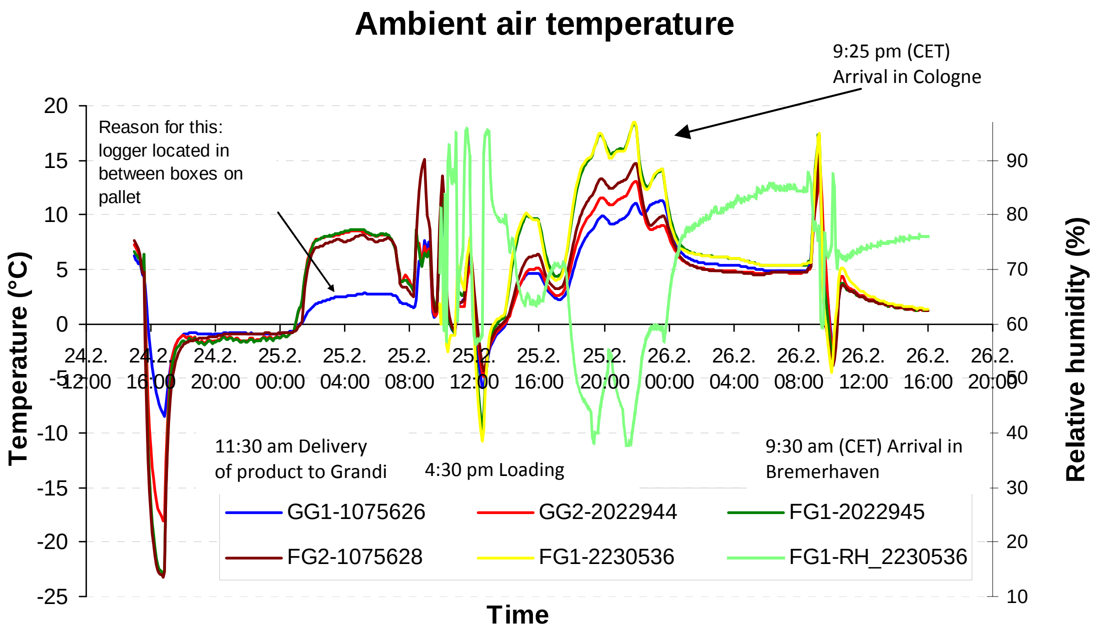

Figure 35. Ambient air temperature on pallet. Four Onset UTBI-001 temperature loggers and one HOBO U12 humidity and temperature logger were placed on pallet surface, top centre, top corner, bottom corner and between boxes. FG1-2230536 refers to the temperature measured by the HOBO logger and FG1-RH_2230536 refers to the relative humidity (RH) measured by the same logger.

Figure 35 reveals hazardous parts of the chain during air freight transport. The ambient temperature was below 0 °C on the route Dalvík - Reykjavík, but when arriving in Reykjavík and until arrival in Bremerhaven ambient air temperature was most of the time above 5 °C. The largest fluctuation and highest temperature was reached during the flight between Reykjavík and Cologne.

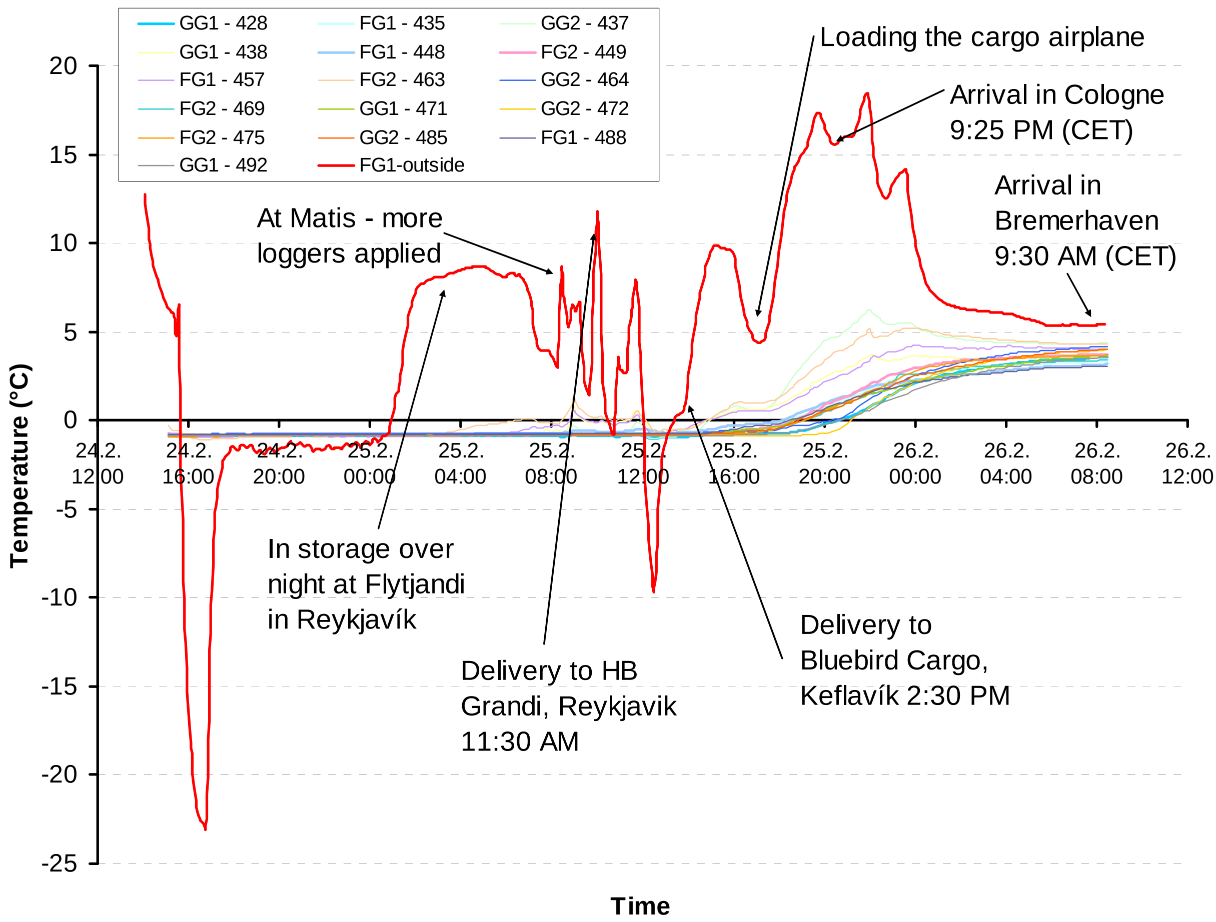

Figure 36. Temperature inside and on the outside surface of EPS boxes during land and air transport. Four DS1922L ibutton temperature data loggers were placed inside each EPS box, with a total of sixteen loggers inside four EPS boxes. Two DS1922L ibutton temperature data loggers were placed on outer surface of the EPS boxes. The average ambient air temperature is calculated from eight ambient loggers.

Figure 36 shows the temperature both inside and outside the boxes during air freight transportation. Rise in product temperature was in agreement with high ambient air temperature. As loading of the airplane began, product temperature started to rise and kept on rising until landing in Cologne.

Transport by sea

Figure 37 shows the temperature inside the boxes during ship transportation, demonstrating the rather steady conditions. Ambient air temperature was below 0 °C most of the time but as ambient air temperature increased, product temperature also raised. A temperature increase was first noticed upon arrival in Esbjerg.

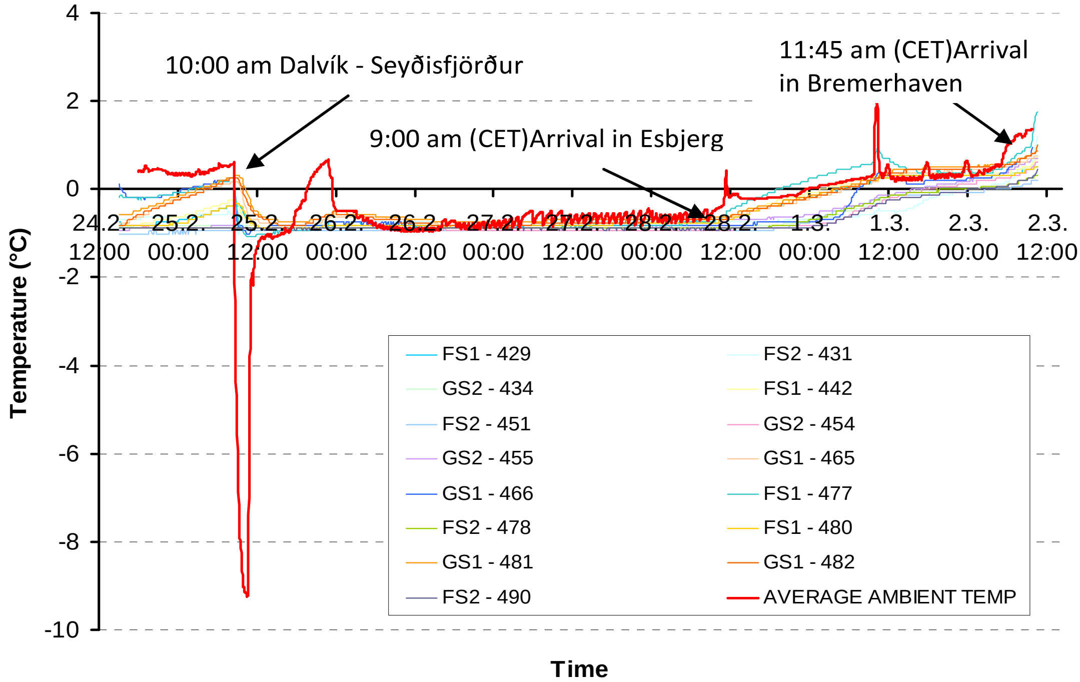

Figure 37. Temperature inside and on the outside surface of EPS boxes during land and sea transport. Four DS1922L ibutton temperature data loggers were placed into each EPS box, with a total of sixteen loggers inside four EPS boxes. Two DS1922L ibutton temperature data loggers were placed on outer surface of the EPS boxes. The average ambient air temperature is calculated from eight ambient loggers.

Figure 38 shows how temperature evolved throughout the two shipments. As can be seen, temperature increased earlier for the air freight. Consequently, the product temperature started to fluctuate after about 42% of total transportation time and reached above zero degrees after about 64% of the time. For the sea transport chain, product temperature started rising after about 66% of total transportation time and reached 0 °C after 81% of the journey time. At the final destination, average product temperature was 3 degrees lower when transported by sea than by air freight.

The results show that hardly any difference was found on temperature between boxes with a gel pack (groups FG and FS) on one hand and without a gel pack (groups GG and GS) on the other hand.

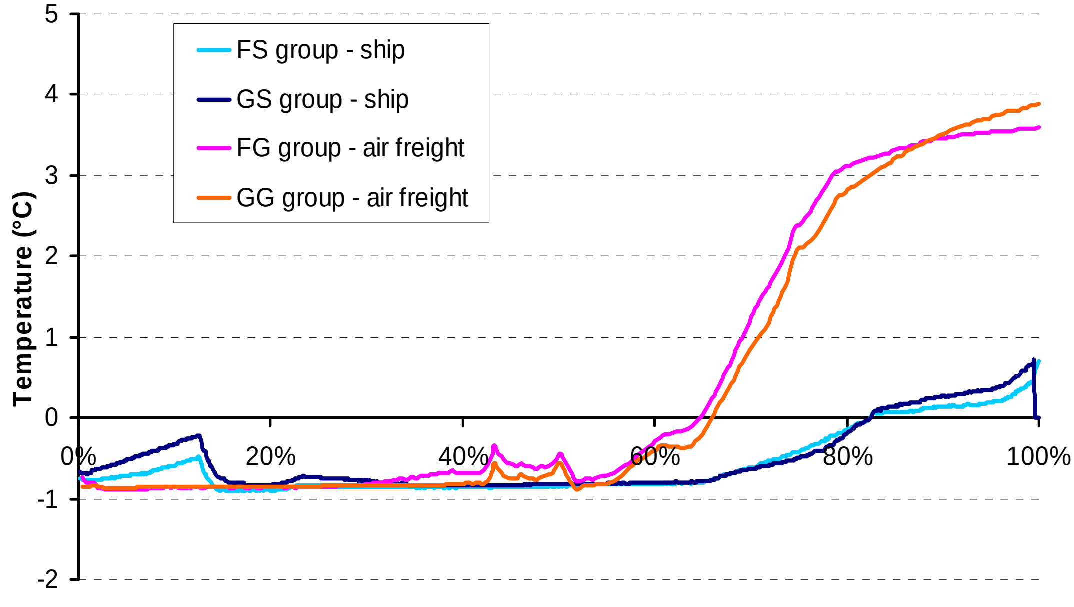

Figure 38. Average temperature inside EPS boxes with or without gel pack. Two groups had a gel pack on top of the fillets (FS and FG) and two had no gel pack (GS and GG). Total of four loggers were inside each box.

The main results from this experiment show that temperature fluctuations were larger and more frequent for product transported by air then by sea. Ambient air temperature fluctuations led to increased product temperature, even exceeding 4 °C in air freight shipped cod loins upon arrival in Bremerhaven.

By comparing the product temperature (at top, centre and bottom inside boxes) between the two transportation methods, it can be seen that it was above 0 °C for 35% of the transport time for air freight and reached 4 °C. During transportation in the refrigerated sea container the product temperature was above 0 °C for 18% of the transport time and never exceeded 1 °C.

At arrival at TTZ in Bremerhaven, the temperature of fish transported by air freight was relatively high (Table 7). For some fillets, the temperatures exceeded 4 °C (rejection point for most fish retailers). Gel packs did not have significant cooling effect in this experiment. Two possible explanations for that are firstly the small size of the gel packs (150 g) and secondly that the fish fillets were well pre-chilled before packaging (-0.9 to -

0.5 °C). The temperature of sea transported fish was significantly lower at arrival at TTZ (Table 8). Surface temperature in boxes with a gel pack was slightly lower than that in boxes without a gel pack.

Table 7. Temperatures (°C) at arrival (26th Feb 2009, 9:30 CET, air freight). P1 to P3 represent three different measuring positions in each box.

Box		**P1**	**P2**	**P3**	**average**
FG 1 (with gel pack)	Surface	4.2	2.3	3.5	**3.3**
	Core	3.9	2.5	3.5	**3.3**
GG 1 (without gel pack)	Surface	4.1	4.2	2.5	**3.6**
	Core	3.3	4.0	3.0	**3.4**

Table 8. Temperatures (°C) at arrival (2nd March 2009, 11:45 CET, sea freight). P1 to P3 represent three different measuring positions in each box.

Box		**P1**	**P2**	**P3**	**Average**
FS 1 (with gel pack)	Surface	1.2	0.9	0.6	**0.9**
	Core	0.9	0.6	0.5	**0.7**
FS 2 (with gel pack)	Surface	2.0	1.6	1.5	**1.7**
	Core	1.1	0.7	0.6	**0.8**
GS 1 (without gel pack)	Surface	2.9	2.7	2.7	**2.8**
	Core	0.9	0.7	0.6	**0.7**
GS9 (without gel pack)	Surface	2.2	1.9	1.6	**1.9**
	Core	1.4	1.4	1.1	**1.3**

The above-mentioned results reveal that temperature control is insufficient in certain parts of the transportation process by air freight between Iceland and Germany. Most critical is the time after delivering products to the airline, during flight and after landing. Temperature abuse was not experienced in the containerized sea transport, but the transport time was significantly longer (around four days) than for air freight.

4.2 Microbial and chemical analysis

Microbial growth in cod loins transported by air freight and retail-packed under air or MA 2 days post process is shown in Figure 39. MA-packaging generally delayed the product microbiota, especially pseudomonads and H_2S-producing bacteria. *Photobacterium phosphoreum* (Pp) growth, evaluated by a quantitative PCR method in few samples obtained from Bremerhaven, showed a similar behaviour between air- and MA-packed products, reaching log 7/g about 11 days after retail-packaging.

Pseudomonad counts in air-stored products were similarly high on day 11, while H_2S-producing bacteria had reached that level 3 days later. Based on the microbiological rejection criterion of log 7/g for spoilage bacteria, the shelf life of these products was probably reached on or close to day 11. Generally little influence resulted from the initial use of the gel pack in EPS boxes (FG groups) during export, with most FG and GG curves evolving together.

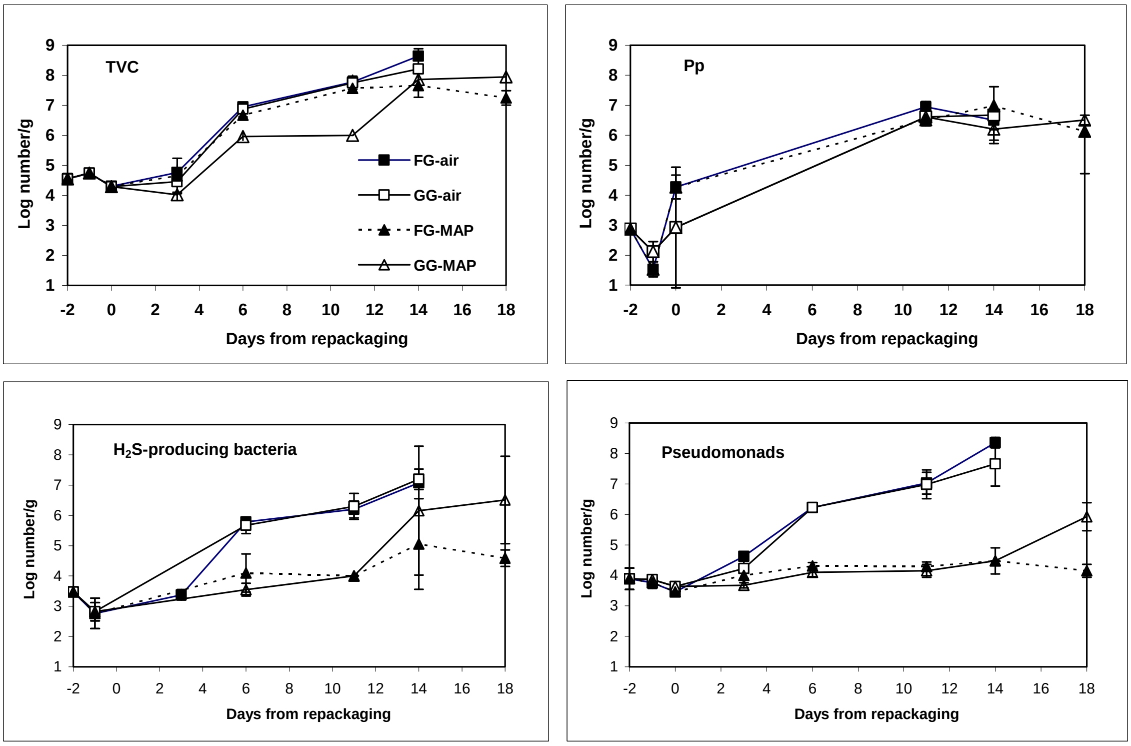

Figure 39. Microbial growth in cod loins transported by air freight and retail-packed under air or MA 2 days post process. Total viable psychrotrophic counts (TVC) and counts of *Photobacterium phosphoreum* (Pp), H_2S-producing bacteria and pseudomonads. Average values of duplicate samples are shown. Error bars show SD.

In cod products exported by sea freight, microbial development was apparently hampered during most of the transport time due to low temperature maintenance (Figure 40). However, a noticeable increase in Pp on the repackaging day (d0) compared to other spoilage bacteria (pseudomonads and H_2S-producing bacteria) indicated that the temperature increase observed in the product (Figures37-38) during late transport influenced Pp especially. After transport, retail-packaging and air-storage at slightly higher temperature led to a rapid growth of spoilage bacteria, reaching critical levels (log

7/g for pseudomonads and H_2S-producing bacteria) after 8 days. The results show that *P. phosphoreum* had reached this level on day 10, again with a similar growth in all groups. However, more sampling points at earlier storage would have given a clearer picture of Pp behaviour. Proliferation of pseudomonads and H_2S-producing bacteria in MA-packed products was delayed resulting in a probably longer shelf life (10-15 days). Pp levels were close to log 7/g on day 15.

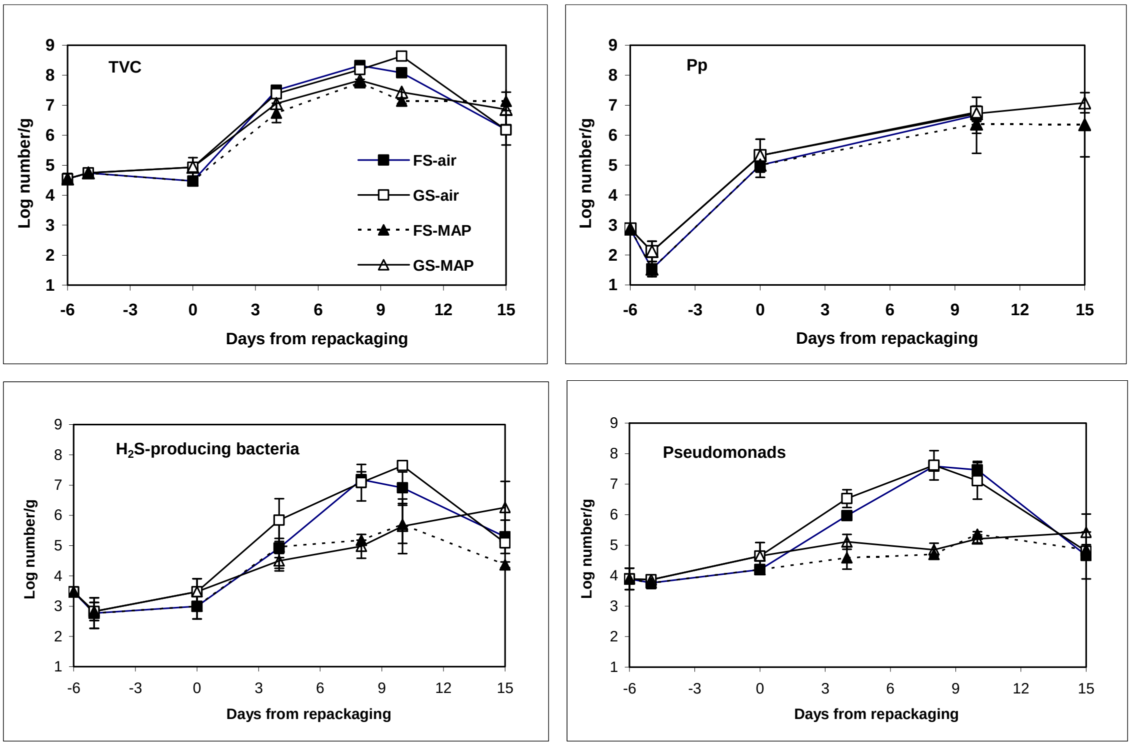

Figure 40. Microbial growth in cod loins transported by sea freight and retail-packed under air or MA 6 days post process. Total viable psychrotrophic counts (TVC) and counts of *Photobacterium phosphoreum* (Pp), H_2S-producing bacteria and pseudomonads. Average values of duplicate samples are shown. Error bars show SD.

In the comparison of both modes of transportation and their effects on the resulting quality loss of fish products, it becomes obvious that the overall steady and low temperature profile of sea freight, in contrast to the fluctuating profile for air freight, may lead to higher quality products at delivery site despite the time difference. This is explained by the physiological state of spoilage bacteria being in their exponential growth phase in air freight fish 6 days post process (4 days after repackaging) but in late lag phase in sea freight fish at the same time point. Based on microbial counts, it is expected that overall shelf life of cod loins from process was around 13 days via air freight, but 13-14 days and 16-21 days for air- and MA-stored fish repackaged after sea transportation, respectively. However, TVB-N content was evaluated at few sampling points and found to be high at late storage (Figure 41). TVB-N production occurred slightly faster in air-stored than MA-stored fish packaged after air freight transport, but not after sea freight (Figure 41). Changes in flesh pH were evaluated more regularly (Figure 42). A similar pH increase was observed in air-packaged fish originating from either transportation mode, reaching about pH 7.1 after 13 (air freight) and 14 (sea freight) days post process. MAP reduced the initial pH of cod loins by about 0.2 unit after which it was found to increase following the accumulation of amine degradation compounds. Generally, a lower pH (6.8-6.9 compared to 7.0-7.1) is found at sensory rejection of MAP products due to the initial buffering effect of CO_2 dissolution into the muscle water phase.

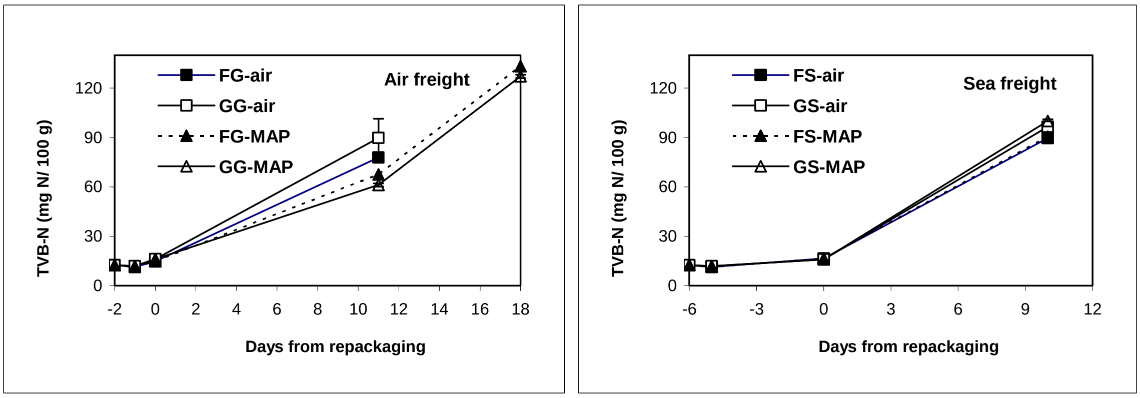

Figure 41. TVB-N content in cod loins transported by air (left) or sea (right) freight and retail-packed under air or MA 2 or 6 days post process. Average values of duplicate samples are shown. Error bars show SD.

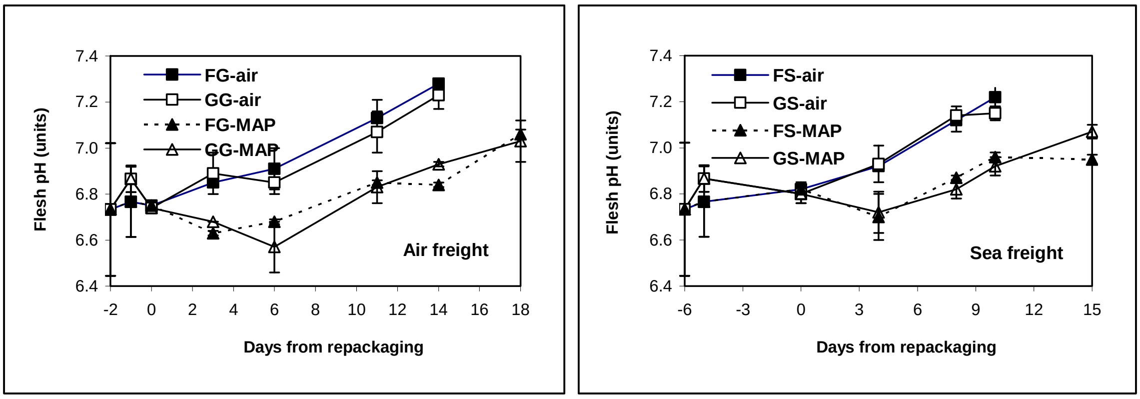

Figure 42. Development of pH in cod loins transported by air (left) or sea (right) freight and retail-packed under air or MA 2 or 6 days post process. Average values of duplicate samples are shown. Error bars show SD.

Based on TVB-N content of 40-50 mg N/100 g at sensory rejection in CBC loins stored under superchilled condition and that of 30 mg N/100 g in abused CBC loins (Figure 26), the shelf life estimation of these products may be expected to be slightly shorter. This is especially true for the fish transported by air freight and stored under air, re-estimating the overall shelf life to 8 and 11 days if TVB-N levels of air- and MA-stored fish, respectively, are compared. Table 9 summarises and compares the findings reported, relating to the shelf life estimation based on the parameters measured.

Table 9. Estimated shelf life and overall shelf life[a] (in days) of retail-packed cod loins as influenced by the transportation mode (air or sea freight) prior to repackaging and the atmospheric condition

Shelf life determination criteria	Air freight		Sea freight	
	Air	MAP	Air	MAP
SSO count: log 7/g	11 (13)	11 (13)	8 (14)	10-15 (16-21)
TVB-N: 30-50 mg N/100 g	6 (8)	9 (11)	5-6 (11-12)	5-6 (11-12)
pH: 7.0-7.1 (air), 6.8-6.9 (MAP)	9-10 (11-12)	11-14 (13-16)	7 (13)	9 (15)

[a] overall shelf life from process time, including transportation time, given in parenthesis.

SSO: specific spoilage organisms

It should be pointed out that cod loins had similar TVB-N values at arrival to TTZ, either air or ship transported. This further supports that the extended transportation time via sea freight is not necessarily detrimental to fish quality if proper temperature control is maintained. In fact, fish transported by sea showed the slowest quality deterioration according to TVB-N values.

5 CONCLUSION

Experiment I.

CBC cooling resulted in a significantly lower temperature profile during the temperature load applied for the first two days of the experiment. The use of a gel pack lowered somewhat the temperature increase in the products when the temperature load was applied and lower temperature was maintained during the entire storage period with a gel pack. As compared to no cooling, the use of liquid cooling contributed to maintaining a lower temperature during the entire storage period, even though the temperatures at packaging were similar.

According to sensory evaluation, immersing the fillets in brine resulted in a shorter freshness period and a shorter maximum shelf life as compared to untreated loins. These results are in agreement with chemical and microbial analysis. Lowest TVB-N and TMA values were obtained in the group NC-RTS-GP where no cooling was applied. Microbial analysis showed that treatment with liquid cooling and CBC led to increase in microbial counts compared to the brine treated group. The brine was quite contaminated with bacteria, especially *Photobacterium phosphoreum.*

According to sensory evaluation, the use of a gel pack was insignificant as it did not result in prolonged freshness period or shelf life as compared to no gel pack. According to microbial and chemical analysis no marked difference was seen whether a gel pack was used or not in CBC-S groups. However, microbial counts were usually somewhat lower in the CBC-RTS groups where a gel pack was used compared to no gel pack. This was confirmed by chemical analysis as the use of a gel pack resulted in a slower formation of TVB-N and TMA in CBC-RTS.

Storage at a steady -1 °C resulted in prolonged shelf life of ca. 3 days compared to storage at simulated air freight temperature conditions according to sensory evaluation.

This was confirmed by microbial and chemical analysis as lower microbial counts, TVB-N and TMA were generally obtained in the CBC-S group than in the CBC-RTS group.

Experiment II.

The storage studies of MA- and air-packed cod loins performed after transport via air and sea freight showed that the overall estimated shelf life of resulting products was not necessarily shorter for sea freight fish, based on microbial and chemical spoilage indicators. It was shown that due to larger and more frequent temperature fluctuations for air than sea freight fish, the quality deterioration process had reached a similar status in either product at delivery in Bremerhaven, i.e. 2 and 6 days post process. In fact, the product temperature of air freight fish was greater than 0 °C for 35% of transportation time in contrast to 18% for sea freight fish. Further, the use of gel pack in 5-kg CBC fish packages did not apparently influence the quality deterioration process of fish transported by either mode. Based on microbial indicators, the estimated shelf life of air-stored products from process (13-14 d) was not influenced by the transportation mode, but shelf life from repackaging was 3 days shorter for sea freight fish. MA-packaging of sea freight fish apparently resulted in a similar or even longer shelf life compared to repacked air freight fish based on microbial data. However, the lack of sensory data makes impossible the evaluation of freshness characteristics of the differently treated products at delivery and after further storage. These findings bring us to conclude that the sea freight transportation mode described in this study is an interesting alternative to air freight, since it does not apparently lead to a faster quality deterioration of CBC cod loins. It can also be foreseen that a greater quality difference may result in fish products that have not received an additional cooling or superchilling treatment in process before being transported by either mode. Nevertheless, a detailed sensory study is required to reveal the quality changes occurring in retail-packed fish products originating from such transportation modes.

6 ACKNOWLEDGEMENTS

This project is a part of the research project the EU-funded Integrated Research Project CHILL-ON (contract FP6-016333-2), Chill-add-on (Kælibót) funded by the AVS research fund under the Ministry of Fisheries (project no. R 061-06), the Technology Development Fund at the Icelandic Centre for Research (project no. 061358006) The financing of this work is gratefully acknowledged.

REFERENCES

AOAC (2000). Assn. Official Analytical Chemists. Official methods of analysis. 17th ed. Washington, D.C. (no. 976.18).

Bonilla AC, Sveinsdottir K, Martinsdottir E. (2007). Development of Quality Index Method (QIM) scheme for fresh cod (Gadus morhua) fillets and application in shelf life study. Food Control 18 (4): 352-358

Dalgaard P, Mejlholm O, Huss HH. (1996). Conductance method for quantitative determination of *Photobacterium phosphoreum* in fish products. J Appl Bacteriol 81:57-64.

Eide O, Børresen T, Ström T. (1982). Minced Fish Production From Capelin (*Mallotus villosus*). A New Method for Gutting, Skinning and Removal of Fat from Small Fatty Fish Species. J Food Sci 47:347-354.

Gram L, Trolle G, Huss HH. (1987). Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int J Food Microbiol 4:65-72.

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

ISO 8586 (1993). Sensory analysis general guidance for the selection, training and monitoring of assessors. Part 1: selected assessors. Geneva, Switzerland: The International Organization for Standardization.

ISO 6496 (1999). Animal feeding stuffs - Determination of moisture and other volatile matter content. Geneva, Switzerland: The International Organization for Standardization.

Lauzon HL. (2003). Notkun Malthus leiðnitækni til hraðvirkra örverumælinga. IFL project report 30-03, 30p (in Icelandic).

Malle P, Tao SH. (1987). Rapid quantitative determination of trimethylamine using steam distillation. J Food Protect 50(9):756-760.

Olafsdottir, G, Lauzon, HL, Martinsdóttir E, Oehlenschläger J and Kristbergsson 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-S109

Shewan JM, Macintosh RG, Tucker CG and Ehrenberg ASC (1953). The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. Journal of Science and Food Agriculture 4 (June), 283-298

Stanbridge LH, Board RG. (1994). A modification of the *Pseudomonas* selective medium, CFC, that allows differentiation between meat pseudomonads and Enterobacteriaceae. Letters in Appl Microbiol 18:327-328.

Stone, H. and Sidel, J.L. (2004). Descriptive analysis. In *Sensory Evaluation Practices*, 3rd Ed. (H. Stone and J.L. Sidel, eds.) pp. 201–244, Elsevier, Amsterdam, the Netherlands.

Sveinsdóttir K, Martinsdóttir E, Green-Petersen D, Hyldig G, Schelvis R, Delahunty C. (2009). Sensory characteristics of different cod products related to consumer preferences and attitudes. Food Qual.Prefer., 20 (2), 120-132.

van Spreekens KJA. (1974). The suitability of Long and Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. Ant. Leeuw 25: 213-219.

7 APPENDIX I

Table A-D show how the sample groups were characterized by sensory attributes.

Table A. Average sensory scores (QDA scale 0-100%) for odour attributes (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C)

Product	sweet	shellfish	meat	vanilla	potatoes	frozen	cloth	TMA	sour	sulphur
p-value	***0,000***	***0,000***	***0,000***	***0,000***	***0,001***	***0,000***	***0,000***	***0,000***	***0,000***	***0,000***
CBC-RTS-GP-d01	58	55	34	38	21	1	1	0	2	1
CBC-RTS-GP-d03	47	43	23	31	22	1	3	2	1	2
CBC-RTS-GP-d06	40	35	19	35	33	1	6	1	4	0
CBC-RTS-GP-d08	33	29	18	28	28	1	9	9	7	1
CBC-RTS-GP-d10	14	13	9	15	29	4	23	24	22	6
CBC-RTS-d01	59	56	30	45	14	0	1	0	1	1
CBC-RTS-d03	46	42	22	33	21	1	2	1	0	0
CBC-RTS-d06	45	38	26	38	30	0	4	1	2	1
CBC-RTS-d08	28	25	17	25	28	1	13	11	11	1
CBC-RTS-d10	18	15	12	17	33	3	28	27	22	4
LC-RTS-GP-d01	57	55	34	40	20	1	1	1	1	1
LC-RTS-GP-d03	49	44	25	36	18	1	1	1	0	0
LC-RTS-GP-d06	39	35	24	35	33	1	11	4	5	1
LC-RTS-GP-d08	26	21	13	20	27	1	17	14	20	2
LC-RTS-GP-d10	15	15	9	16	28	2	40	36	40	14
NC-RTS-GP-d01	60	56	32	39	14	1	1	1	2	0
NC-RTS-GP-d03	48	40	23	34	22	1	1	1	2	1
NC-RTS-GP-d06	47	37	25	42	27	1	2	0	1	1
NC-RTS-GP-d08	29	26	17	25	26	1	8	9	8	1
NC-RTS-GP-d10	25	22	14	21	30	1	16	13	14	3
CBC-S-GP-d02	57	54	30	42	21	1	2	2	0	0
CBC-S-GP-d07	34	28	20	26	27	2	10	8	7	1
CBC-S-GP-d09	31	26	16	25	29	1	14	8	8	0
CBC-S-GP-d13	20	15	10	20	32	2	21	24	16	6
CBC-S-d02	56	55	28	41	20	1	1	1	0	1
CBC-S-d07	34	32	20	24	25	1	5	4	3	0
CBC-S-d09	31	27	17	23	31	1	10	8	9	0
CBC-S-d13	19	13	10	20	29	2	21	24	18	7

Table B. Average sensory scores (QDA scale 0-100%) for appearance attributes (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

Product	dark	discoloured	precipitation
p-value	0,098	***0,045***	***0,015***
CBC-RTS-GP-d01	25	23	16
CBC-RTS-GP-d03	29	26	18
CBC-RTS-GP-d06	27	31	20
CBC-RTS-GP-d08	30	29	21
CBC-RTS-GP-d10	37	37	31
CBC-RTS-d01	26	24	15
CBC-RTS-d03	27	23	23
CBC-RTS-d06	23	26	23
CBC-RTS-d08	20	24	24
CBC-RTS-d10	38	39	27
LC-RTS-GP-d01	29	28	21
LC-RTS-GP-d03	19	19	18
LC-RTS-GP-d06	27	28	27
LC-RTS-GP-d08	33	31	24
LC-RTS-GP-d10	36	37	28
NC-RTS-GP-d01	22	24	18
NC-RTS-GP-d03	26	24	21
NC-RTS-GP-d06	24	24	21
NC-RTS-GP-d08	22	26	24
NC-RTS-GP-d10	27	31	29
CBC-S-GP-d02	24	24	19
CBC-S-GP-d07	29	28	23
CBC-S-GP-d09	29	30	27
CBC-S-GP-d13	26	27	23
CBC-S-d02	30	26	16
CBC-S-d07	29	28	24
CBC-S-d09	28	28	30
CBC-S-d13	25	28	24

Table C. Average sensory scores (QDA scale 0-100%) for flavour attributes (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

Product	salt	metallic	sweet	meat	frozen	pungent	sour	TMA	off
p-value	0,796	***0,000***	***0,000***	***0,000***	***0,008***	***0,000***	***0,000***	***0,000***	***0,000***
CBC-RTS-GP-d01	9	56	58	35	1	1	2	1	0
CBC-RTS-GP-d03	15	40	41	24	1	0	2	2	1
CBC-RTS-GP-d06	11	35	38	20	1	2	3	3	2
CBC-RTS-GP-d08	9	24	24	19	1	6	7	8	8
CBC-RTS-GP-d10	7	17	18	13	5	14	16	13	18
CBC-RTS-d01	7	55	63	32	1	0	0	0	0
CBC-RTS-d03	15	40	42	24	1	3	0	2	2
CBC-RTS-d06	11	34	38	23	1	3	2	1	2
CBC-RTS-d08	10	25	26	18	2	6	7	10	8
CBC-RTS-d10	6	17	18	14	4	14	18	22	24
LC-RTS-GP-d01	9	52	56	32	2	0	2	1	1
LC-RTS-GP-d03	12	42	48	26	0	0	0	2	0
LC-RTS-GP-d06	11	28	33	20	2	2	6	3	3
LC-RTS-GP-d08	9	22	24	15	2	10	15	12	11
LC-RTS-GP-d10	6	12	14	11	5	21	34	34	35
NC-RTS-GP-d01	7	59	59	35	1	1	1	1	0
NC-RTS-GP-d03	11	39	40	24	1	1	2	1	3
NC-RTS-GP-d06	7	37	38	26	1	2	2	1	2
NC-RTS-GP-d08	9	26	24	17	2	6	6	8	7
NC-RTS-GP-d10	5	18	21	16	3	7	8	8	13
CBC-S-GP-d02	11	51	55	28	1	0	1	1	2
CBC-S-GP-d07	12	27	30	22	2	5	6	5	5
CBC-S-GP-d09	14	25	25	18	1	4	9	7	11
CBC-S-GP-d13	9	13	14	11	4	11	17	22	22
CBC-S-d02	11	51	52	24	1	1	1	2	1
CBC-S-d07	13	30	34	20	1	2	6	4	3
CBC-S-d09	13	26	28	15	2	3	6	9	9
CBC-S-d13	13	13	18	11	3	11	17	22	23

Table D. Average sensory scores (QDA scale 0-100%) for texture attributes (CBC: Combined blast and contact cooling, RTS: Real temperature simulation LC: Liquid cooling, NC: No cooling, GP: Gel pack, S: Storage at -1 °C).

Product	flaky	soft	juicy	tender	mushy	meaty	clammy	rubbery
p-value	0,099	***0,035***	***0,000***	0,150	0,574	0,079	0,911	0,901
CBC-RTS-GP-d01	51	70	61	61	32	40	14	11
CBC-RTS-GP-d03	56	65	70	71	41	31	12	8
CBC-RTS-GP-d06	57	62	62	63	30	37	14	8
CBC-RTS-GP-d08	53	60	58	62	33	34	16	11
CBC-RTS-GP-d10	53	60	52	56	33	35	15	11
CBC-RTS-d01	46	74	66	66	33	32	14	10
CBC-RTS-d03	55	60	68	62	35	37	15	16
CBC-RTS-d06	54	61	62	61	27	39	13	9
CBC-RTS-d08	54	61	59	63	36	33	13	12
CBC-RTS-d10	53	64	52	60	36	33	19	16
LC-RTS-GP-d01	53	74	66	67	38	32	13	9
LC-RTS-GP-d03	40	61	68	65	43	35	18	11
LC-RTS-GP-d06	57	61	63	64	31	33	15	10
LC-RTS-GP-d08	52	60	58	59	36	31	16	11
LC-RTS-GP-d10	50	61	48	58	37	29	14	10
NC-RTS-GP-d01	59	66	64	61	24	42	14	12
NC-RTS-GP-d03	54	60	63	61	29	40	17	14
NC-RTS-GP-d06	56	65	59	59	26	40	15	11
NC-RTS-GP-d08	52	55	56	56	29	40	18	16
NC-RTS-GP-d10	51	56	48	51	30	43	19	15
CBC-S-GP-d02	55	72	65	67	34	33	15	10
CBC-S-GP-d07	56	61	59	63	30	36	13	9
CBC-S-GP-d09	50	57	59	58	30	31	11	11
CBC-S-GP-d13	57	58	55	58	40	27	19	12
CBC-S-d02	60	70	64	64	27	37	13	15
CBC-S-d07	56	64	62	64	30	36	10	8
CBC-S-d09	53	55	57	58	30	31	11	9
CBC-S-d13	60	54	55	56	32	32	19	13

Generally, sweet and shellfish odours were very characteristic for all groups at the beginning of storage, but decreased with the storage time. Odour of vanilla/warm milk was detected the beginning and during storage, but decreased at the end of storage. A hint of boiled potatoes odour of was detected at the beginning of storage, but increased somewhat with storage time. Frozen storage odour was not detected, and only a hint of sulphur odour was detected in LC-RTS-GP after 10 days of storage. Odour of table cloth, TMA and sour odours were not detected in the samples at the beginning of storage. These attributes were however detected in CBC-RTS-GP and CBC-RTS after 10 days, after eight days in LC-RTS-GP and 13 days in CBC-RTS-GP and CBC-RTS. Only a hint of these attributes was detected in NC-RTS-GP after 10 days of storage.

The groups generally had light and even colour, but became somewhat more discoloured with storage time, with more white precipitation on the surface.

Frozen storage flavour was not detected in the sample groups, but a hint of salt flavour. At the beginning of storage metallic and sweet were very characteristic of the flavour, which decreased with storage time and were hardly detected at the end of storage. Meaty flavour was evident at the beginning of storage, but decreased with storage time. Pungent, sour, TMA flavours and off-flavour were not detected at the beginning of storage, and only a hint was detected in CBC-RTS-GP and NC-RTS-GP on day 10, while these characteristics were evident in CBC-RTS and LC-RTS-GP on day 10, CBC-S-GP and CBC-S on day 13.

At the beginning of storage, all sample groups were described with soft texture, but decreasingly with storage time. Similar trend was observed for juicy texture.