Nýsköpun & neytendur
Consumers & Products

Vinnsla & virðisaukning
Value Chain & Processing

Erfðir & eldi
Genetics & Aquaculture

Líftækni & lífefni
Biotechnology & Biomolecules

Mælingar & miðlun
Analysis & Consulting

Öryggi & umhverfi
Food Safety & Environment

matís

Effect of improved design of wholesale EPS fish boxes on thermal insulation and storage life of cod loins – simulation of air and sea transport

Björn Margeirsson
Hélène L. Lauzon
Kolbrún Sveinsdóttir
Eyjólfur Reynisson
Hannes Magnússon
Sigurjón Arason
Emilía Martinsdóttir

Vinnsla, virðisauki og eldi

Skýrsla Matís 29-10
Ágúst 2010

ISSN 1670-7192

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D

Report summary

ISSN: 1670-7192

Titill / Title	**Effect of improved design of wholesale EPS fish boxes on thermal insulation and storage life of cod loins – simulation of air and sea transport**		
Höfundar / Authors	Björn Margeirsson, Hélène L. Lauzon, Kolbrún Sveinsdóttir, Eyjólfur Reynisson, Hannes Magnússon, Sigurjón Arason, Emilía Martinsdóttir.		
Skýrsla / Report no.	29-10	*Útgáfudagur / Date:*	August 2010
Verknr. / Project no.	1682/1852	2. útgáfa mars 2011	
Styrktaraðilar / funding:	*EU (contract FP6-016333-2) Chill-on, AVS Fund of Ministry of Fisheries in Iceland (project no. R037-08), Technology Development Fund of the Icelandic Centre for Research (project. no. 081304508), University of Iceland Research Fund*		

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D
Report summary

ISSN: 1670-7192

Ágrip á íslensku:	Markmið tilraunanna var að rannsaka hve vel tvær tegundir frauðkassa verja þorskhnakkastykki fyrir dæmigerðu hitaálagi í flugflutningskeðju frá framleiðanda á norðanverðu Íslandi til kaupanda í Evrópu. Notast var við hitamælingar, skynmat, efna- og örverumælingar til að bera frauðkassana saman og kanna mikilvægi staðsetningar flakabita innan kassa (horn og miðja). Að lokum var geymsluþol hnakkastykkja, sem urðu fyrir dæmigerðu flugflutningshitaálagi, borið saman við geymsluþol hnakkastykkja við stöðuga -1 °C geymslu sem er raunhæfur möguleiki við gámaflutninga með skipum. Nýi frauðkassinn, sem hannaður var með FLUENT varmaflutningslíkani, reyndist betri en eldri kassinn með tilliti til varmaeinangrunar. Hitaálagið á fyrsta degi tilraunarinnar olli því að hæsti vöruhiti í hornum hækkaði í 5.4 °C í eldri gerðinni en einungis í 4.5 °C í þeirri nýju. Munur milli hæsta vöruhita í miðjum og hornum kassa var um 2 til 3 °C. Með skynmati var sýnt fram á að geymsla í nýja frauðkassanum leiddi til tveggja til þriggja daga lengra ferskleikatímabils og eins til tveggja daga lengra geymsluþols m.v. geymslu í eldri frauðkassanum. Munurinn milli kassanna var þó ekki staðfestur með efna- og örverumælingum. Staðsetning innan kassa (horn og miðja) hafði ekki marktæk áhrif á niðurstöður skynmats og var einungis um lítinn mun að ræða milli staðsetninga í mælingum á TVB-N og TMA. Hermun flug- og sjóflutnings (hitasveiflur og stöðugur hiti) leiddi í ljós að fyrir vel forkælda þorskhnakka má vænta eins til fimm daga lengra ferskleikatímabils og um þriggja til fimm daga lengra geymsluþols í vel hitastýrðum sjóflutningi miðað við dæmigerðan flugflutningsferil frá Norðurlandi. Þar sem sjóflutningur frá Íslandi tekur oft um fjórum til fimm dögum lengri tíma en flugflutningur (háð m.a. vikudegi og staðsetningu vinnslunnar) sýnir þetta að sjóflutningur er raunhæfur möguleiki fyrir íslenska ferskfiskframleiðendur. Með notkun á nýju frauðkössunum í flugflutningi á fiskurinn þó eftir lengra ferskleikatímabil þegar hann kemst í hendur kaupenda erlendis en í skipaflutningi. 2. útgáfa, mars 2011 Í fyrri útgáfu skýrslunnar þótti ekki nógu skýrt koma fram að sá umhverfishitaferill, sem líkja átti eftir sjóflutningi, miðaðist í raun við nokkurn veginn bestu mögulegu aðstæður í sjóflutningskeðjum ferskra fiskafurða frá Íslandi. Hitamælingar í kæliverkefnunum *Hermun kæliferla* og *Chill-on* hafa sýnt fram á að forflutningi innanlands fylgir oft óæskilegt hitaálag í nokkrar klst. hvort sem um er að ræða flug- eða sjóflutningskeðjur. Til þessa hitaálags var tekið tillit í tilfelli flugkeðjunnar en ekki sjóflutningskeðjunnar í fyrstu útgáfu skýrslunnar. Mest áhersla var á lengd geymsluþols í fyrri útgáfu skýrslunnar en bætt er við umfjöllun um ferskleikatímabil í nýrri útgáfu hennar.
Lykilorð á íslensku:	*Frauðplastkassar, flutningsmáti, þorskhnakkar, ferskleiki, geymsluþol, skemmdarbakteríur*

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D

Report summary

ISSN: 1670-7192

Summary in English:	The aim of the study was to investigate the performance of two different types of EPS boxes in protecting pre-chilled, fresh fish products subjected to temperature conditions, which are likely to occur during air- and land based, multimodal transport from a processor in North-Iceland to a wholesaler in Europe. The performance of the EPS boxes was evaluated by means of temperature monitoring, chemical- and microbial measurements and finally sensory evaluation. Furthermore, effect of fillet positions inside the wholesale fish packages (corner vs. middle) were investigated by means of the aforementioned methods. Finally, the shelf life of the air-transported simulation fish loins was compared to the shelf life of fish loins stored at around -1 °C, which can be achieved during non-interrupted and well temperature-controlled, containerised sea transport. The new box, designed with a numerical FLUENT heat transfer model, proved to be better with regard to thermal insulation than the old box. The thermal load during the first day of the experiment caused the maximum product temperatures in the bottom corners of the top and second top to rise to 5.4 °C and 4.5 °C for the original and new boxes, respectively. The maximum temperature in the middle of the boxes was around 2 to 3 °C lower than the maximum temperature in the bottom corners. According to sensory evaluation, storage in the new boxes resulted in approximately two to three days longer freshness period and one to two days longer shelf life than storage in the old boxes. The difference between the two box types is not as clear with regard to chemical and microbial measurements. The sampling location (corner versus middle), did not significantly affect the sensory quality and only minor differences were noticed in TVB-N and TMA between sampling locations in the new box. Comparing the steady and dynamic storage in the old boxes it can be concluded that the increased freshness period (around 1-5 days) and shelf life (around 3-5 days) at steady temperature could compensate for the longer transport time by sea instead of air freight. This makes containerised sea transport a worthy choice for Icelandic fresh fish manufacturers depending on the week day and location of processing. However, for maximum remaining freshness period at the time of delivery to the buyer in Europe the results showed that air transport with the new boxes is the more advantageous transport mode relying on shorter transport time and improved thermal protection of the new boxes.
English keywords:	*EPS boxes, transport mode, cod loins, freshness, shelf life, spoilage bacteria*

CONTENTS

1 INTRODUCTION

The combined blast and contact (CBC) cooling technique (by Skaginn hf.) has proved efficient for pre-chilling fresh fish loins/fillets before packaging and prolonging shelf life (Magnússon and others 2009; Gao 2007; Martinsdóttir and others 2004, 2005). One of the main advantage of the CBC technique is a quick reduction of the product temperature to around -1.0 to -0.5 °C before packaging, causing around 5 to 15% of the water of the fish muscle to be frozen (Rha 1975) when packaged. This means that extra energy is needed for melting the partly frozen water, therefore the pre-chilled, packaged fish products can withstand more severe thermal load than un-pre-chilled products in similar packaging can do. This could be related to the fact that by comparing the results of Magnússon and others (2009) and Gao (2007), the pre-chilling is probably more important for products subjected to thermal loads during transport and storage than for products kept at steady temperature conditions. Dynamic temperature conditions are much more likely to be experienced during air freight than during sea freight according to Mai and others (2010).

The volume of fresh fish product export during the last two decades from Iceland is shown in Figure 1 (Statistics Iceland 2010). As is shown in Figure 1, the majority has been air transported during the last decade, however, the proportion of sea to air export has been increasing, see Figure 2. Common transport time from Icelandic processors to retailers in Europe is around five to seven days for the sea transport chains and around two days in case of transport by air (Mai and others 2010). For processors situated near Keflavík airport the air transport chain can, however, be a whole day shorter, i.e. only one day. This means that the transport time is around three to six days longer for the sea transport depending on the week day and location of processing.

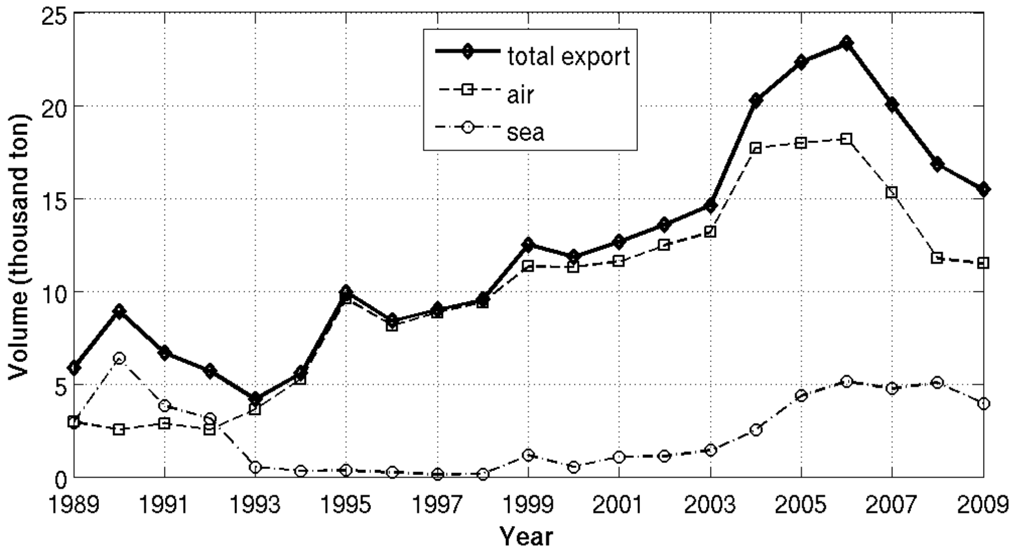

Figure 1. Export of fresh fish fillets and loins by air and sea from Iceland between 1989 and 2009.

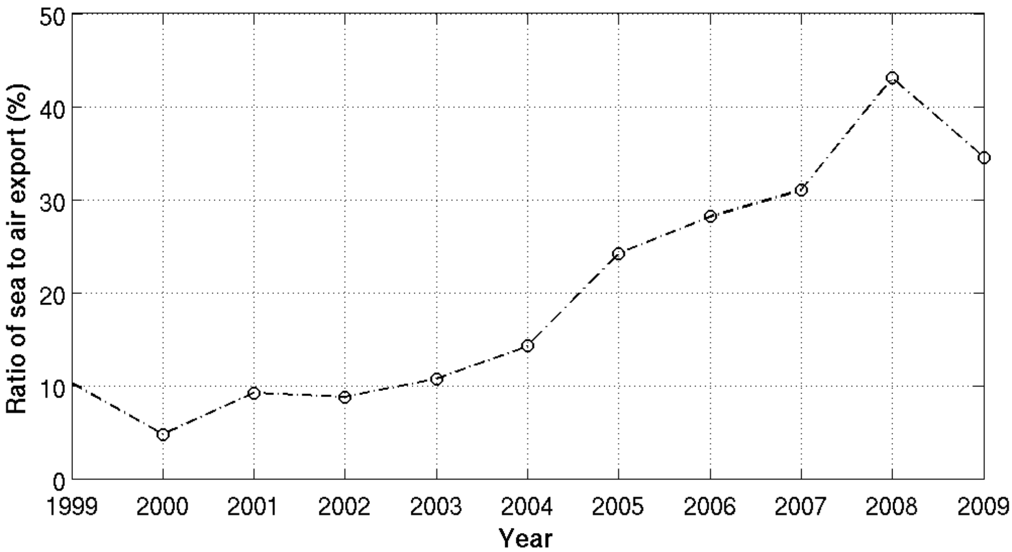

Figure 2. Ratio of sea to air exported fresh fish products from Iceland between 1999 and 2009.

Thermally insulated packaging and phase change materials such as ice packs are other possible mechanisms for protecting the perishable fish products against thermal loads during transport and storage. Margeirsson and others (2009) showed that expanded polystyrene (EPS) wholesale boxes are better insulated than corrugated plastic (CP) wholesale boxes but the need for thermal insulation is less for multiple boxes packaged on a pallet compared to free standing boxes. Furthermore, they found that placing a 250 g ice pack on top of 3 kg of fillets inside wholesale fish boxes significantly reduced the product temperature increase during thermal load. Margeirsson and others (2009) only used one type of EPS box and the fish fillets were not pre-chilled before being thermally loaded.

The aim of the current study was to investigate the performance of two different types of wholesale EPS fish boxes in protecting pre-chilled, fresh fish products subjected to temperature conditions, which are likely to occur during air- and land based, multimodal transport from a processor in North-Iceland to a wholesaler in Europe. One of the box types was designed with a numerical FLUENT heat transfer model in collaboration between Matís and University of Iceland, Reykjavík, Iceland, Promens Tempra, Hafnarfjörður, Iceland and Wessex Institute of Technology, Southampton, UK. The performance of the EPS boxes was evaluated by means of temperature monitoring, chemical- and microbial measurements and finally sensory evaluation. Furthermore, effect of loins positions inside the wholesale fish packages (corner vs. middle) were investigated by means of the aforementioned methods. Finally, the shelf life of the air transported simulation fish loins was compared to the shelf life of fish loins stored at around -1 °C, which can be achieved during non-interrupted and well temperature-controlled, containerised sea transport.

2 MATERIALS AND METHODS

2.1 Experimental design

The raw material used in the experiments was trawler caught south of Kolbeinsey of Iceland on 8 March 2010 by the fishing trawler Björgúlfur EA-312. After bleeding in cold seawater, gutting and washing, the cod was packed and stored with about 5 layers of ice (fish to ice ratio approximately 3:1) in 460 L tubs on board the vessel. The tubs were kept in a chilled hold on board the trawler. The product was landed in Dalvík in North-Iceland and transported in the tubs by a forklift directly after weighing tubs on harbour scale to the main processing cold storage in Dalvík (less than 250 meters from the vessel at quay).

During processing between 11 and 12 AM on 9 March 2010, fillets were superchilled with a CBC (combined blast and contact) cooler by Skaginn hf. (www.skaginn.is) before skinning, portioning and packaging into two different types of 5 kg EPS (expanded polystyrene) boxes.

One frozen gel pack at around -18 °C (weight: 125 g) was put on top of the loins in each fish box in all experimental groups except for the steady storage temperature group simulating well controlled containerised sea transport. For the steady temperature group, around 0.4 kg of freshwater ice was put on top of the loins as is commonly done for sea transported fresh fish products.

The five experimental groups are shown in Table 1. Sampling on day 0 represents the day of processing and packaging (one day post-catch).

Table 1. Overview of the experimental groups and the corresponding sampling days. Legends: O: original EPS box type, N: new EPS box type, ST: steady storage temperature, DT: dynamic storage temperature, SC: superchilled (-1 °C) storage temperature following the dynamic temperature storage, Co: loins taken from box corners, Mi: loins taken from the middle of the box

Group	Sampling days – sensory evaluation	Sampling days – microbial and chemical measurements
O-ST-Co	0, 2, 6, 10, 13	0, 1, 2, 6, 10, 13
O-DT-Co	0, 2, 6, 10	0, 1, 2, 6, 10, 13
N-DT-Co	0, 2, 6, 10	0, 1, 2, 6, 10, 13
N-DT-Mi	0, 2, 6, 10	0, 1, 2, 6, 10, 13
O-DT-SC-Co		0, 6, 10, 13

The original (O) EPS box is manufactured by Plasteyri (Akureyri, Iceland) with outside dimensions of 400 x 265 x 159 mm (length x width x height) and weight about 205 g, see Figure 3 and Figure 4. The original box used for the steady temperature group was equipped with drainage holes for melting ice water, see Figure 3. This group had ice on top of the loins but other groups were equipped with one frozen gel pack each, see Figure 4.

The new (N) EPS box is an improved version of the 5 kg EPS box manufactured by Promens Tempra ehf. (Hafnarfjörður, Iceland), see Figure 4. Its outside dimensions are 400 x 265 x 133 mm (length x width x height) and weight about 183 g, i.e. about 26 mm lower and 22 g lighter than the original box.

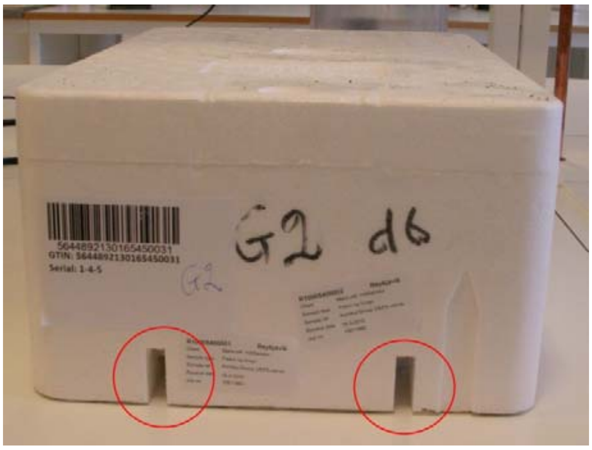

Figure 3. Drainage holes at bottom of the original EPS boxes used for the O-ST-Co experimental group.

Figure 4. Frozen gel packs on top of loins in two types of EPS boxes. Above: the original box, below: the new box with rounded corners.

After inserting the temperature data loggers (see Section 2.2), the boxes were palletised and kept in chilled and frozen storage rooms before being land transported in a mechanically refrigerated truck from the processor’s storage to Matís facilities in Reykjavík between 4 PM and approximately midnight on 9 March 2010. At Matís, the boxes were stored in temperature controlled air climate chambers with each experimental group divided into two free standing piles as shown in Figure 5. The dynamic temperature conditions for the DT experimental groups were obtained by storing the fish boxes in a warm up air climate chamber at around 9 °C for 9 h from arrival at Matís. After that, all the DT groups were chilled at 0 – 4 °C for 3 h at the same chamber before being thermally loaded at around 13 °C for 4 h. The SC-group was then transferred to the steady, chilled air climate chamber at -1 °C and the other DT groups were chilled at around 2 °C for the rest of the experiment.

The purpose of the empty bottom box in each pile was to thermally insulate the experimental groups from the floor, thereby balance the thermal load on the fish boxes. This configuration still yields maximal thermal load on the top and bottom boxes of each pile while positioned in the warm up climate chamber. The reason is that the top surface of the top box is not insulated from the ambient air as is true for the lower boxes and the temperature of the empty boxes at the bottom was equal to the warm air temperature (around 10 – 14 °C) in the beginning of the warm up periods. For the dynamic storage temperature groups, temperature was monitored for the bottom fish boxes (standing on top of the empty boxes), the top boxes and the second top boxes in order to compare the temperature evolution at different heights in the box piles.

Figure 5. EPS fish box piles during storage in an air climate chamber.

2.2 Temperature measurements

IButton temperature loggers (Micro-T DS1922L) from Maxim Integrated Products distributed by NexSens Technology (Dayton, OH, USA, see Figure 6) were used for all temperature monitoring in the trial. This logger has an accuracy of ±0.5 °C and 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. All temperature loggers were factory calibrated and re-calibrated by the authors in thick mixture of fresh crushed ice and water.

Figure 6. IButton DS1922L temperature loggers.

After packaging, product and surface temperatures were monitored for three boxes in each of the five experimental groups except for the steady storage temperature group O-ST-Co, in which only two boxes were temperature monitored. Six temperature loggers were put in each of the monitored EPS boxes and one on the outside of each box, see Figure 7. For measuring the product temperature, the loggers were placed in plastic bags (externally aseptic), in order to avoid microbial contamination. In addition to the product and surface temperature loggers, ambient air temperature was monitored at five to eight different positions inside each climate chamber.

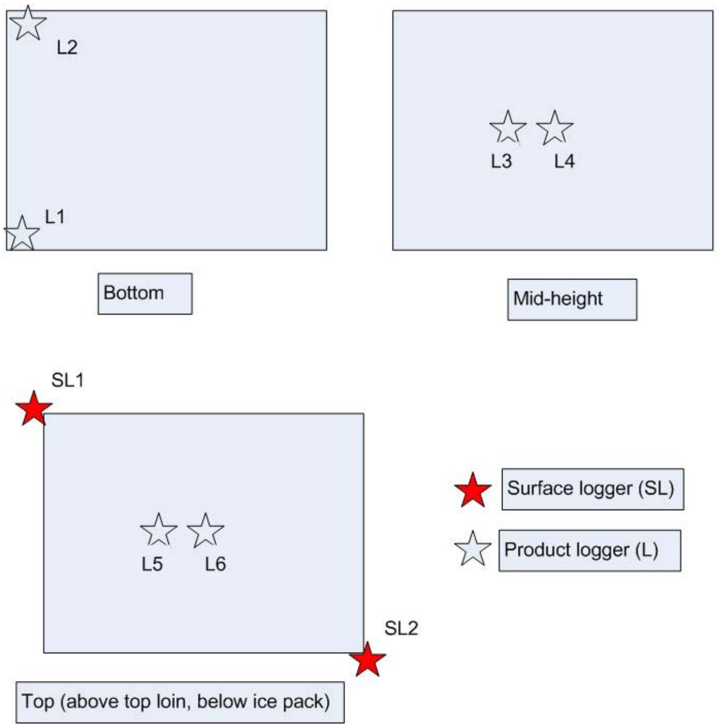

Figure 7. Positions of surface temperature logger and product temperature loggers at three levels on the outside and inside the temperature monitored EPS boxes.

2.3 Sampling

For groups O-ST-Co, O-DT-Co, N-DT-Co and O-DT-SC-Co, pieces of loins were aseptically cut from each corner of a box and transferred to a cutting board. From each corner, three 40 g pieces from loins were then cut to use for sensory evaluation, totally 12 pieces from each box. Simultaneously, cut-offs from the sampling for sensory analysis were pooled to use for microbiology counts. For group N-DT-Mi, three loins were taken from the centre of the box. From each loin, four 40 g pieces were cut to use for sensory evaluation. The rest was pooled into a sample and minced for microbiological and chemical analyses. No sensory evaluation was done on group O-DT-SC-Co. Two boxes were used for each group resulting in duplicate samples.

2.4 Sensory evaluation

Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (2004), and the Torry freshness score sheet (Shewan and others 1953) were used to assess cooked samples of cod (Table 2). Nine 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 and Torry method and experienced in sensory analysis of cod. One session was used for training prior to the sensory evaluation. For the QDA method, 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 (Sveinsdóttir and others 2009; Magnússon and others 2006). The sensory attributes were 30 and are described in Table 2.

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 in a pre-warmed oven to a core temperature of 67°C (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.

.

Table 2. 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 whole warm boiled potatoes
frozen storage	o-frozen	Freezer storage odour, refridgerator
dishcloth	o-cloth	Reminds of a dishcloth (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
heterogeneous	a-hetero	Left end: homogeneous, even colour. Right end: heterogeneous, discoloured, 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 very fresh (boiled) cod
meaty	f-meaty	Reminds of boiled meat
frozen storage	f-frozen	Freezer storage flavour, refridgerator
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

2.5 Microbial evaluation

Minced flesh (20 g) was mixed with 180 g of cooled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 minute. Successive 10-fold dilutions were done with cooled MRD as required. Total viable psychrotrophic counts (TVC) and counts of H_2S-producing bacteria were evaluated on iron agar (IA) as described by Gram and others (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. Plates were incubated at

17 °C for 4-5 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 *Pseudomonas* spp. 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. In all the above counts surface-plating and aerobic incubation were performed.

On days 0, 6 and 13 from packaging, *Salmonella*, faecal coliforms and *Listeria* were evaluated in one experimental group (O-DT-Co) (NMKL 1999; NMKL 2005; NMKL 2007).

Counts of *Pseudomonas* spp. were also done by a quantitative PCR method along with counts of *Photobacterium phosphoreum*. 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 of *Photobacterium phosphoreum* was previously calibrated against the PPDM-Malthus conductance method (Dalgaard and others 1996) using fish samples from storage trials. DNA standard for *Pseudomonas* was calibrated against cultivation on CFU media using the same samples.

Groups O-ST-Co, O-DT-Co and N-DT-Co were furthermore analysed close to the sensory rejection point (d13, d10 and d10, respectively) for the dominating bacterial composition by 16S clone analysis as previously described (Reynisson and others 2010). About thirty clones were analysed for each sample.

2.6 TVB-N, TMA and pH measurements

The method of Malle and Tao (1987) was used for measurements of Total Volatile Base-Nitrogen (TVB-N) and Trimethylamine (TMA). 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 solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulphuric acid solution. TMA was measured in trichloroacetic acid (TCA) extract by adding 20 ml of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine.

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). All measurements were done in duplicate and results presented as an average.

2.7 Statistical 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 sensory, chemical and microbial 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.

3 RESULTS AND DISCUSSION

3.1 Temperature measurements

The mean surface and product temperatures for the five experimental groups are presented in Table 3. The table reveals a very stable product temperature for the steady storage temperature group O-ST-Co. The main reason for the variance of the environmental (surface) temperature is the frozen storage at the processor and the relatively high (1 to 5 °C) temperature during the land transport from the processor to Matís in Reykjavík.

Since the shelf life was not determined by sensory evaluation for the group O-DT-SC-Co, the mean surface and product temperatures were calculated for two different storage periods as is shown in Table 4. Very similar mean product temperatures were yielded between the DT-groups and it should be noted that since the thermal load for these groups started only around 12 h after packaging, the length of the period studied should be taken into consideration when comparing the DT-groups.

Table 3. Mean surface and product temperature with ± one standard deviation during the specified storage period. Legends: O: original EPS box type, N: new EPS box type, ST: steady storage temperature, DT: dynamic storage temperature, SC: superchilled (-1 °C) storage temperature following the dynamic temperature storage, Co: loins taken from box corners, Mi: loins taken from the middle of the box

Group	Surface temperature (°C)	Product temperature (°C)	Period studied from packaging (days)
O-ST-Co	-1.1 ± 1.5	-1.1 ± 0.1	11.5
O-DT-Co	2.1 ± 3.4	1.6 ± 0.8	6.5
N-DT-Co	2.1 ± 3.3	1.3 ± 0.7	8
N-DT-Mi	2.1 ± 3.2	1.2 ± 0.8	8.5
O-DT-SC-Co	-0.4 ± 3.3	-0.3 ± 1.1	8
O-DT-SC-Co	-0.7 ± 2.8	-0.5 ± 0.9	11.5

The mean surface and product temperatures were calculated for different vertical box positions, i.e. at different heights in the box piles, see Table 4. Very similar surface temperatures were obtained at different heights and interestingly, the product temperature in bottom and top boxes was not notably higher than the product temperature in the boxes in the second top row.

For comparing the thermal protection of the EPS box types, the results from the surface and product temperature measurements during a fixed storage time should be considered. The mean product temperature for the N-DT-Co group was calculated as 1.4 ± 0.8 °C for the top box during 6.5 days storage, which is identical to the results for the corresponding O-DT-Co top box. A more thorough comparison between the temperature evolution of the original and new boxes during the dynamic temperature periods will be given below.

Table 4. Mean surface and product temperature at different box positions with ± one standard deviation during the storage period. Legends: O: original EPS box type, N: new EPS box type, ST: steady storage temperature, DT: dynamic storage temperature, SC: superchilled (-1 °C) storage temperature following the dynamic temperature storage, Co: loins taken from box corners, Mi: loins taken from the middle of the box

Group	Position of box in pile	Surface temperature (°C)	Product temperature (°C)	Period studied from packaging (days)
O-DT-Co	top	2.2 ± 3.4	1.4 ± 0.8	6.5
O-DT-Co	bottom	2.1 ± 3.3	2.0 ± 0.8	6.5
O-DT-Co	2nd top	2.1 ± 3.4	1.5 ± 0.8	6.5
N-DT-Co	top	2.1 ± 3.3	1.5 ± 0.8	8
N-DT-Co	bottom	2.1 ± 3.3	1.2 ± 0.7	8
N-DT-Co	2nd top	2.2 ± 3.2	1.2 ± 0.7	8
N-DT-Mi	top	2.0 ± 3.2	1.4 ± 0.7	8.5
N-DT-Mi	bottom	2.0 ± 3.2	1.1 ± 0.9	8.5
N-DT-Mi	2nd top	2.2 ± 3.1	1.1 ± 0.8	8.5

3.1.1 Environmental temperature

The surface temperature during the total storage of the five experimental groups is shown in Figure 8. The influence of the aforementioned frozen storage (see Section 2.1) is evident in the figure (mean surface temperature of -21 °C for around 1.6 h). Following the frozen storage at the processor was land transport lasting for around 7.5 h with mean surface temperature around 1 °C and maximum surface temperature around 6 °C in the beginning of the land transport. It should be noted that the thermal load on the DT-groups (around 9 °C for 9 h + around 13 °C for 4 h) is not excessive with regard to the temperature mapping results of airborne supply chains of Mai and others (2010). It should be noted that Mai and others (2010) investigated airborne supply chains from North-Iceland, which includes around 6 hours land transport in addition to storage overnight. Less ambient thermal load can be expected for products from processors closer to Keflavík airport. In other words, the thermal load on the DT-groups is not more than what can be expected for fresh fish transport by air from North-Iceland. Regarding the comparison between the air and sea environmental temperature profiles, it should be noted that the sea temperature profile (O-ST) represents an almost perfect sea transport chain with no pre-land transport as often is the case. Further, environmental temperature of around 2 °C on day 5 or 6 after packaging (at the end of the sea transport itself) is more likely to be experienced than -1 °C in most sea transport chains from Iceland. The ambient temperature distribution inside the steady temperature air climate chamber (containing the O-ST-Co group for the whole storage time at Matís and the O-DT-SC-Co group after the temperature fluctuations on day 1) was relatively homogeneous as is displayed in Figure 9.

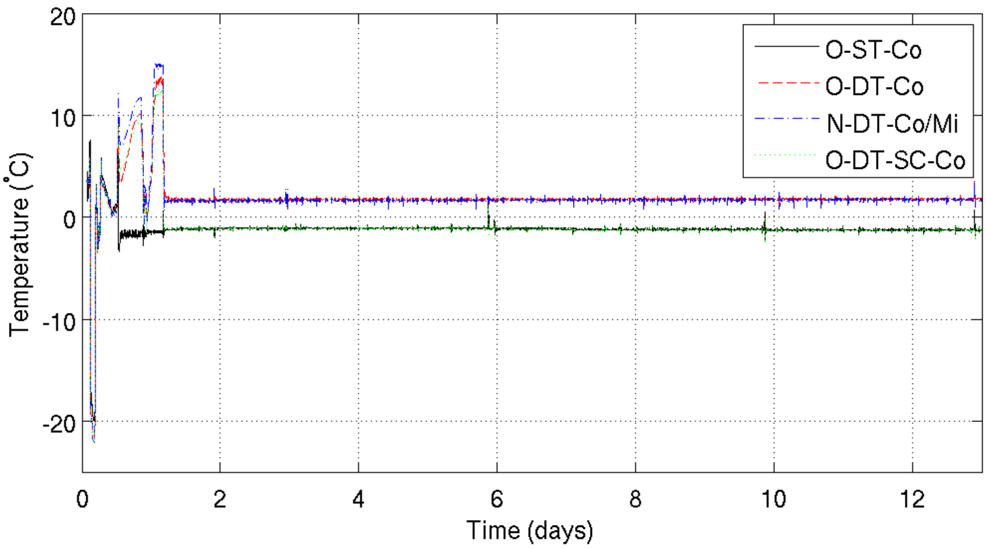

Figure 8. Environmental temperature for the five experimental groups. The blue dash-dotted line represents both the N-DT-Co and the N-DT-Mi groups since they represent the same boxes.

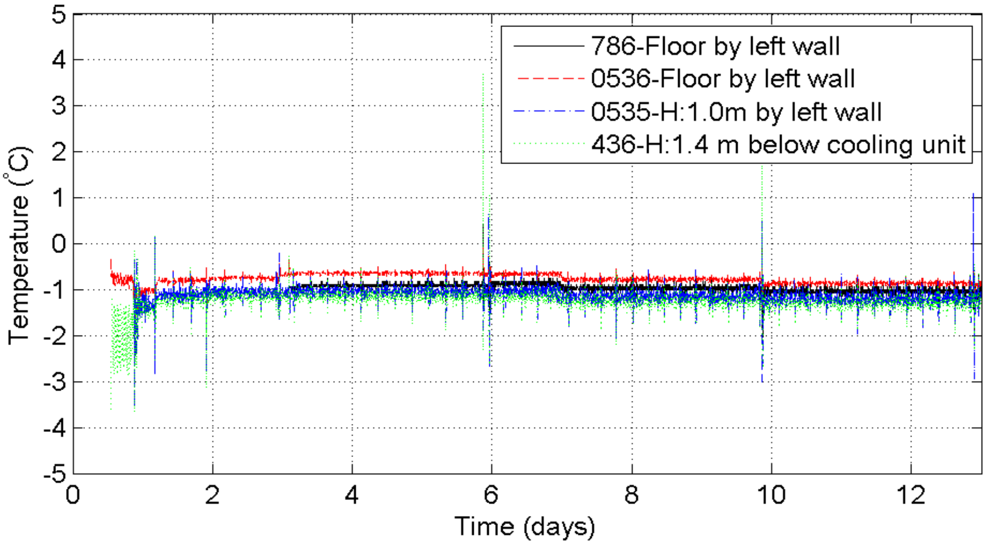

Figure 9. Ambient temperature inside the steady temperature air climate chamber after arrival of the boxes at Matís.

3.1.2 Product temperature

Very steady product temperature was experienced for group O-ST-Co as is shown for two samples in Figure 10. The undesirable product temperature fluctuations in the beginning are explained by the frozen storage at the processor on one hand and a moderate temperature abuse during the land transport from the process to Matís on the other hand (see Section 3.1.1).

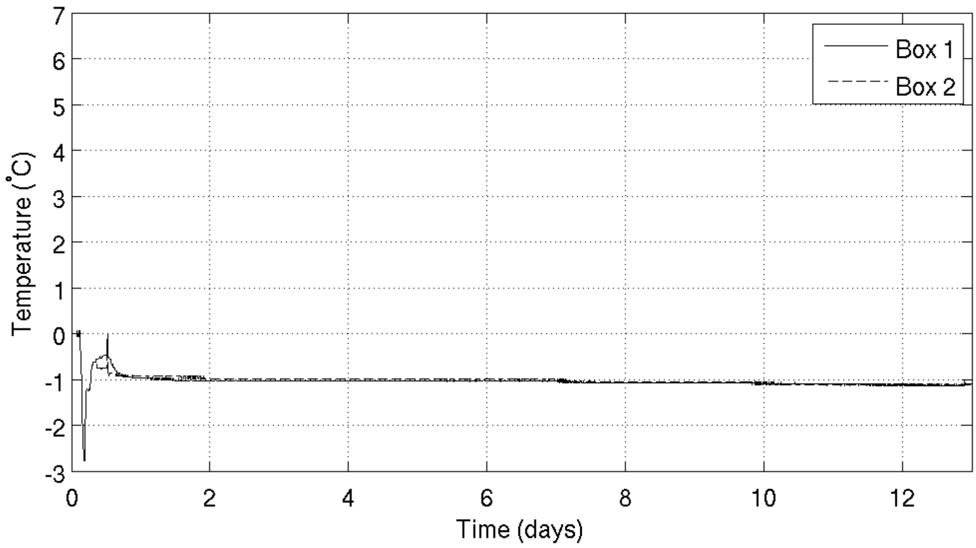

Figure 10. Product temperature of two O-ST-Co boxes.

Different product temperature profiles were obtained for the dynamic groups as is shown in Figure 11 to Figure 13 for samples in the top box, second top box and bottom box, respectively.

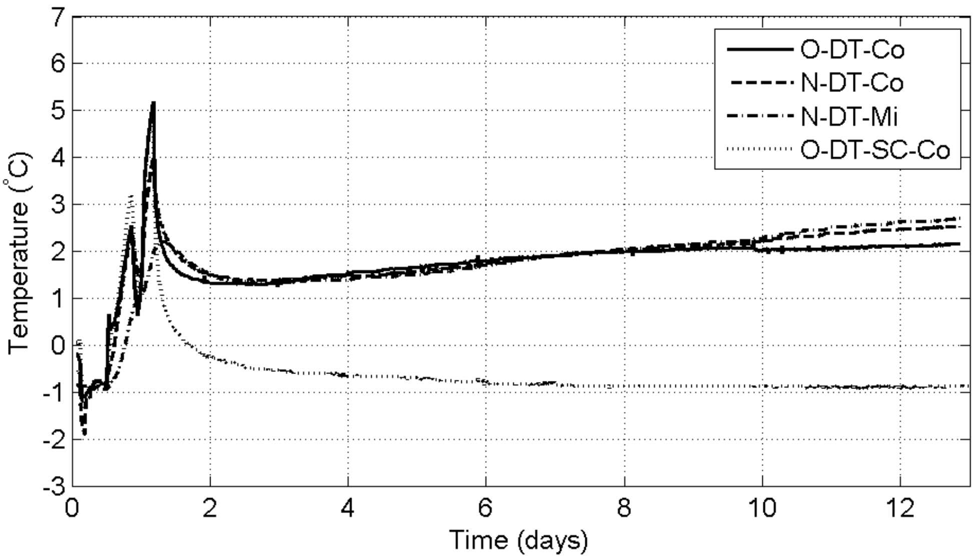

Figure 11. Product temperature from packaging in bottom corners in the top box of each pile (unless otherwise stated).

Judging from Figure 12 and Figure 13, there was a noticeable time delay of the temperature increase in the middle of the new EPS boxes (group N-DT-Mi) compared to the corners of both the new (N-DT-Co) and original (O-DT-groups) boxes. As could be expected, the product temperature in the superchilled group O-DT-SC-Co stabilized at around -1 °C after the dynamic temperature period since it was then stored at around -1 °C inside the steady air climate chamber (see Figure 9).

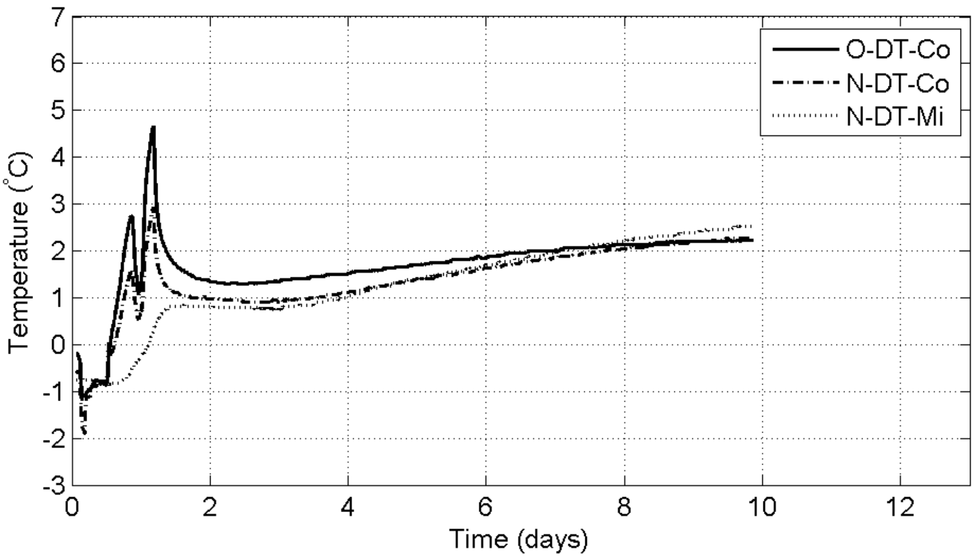

Figure 12. Product temperature from packaging in bottom corners of the second top box of each pile.

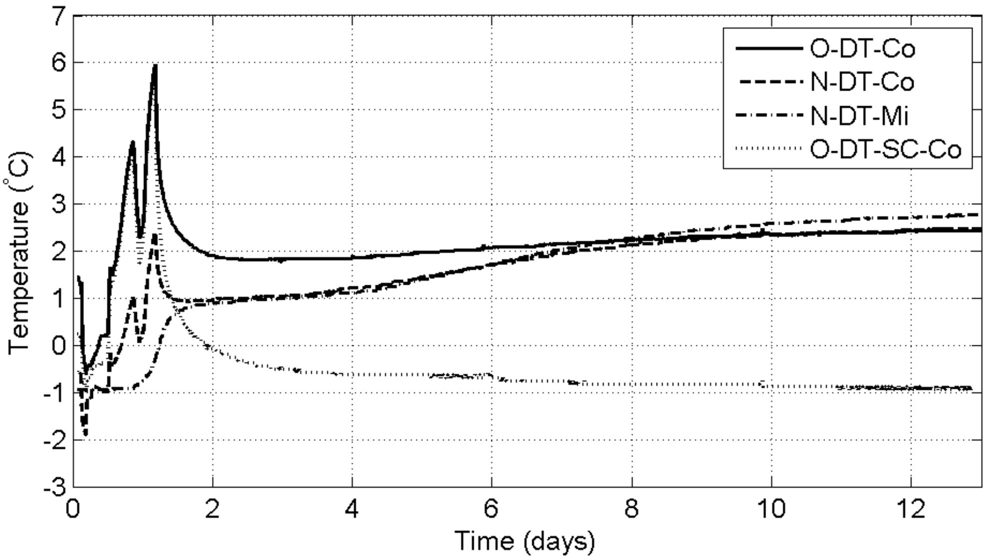

Figure 13. Product temperature from packaging in bottom corners of the bottom box of each pile.

Furthermore, Figure 11 - Figure 13 show that the product temperature rise at the corners of the new box (N-DT-Co) was slower during the dynamic period than the corresponding product temperature rise observed at the corners of the original boxes (O-DT-Co and O-DT-SC-Co). This is clearly demonstrated in Figure 14 - Figure 16 which are zoom-ups of the corresponding Figure 11 - Figure 13, only showing the first 48 h of the experiment. However, a slower chilling process of the fish bulk occurred in the corners of the new boxes almost totally exposed to the environment (positioned at the top of the pile) compared to the original boxes (see Figure 14). This was not the case for the boxes positioned at lower levels. To the contrary, the temperature rise was less pronounced and accompanied with a corresponding temperature fall in the new boxes upon proper storage compared to the original ones.

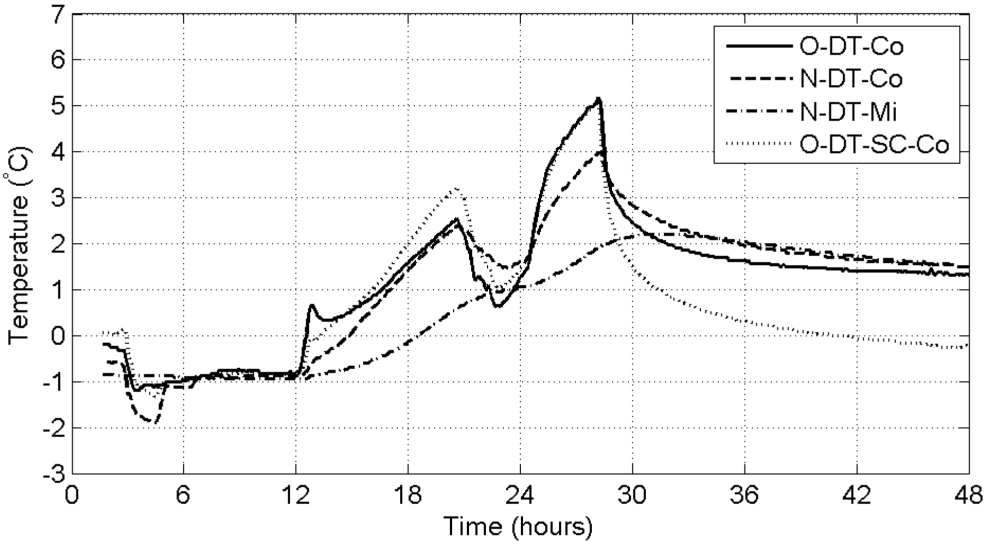

Figure 14. Product temperature from packaging in bottom corners of the top box of each pile during the first 48 h.

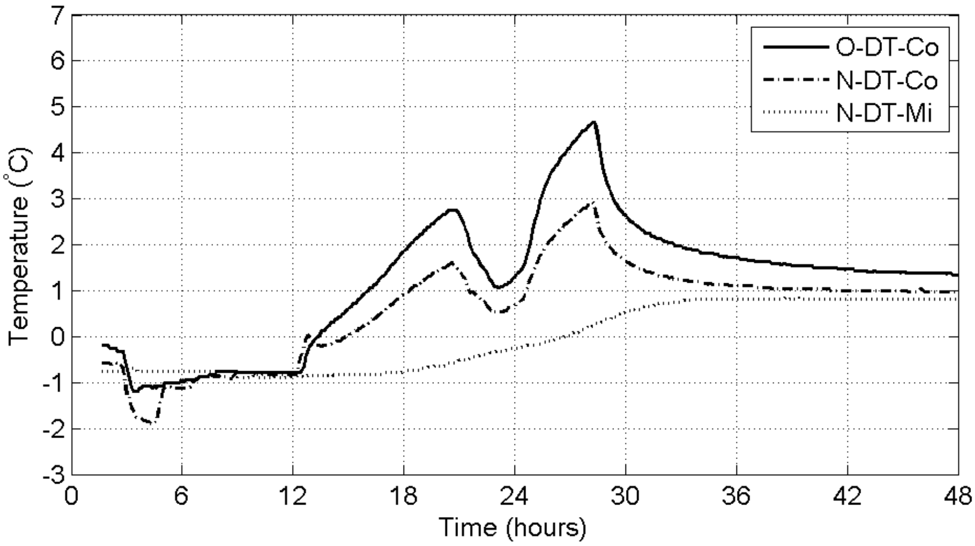

Figure 15. Product temperature from packaging in bottom corners of the second top box of each pile during the first 48 h.

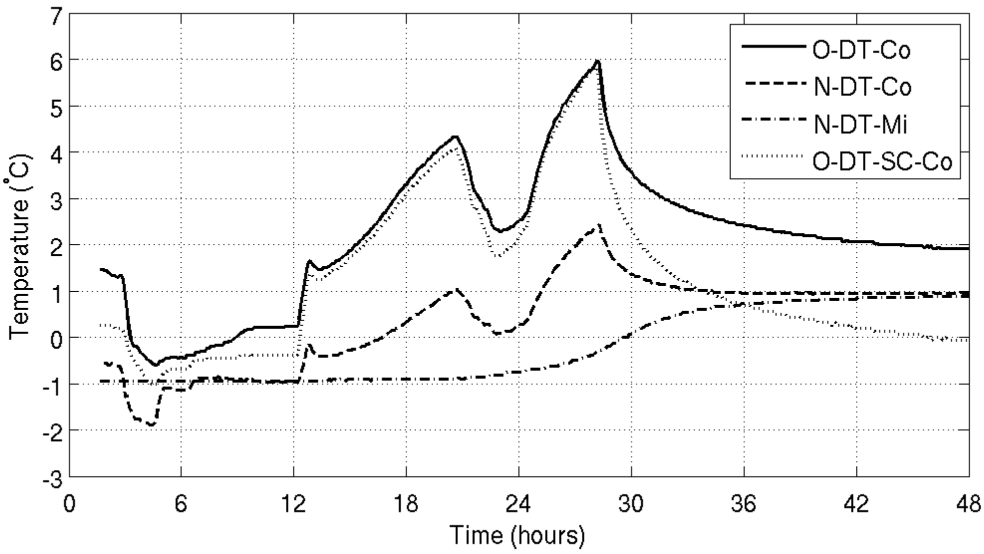

Figure 16. Product temperature from packaging in bottom corners of the bottom box of each pile during the first 48 h.

In order to get a fair comparison between the thermal performances of the original and new boxes, the mean product temperature at four bottom corner positions (positions no. 1 and 2 in Figure 7) in the top and the second top new boxes (group N-DT-Co) was compared to that calculated from the corresponding positions for the original boxes in the O-DT-Co group. The bottom boxes were therefore not included in the calculations for the mean product temperature during the dynamic temperature period since the product temperature in the bottom O-DT-Co box was around 1.2 °C higher than the corresponding product temperature in the bottom N-DT-Co box at midnight (12 h after packaging) on 9 March (see Figure 16). At that time point, the mean temperature with one standard deviation was calculated as -0.8 ± 0.1 °C for the original boxes and -0.9 ± 0.1 °C for the new boxes. Shortly after midnight, a dynamic temperature period started, as is displayed in

Figure 17, which firmly confirms that the two original boxes under consideration were similarly thermally abused to the two new boxes.

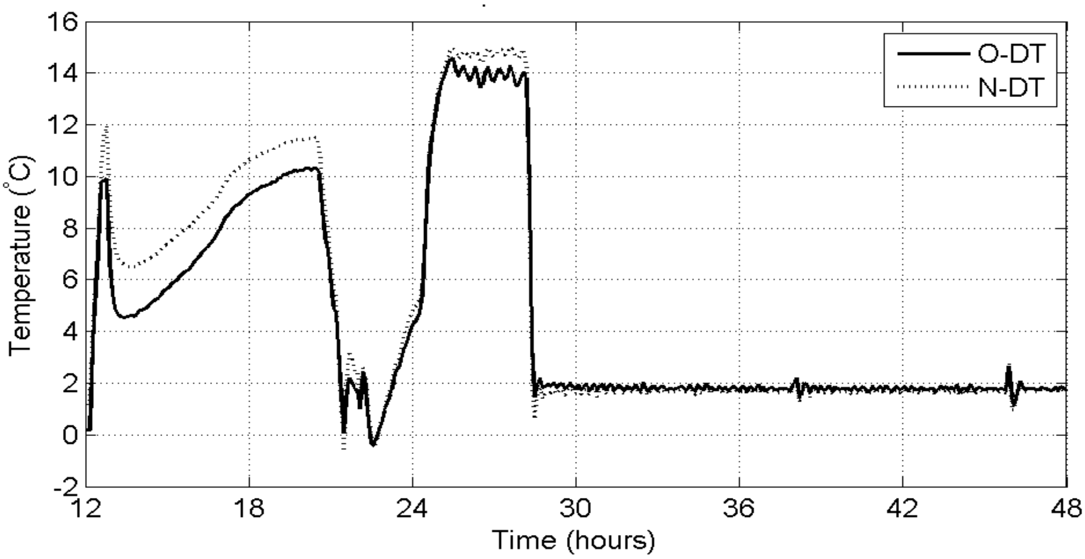

Figure 17. Mean surface temperature for the original (O) and the new (N) EPS boxes during a dynamic temperature period. The mean surface temperature was calculated from the top and second top boxes for each group.

The mean product temperature evolution during the dynamic temperature period is shown in Figure 18, clearly revealing the faster fish temperature increase in the corners of the original boxes compared to the new boxes. The maximum temperatures measured in the bottom corners of the top and second top original and the new boxes were 5.4 °C and 4.5 °C, respectively. The mean product temperatures during the period from 12 h to 48 h were 1.8 ± 1.0 °C and 1.4 ± 0.8 °C for the original and new boxes, respectively. Calculation of the corresponding mean product temperatures during the whole first two days of the experiment, i.e. from packaging until the samples were collected on day 2, yields 1.2 ± 1.4 °C and 0.9 ± 1.3 °C for the original and new boxes, respectively. Less difference was obtained between the product temperatures in the middle of the two box types, see Figure 19. Still, the mean product temperature during the period from 12 h to 48 h was higher in the original box, 0.9 ± 1.0 °C versus 0.7 ± 0.8 °C for the new box. The maximum temperatures measured in the middle of the top and second top original and the new boxes were 2.5 °C and 2.6 °C, respectively. This implies that the maximum temperature in the middle of the boxes was around 2 to 3 °C lower than the maximum temperature in the bottom corners.

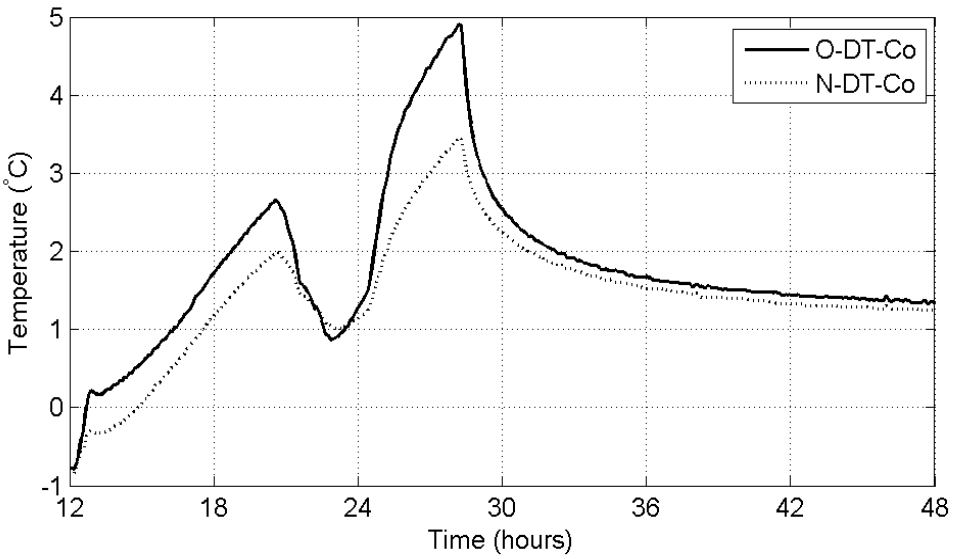

Figure 18. Mean product temperature at the bottom corners of the original (O) and the new (N) EPS boxes during a dynamic temperature period. The mean product temperature was calculated from four bottom corner positions in the top and second top boxes for both the original and the new boxes.

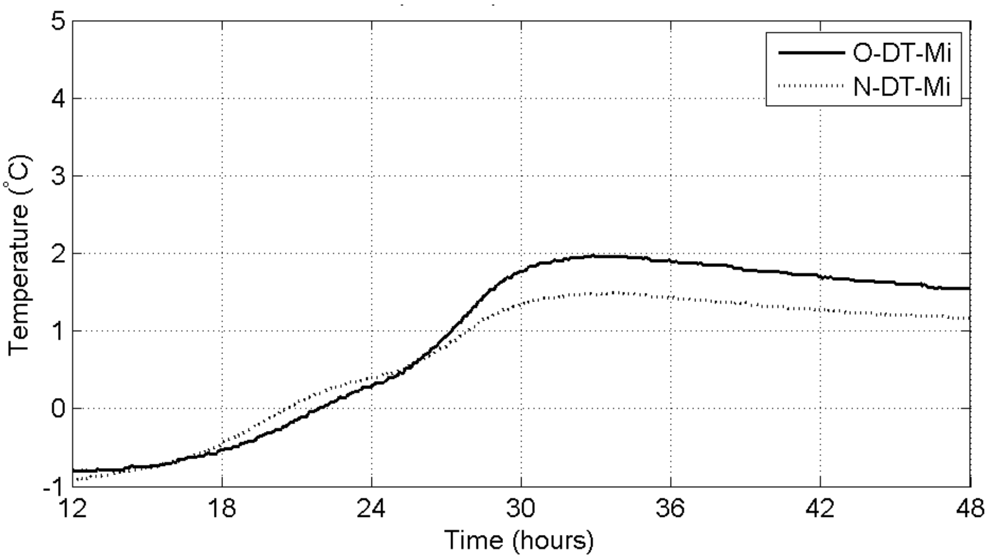

Figure 19. Mean product temperature in the middle of the original (O) and the new (N) EPS boxes during a dynamic temperature period. The mean product temperature was calculated from four middle positions in the top and second top boxes for both the original and the new boxes.

The slower temperature increase in the new EPS boxes indicates their better thermal performance compared to the original EPS boxes, meaning that the new boxes provide a better insulation than the original EPS boxes do. As already has been noted in Section 2, the original box is around 22 g heavier, which makes the better thermal protection of the new boxes even more meaningful.

3.2 Sensory evaluation

The sensory changes of the samples with storage time were monitored by two methods, one to evaluate fish freshness and the other providing a more detailed analysis of sensory attributes. Figure 20 shows how the samples were characterised by the sensory attributes. Altogether 88% of the sensory variation was explained by the first two principal components (PC1 and PC2). 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 left in the upper part of Figure 20B describing samples after 0 - 2 days of storage (Figure 20A). As storage time progressed, these sensory attributes become less evident but the vanilla odour and juicy texture become more characteristic, and then more dark and discoloured appearance and potato odour (lower part of Figure 20B). The sensory attributes characteristic for cod at the end of storage, such as sour flavour, off-flavour and especially TMA flavour and odour, located to the left in the upper region were used to describe the samples at the end of the storage period. The sample group O-ST-Co appeared to retain the freshness characteristics longer than other sample groups (Figure 20A). However, O-DT-Co lost its freshness characteristics faster, being prominently described by spoilage attributes after six and 10 days of storage compared to other groups at the same storage time.

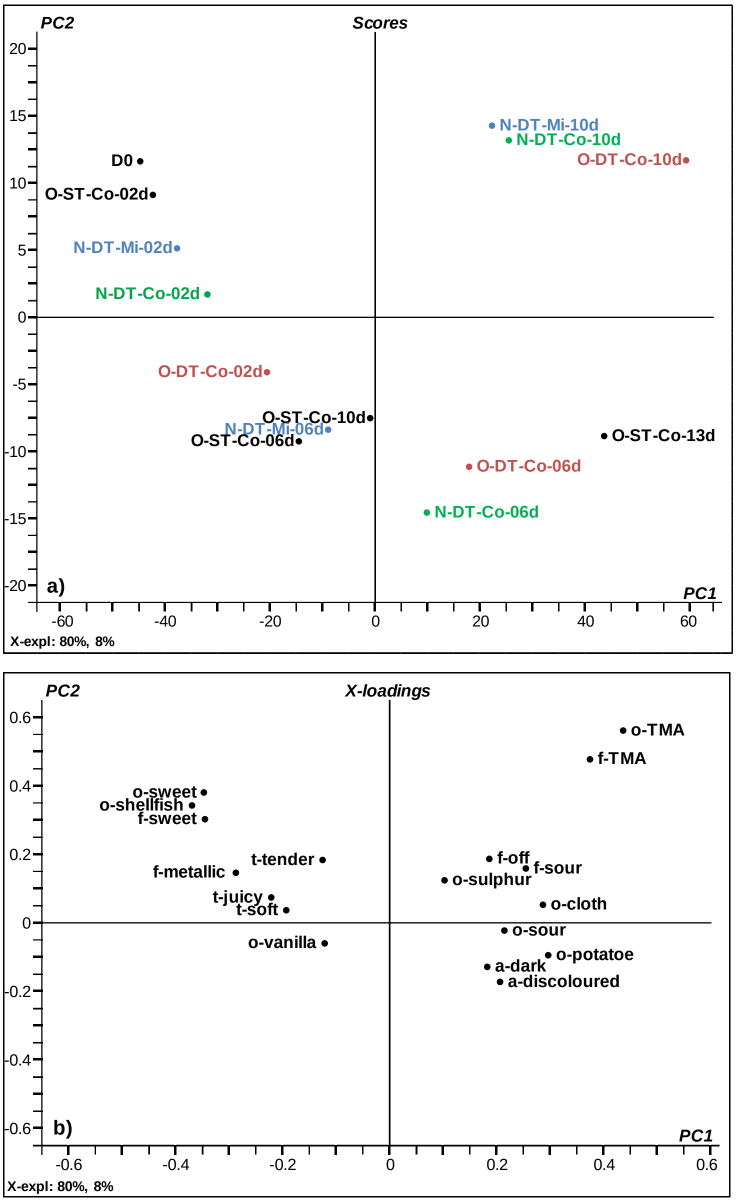

Figure 20. PCA describing sensory quality, odour (o-), appearance (a-), flavour (f-) and texture (t-) of the sample groups with storage time (d). PC1 vs PC2 (X-expl.: 80% and 8%). A) upper figure with scores, B) lower figure with X-loadings.

Figure 21 shows how the Torry freshness score changes with storage time. On the processing day the average Torry score was already below 9. A Torry score around seven indicates that the fish has lost most of its freshness odour and flavour characteristics and has a rather neutral odour and flavour (Shewan and others 1953). The time elapsed from processing until a Torry score of seven is reached is called the freshness period. This score was obtained after 2 - 3 days for O-DT-Co, after 5 - 6 days for N-DT-Co and N-DT-Mi, but after 6 - 7 days for O-ST-

Co. When the average Torry score is around 5.5, most of the sensory panellists detect spoilage attributes and this score has been used as the limit for consumption at Matís (Martinsdóttir and others 2001). According to this, the maximum shelf life of O-DT-Co was six days, N-DT-Co eight days, N-DT-Mi 8 - 9 days, and O-ST-Co 11 days. Results from comparable experiments performed at the end of February 2009 (Martinsdóttir and others 2010) where the fish loins were processed at the same processing factory two days after catch and stored at steady ambient temperature of -1.2 ± 0.2 °C at Matís showed a similar freshness period (7-8 days) and maximum total shelf life of 12 days. This freshness period of six to eight days post-processing in the steady superchilled loins is shorter compared to earlier studies which showed that comparable fillets retained their freshness characteristics for up to 9-10 days under superchilled storage (Martinsdóttir and others 2005). The total shelf life in the earlier studies of CBC fillets processed fish one day to 3 days after catch and stored under superchilled conditions was up to 14 to 16 days (Martinsdóttir and others 2004, 2005). This variation in quality may be explained by seasonal differences, since the earlier trials were performed from October to December. The biological condition of the fish in connection to the reproduction cycle in late winter time is a possible explanation for the differences in shelf life. Earlier research on seasonal variation (May and October) on shelf life of whole haddock have shown a shelf life of 9 -10 days for the May experiment and 14-15 days for October experiment reflecting biological condition of the fish at each season (Tryggvadóttir and Ólafsdóttir, 2000).

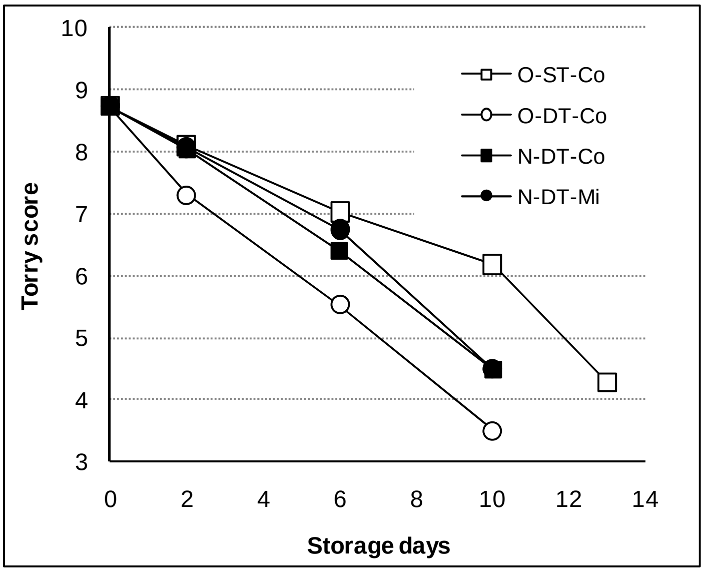

Figure 21. Average Torry freshness scores. O: Original EPS box type, N: New EPS box type, ST: Steady storage temp., DT: Dynamic storage temp., Co: Corner box samples, Mi: Middle box samples.

Figure 22 to Figure 25 show how odour and flavour attributes related to deteriorative changes 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 20 (on the scale 0 to 100), most panellists detect them (Bonilla and others 2005; Magnússon and others 2006). According to this criterion, O-DT-Co had a maximum shelf life of 6 - 7 days, N-DT-Co eight days, N-DT-Mi nine days, but O-ST-Co 11 - 12 days. These results are in agreement with those from the Torry evaluation.

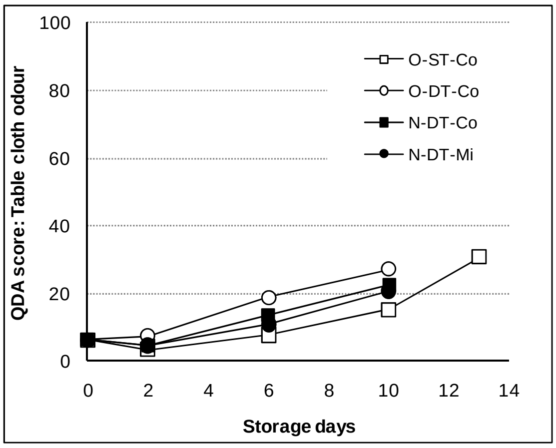

Figure 22. Average QDA scores of table cloth odour.

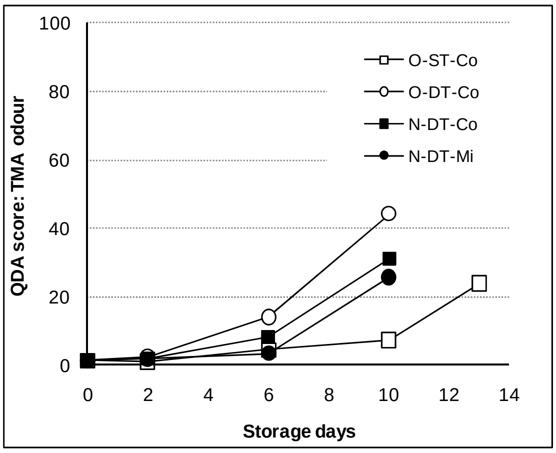

Figure 23. Average QDA scores of TMA odour.

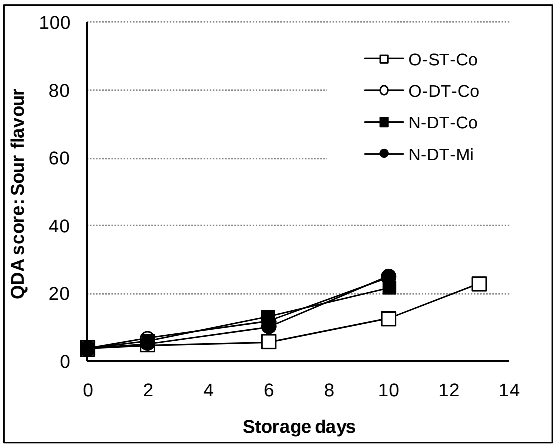

Figure 24. Average QDA scores of sour flavour.

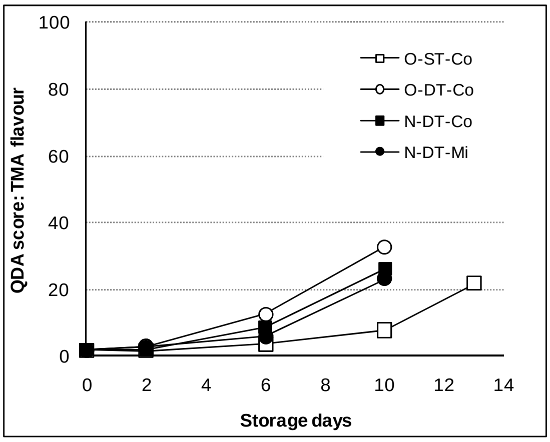

Figure 25. Average QDA scores of TMA flavour.

A comparison of the freshness period and the maximum shelf life among groups is shown in Table 5.

Table 5. Freshness period and shelf life according to sensory evaluation. O: Original EPS box type, N: New EPS box type, ST: Steady storage temp., DT: Dynamic storage temp., Co: Corner box samples, Mi: Middle box samples

Group	Freshness period (days)	Shelf life (days)
O-ST-Co	6 -7	11-12
O-DT-Co	2-3	6-7
N-DT-Co	5	8
N-DT-Mi	5	8-9

Comparison of samples treated and sampled in the same way, stored in the original and the new boxes under dynamic temperature conditions (O-DT-Co versus N-DT-Co) showed that according to sensory evaluation, storage in the new boxes resulted in approximately 2 - 3 days longer freshness period and about two days longer shelf life. Further, the sampling location (corner (Co) versus middle (Mi)) within the new boxes did not affect the sensory quality significantly.

Comparing the steady and dynamic storage in the old boxes it can be concluded that the increased freshness period (around 4 days) and shelf life (around 5 days) at steady temperature could compensate for the longer transport time by sea instead of air freight. However, the freshness period at steady temperature is only 1-2 days longer if the new boxes are used for the dynamic air transport simulation groups. This shows that by using the new boxes, the deteriorating effects of thermal load in air transport chains can be reduced such that air transport is the more advantageous transport mode for maximising the valuable remaining freshness period at the time of delivery to the buyer in Europe.

3.3 Microbial evaluation

The initial microbiological quality of the raw material was satisfactory (TVC < log 5/g) but could have been better considering the fact that the fish was processed one day post-catch. On days 0, 6 and 13 from packaging, *Salmonella*, faecal coliforms and *Listeria* were evaluated in one experimental group (O-DT-Co). These bacteria were not detected in any of the samples.

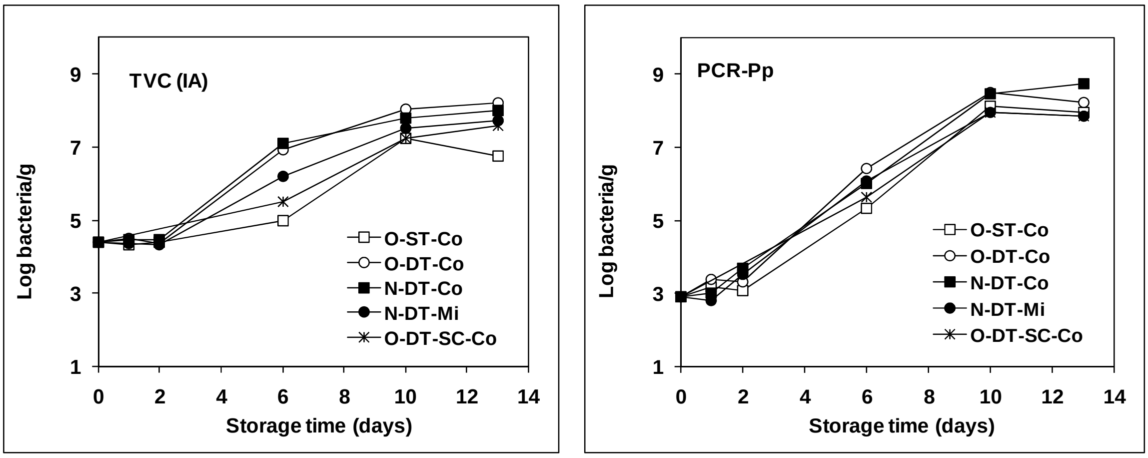

Figure 26. Total psychrotrophic viable counts (left) and *Photobacterium phosphoreum* counts estimated by PCR method (right) in cod loins over storage time. O: Original EPS box type, N: New EPS box type, ST: Steady storage temp., DT: Dynamic storage temp., SC: Superchilled (-1 °C) storage followed by dynamic temp., Co: Corner box samples, Mi: Middle box samples.

High levels of pseudomonads and *Photobacterium phosphoreum* (Pp) were observed at packaging (Figure 26 and Figure 27). Microbial growth was generally slow during the first days of storage in all groups despite the temperature abuse applied to four of them. This is most likely because of the built-in cold (superchilled condition) the CBC process provides to the loins. TVC progressed more slowly in groups stored mainly (O-DT-SC-Co) or solely (O-ST-Co) at -1 °C, with significantly lower counts first observed after 6 days of storage (Figure 26). As expected, slower bacterial growth was observed in samples collected in the middle of the box (N-DT-Mi) as compared to the corners (N-DT-Co) where the weakest insulation is to be found. The difference was though only significant on day 6. A rapid Pp proliferation was seen in all groups during storage, with the slowest growth occurring in O-ST-Co group which was not temperature abused. Pp levels were only significantly lower in O-ST-Co group on day 6 compared to O-DT-Co. It is noteworthy that Pp counts were seen to surpass TVC by at least 0.5 log units after 10 days storage. This indicates that the TVC medium (IA) did not sustain Pp growth. This has been observed in earlier trials involving modified atmosphere storage of cod loins (Lauzon and others 2009) as well as recently in EPS-packed cod loins from the same producer (H.L. Lauzon, unpublished data). Long & Hammer's (LH) medium used during these studies provided higher counts than IA. Pp is known to be able to grow well on LH (Dalgaard and others 1997).

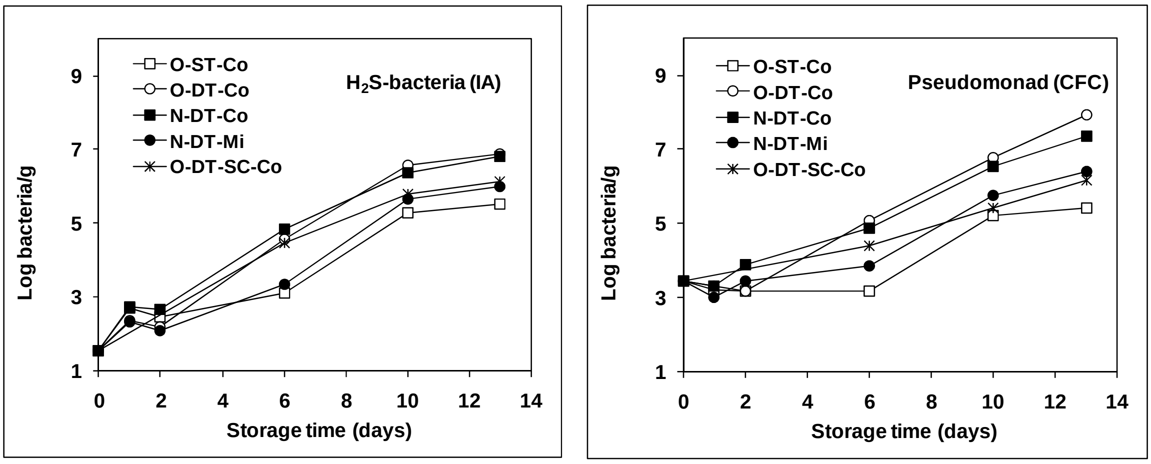

Figure 27. Counts of H_2S-producing bacteria (left) and pseudomonads (right) in cod loins over storage time, evaluated by conventional methods. O: Original EPS box type, N: New EPS box type, ST: Steady storage temp., DT: Dynamic storage temp., SC: Superchilled (-1 °C) storage followed by dynamic temp., Co: Corner box samples, Mi: Middle box samples.

Growth of other spoilage bacteria shown in Figure 27 progressed more slowly than for Pp. A comparison on day 10 indicates a 1000-fold difference in counts of Pp and other spoilage bacteria evaluated in the O-ST-Co group in contrast to a 100-fold difference in the other groups. This suggests that rather cold-tolerant Pp were developing in the cod products as this bacterium has been reported to tolerate superchilling conditions less well than the other spoilage bacteria (Olafsdottir and others 2006; Lauzon and others 2009). Again, slower bacterial growth was observed in samples collected in the middle of the box as compared to the corners, with a significant difference only observed on day 6. Overall, no significant difference was observed for any of the microbial measurements obtained from samples taken at each sampling point in the old (O-DT-Co) and new (N-DT-Co) box types.

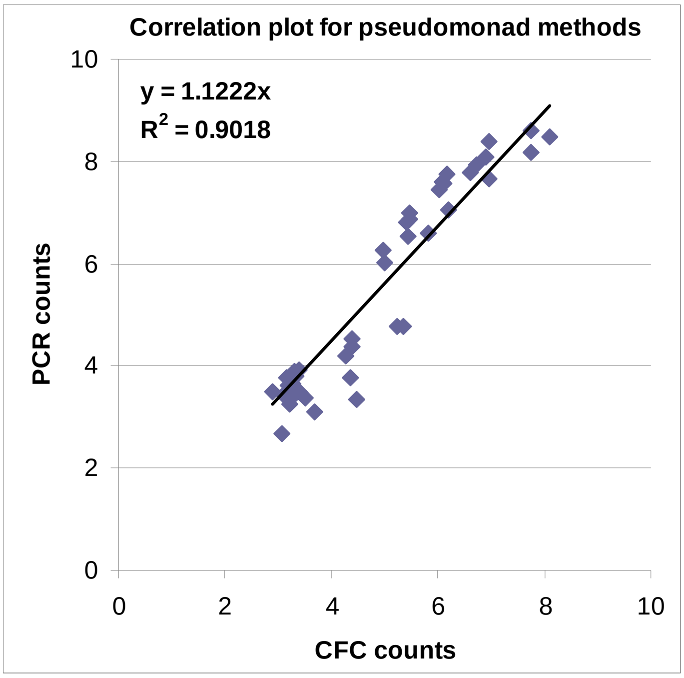

Figure 28. Comparison of methods estimating pseudomonad counts: CFC counts vs. PCR counts.

Two methods were used to estimate pseudomonad counts, the conventional plating on the modified CFC medium and the quantitative PCR method developed by Reynisson and others (2008). Figure 28 is a correlation plot showing the good congruence (R^2=0.9) of the counts obtained. The plot reveals that at low levels, CFC medium tends to overestimate the counts while at higher levels, the counts are very similar to PCR results or slightly underestimated by the plating method. This is likely to occur as reading of the pink colonies on the modified CFC medium can be ambiguous on overloaded plates since the whole surface of the agar turns pink.

Analysis of total bacterial composition using a molecular cloning technique (Reynisson and others 2009, 2010) provided an additional insight on other bacterial groups that were of importance during storage (Figure 29). The results showed that *P. phosphoreum* was the only bacteria detected during stable temperature storage indicating its high dominance in this group (O-ST-Co). The other two groups, stored at dynamic temperature in new and old boxes, showed dominance by *Psychrobacter* sp. and also *Flavobacterium* sp. but to a lesser extent. These results may seem to disagree with the findings reported in Figure 26 and Figure 27 for the two abused groups. However, as TVC do not apparently provide the correct assessment of the cultivable microbiota, one can expect that other bacteria may dominate over

Pp. This is especially true in temperature abused products evaluated at overt spoilage where a wider microbiota may flourish. It is interesting that the detected genera were also the dominating groups in previous studies on brined cod loins and whole, gutted haddock (Reynisson and others 2009, 2010), which may indicate their importance in the microbial succession during fish storage. However, the role of these genera in the spoilage process of cod loins or fish in general should be further investigated.

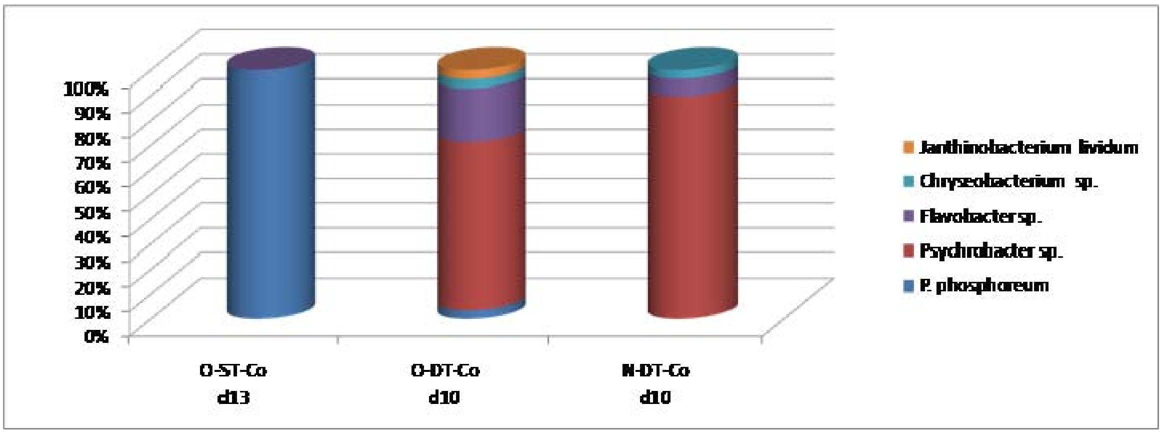

Figure 29. Dominating bacterial community evaluated by16S clone analysis in three sample groups just passed sensory rejection. O: Original EPS box type, N: New EPS box type, ST: Steady storage temp., DT: Dynamic storage temp., Co: Corner box samples.

3.4 TVB-N, TMA and pH measurements

TVB-N and TMA content in differently treated cod loins during storage is illustrated in Figure 30. From day 6 onwards, formation of both TVB-N and TMA was slower in the two groups where superchilling (about -1 °C) was applied (O-ST-Co and O-DT-SC-Co) for most or all storage period, the difference being significant from day 10. Comparing samples from the old and new box types (O-DT-Co and N-DT-Co), only a small difference was noticed between the groups. However, a significant difference in TVB-N content between these box types was observed on day 13. Minor differences were noticed in TVB-N and TMA whether samples were taken from the corner of the new box (N-DT-Co) or from the middle (N-DT-Mi). Slightly lower values were though obtained from day 6 onwards in samples from the middle of the box, with a significant difference observed on day 13 for TVB-N content. The lower TMA values observed during superchilled storage are in agreement with slower *P. phosphoreum* growth in these two groups, although less distinguishable. Good agreement was found between profiles of TVB-N/TMA production and pH values (Figure 31) in cod loins over storage time. The pH measurements did not indicate any deviations from what is generally observed in cod products (initial pH = 6.7, rising to about 7 when approaching sensory rejection). As expected, high values were observed at overt spoilage.

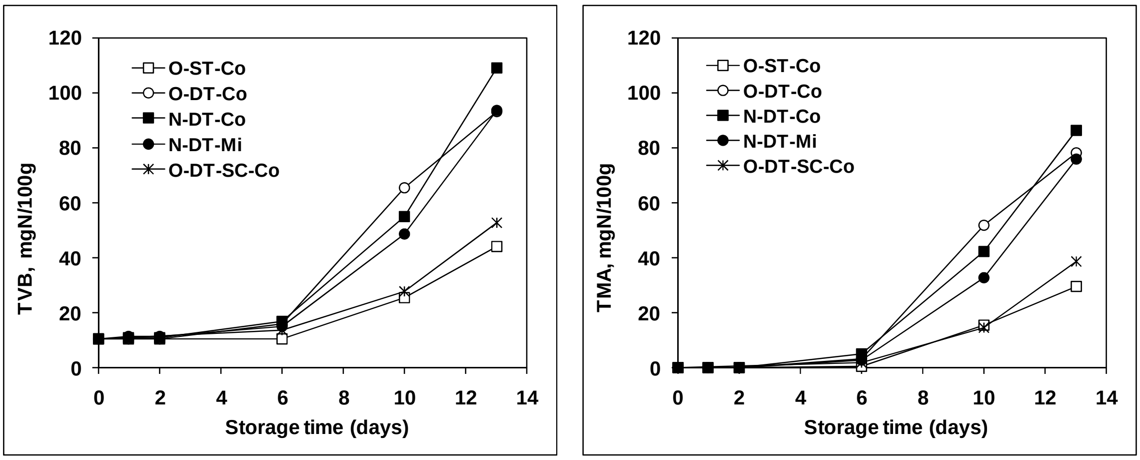

Figure 30. Total volatile base nitrogen (TVB-N, left) and trimethylamine (TMA, right) formation in cod loins. O: Original EPS box type, N: New EPS box type, ST: Steady storage temp., DT: Dynamic storage temp., SC: Superchilled (-1 °C) storage, Co: Corner box samples, Mi: Middle box samples.

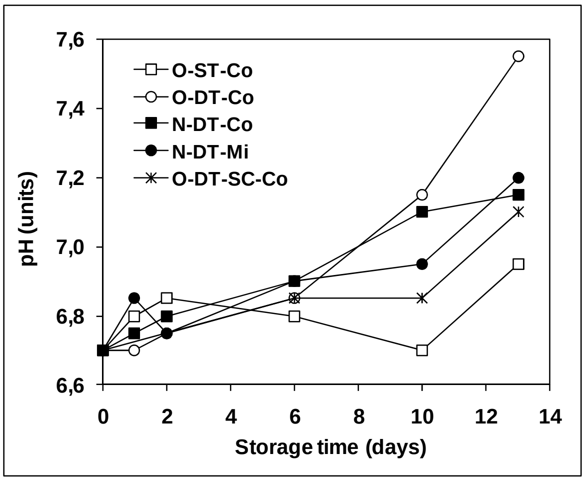

Figure 31. Development of pH in cod loins. O: Original EPS box type, N: New EPS box type, ST: Steady storage temp., DT: Dynamic storage temp., SC: Superchilled (-1 °C) storage, Co: Corner box samples, Mi: Middle box samples.

3.5 Overall analysis

The mean product temperature evolution during the dynamic temperature period revealed faster fish temperature increase in the corners of the original boxes compared to the new boxes. The mean product temperatures during the period from 12 h to 48 h were 1.8 ± 1.0 °C and 1.4 ± 0.8 °C for the original and new boxes, respectively. The thermal load during the first day of the experiment caused the maximum product temperatures in the bottom corners of the top and second top to rise to 5.4 °C and 4.5 °C for the original and new boxes, respectively. Less difference was obtained between the product temperatures in the middle of the two box types. The slower temperature increase in the new EPS boxes indicates their better thermal performance compared to the original EPS boxes, meaning that the new boxes provide a better insulation than the original EPS boxes do. The new type of boxes weighs 22 g less than the original ones making the better thermal protection of the new boxes even more meaningful.

Keeping the samples under superchilled conditions resulted in longest shelf life of 11 - 12 days. According to sensory evaluation, storage in the new boxes resulted in approximately two to three days longer freshness period and one to two days longer shelf life than storage in the old boxes. Further, the sampling location versus middle within the new boxes did not affect the sensory quality significantly.

Microbial growth was generally slow during the first days of storage in all groups despite the thermal load applied to some of them. This is most likely because of the built-in cold (superchilled condition) the CBC process provides to the loins. Slower bacterial growth was observed in samples collected in the middle of the box as compared to the corners, with a significant difference only observed on day 6. Comparing the old and new box types, no significant difference was observed for any of the microbial measurements made in any sample collected. Comparing TVB-N and TMA in samples from the old and new box types groups only a small difference was noticed. Minor differences were noticed in TVB-N and TMA whether samples were taken from the corner of the new box or from the middle. Slightly lower values were though obtained from day 6 onwards in samples from the middle of the box, with a significant difference observed on day 13 for TVB-N content. The lower TMA values observed during superchilled storage are in agreement with slower *P. phosphoreum* growth in these two groups, although less distinguishable.

Table 6 gives an overview of the estimated values of the main parameters at sensory rejection. *Photobacterium phosphoreum* was the main spoilage bacterium evaluated with a concentration range of about log 7-8 CFU/g at sensory rejection of the cod products under investigation, corresponding to the high TVB-N (35 mg N/100 g) and TMA (20-25 mg N/100 g) content observed in all groups except O-DT-Co. This is in agreement with the findings from 2009 (Martinsdóttir and others 2010). The shelf life after processing (one day post-catch) was slightly shorter or 11 days in 2010 compared to 12 days (two days post-catch) in 2009 according to the Torry rejection score of 5.5. TVB-N content of the fish at the start of the storage was 10.7 mg N/100g in 2010 and 12.5 mg N/100g in 2009, indicating a similar freshness at the start of the storage study.

Table 6. Comparison of microbiological and chemical values (estimate) at the end of shelf life as judged by sensory evaluation (QDA and Torry score). O: Original EPS box type, N: New EPS box type, ST: Steady storage temp., DT: Dynamic storage temp., Co: Corner box samples, Mi: Middle box samples

Analysis/Groups	**O-ST-Co**	**O-DT-Co**	**N-DT-Co**	**N-DT-Mi**
Shelf life (days) [1]	11-12	6-7	8	8-9
TVC (IA, $\log_{10}$ CFU/g)	6.9	6.9	7.4	7.5
H_2S-producing bacteria (IA)	5.5	4.8	5.8	4.8
Pseudomonads (CFC medium)	5.4	5.4	5.7	5.0
Pseudomonads (PCR method)	6.3	5.2	6.2	5.9
P. phosphoreum (PCR method)	8.0	6.6	7.1	7.1
TVB-N (mg N/100 g)	33	21	35	35
TMA (mg N/100 g)	21	<10	24	21
pH	6.8	6.9	7.0	6.9

[1]Shelf life from processing (one day post-catch)

Comparing the steady and dynamic storage in the old boxes it can be seen that the increased freshness period (4 days) and shelf life (5 days) at steady temperature could compensate for the longer transport time by ship instead of air freight. It can be anticipated that the higher sensory quality of fish products stored in the new boxes is also likely to be observed at the distribution point after ship or air transport. This would contribute to the delivery of product of higher quality to the final buyer or consumer. These results are generally supported by microbiological and chemical analyses as growth of the bacteria is slowest in the group kept at steady storage -1°C and the group kept at -1°C after the temperature abuse compared to the other groups. Further, formation of both TVB-N and TMA was slower in the two groups where superchilling was applied for most or all of the storage period.

4 CONCLUSIONS

The overall conclusion for the packaging comparison is that the improved thermal protection of the new box design unarguably results in prolonged shelf life and freshness period, in particular. However, the difference between the two box types is not as clear with regard to chemical and microbial measurements. Thus, further study should be conducted on this.
The overall conclusion for the transport simulation is that the advantage of shorter transport time for air transport is mostly lost due to the hardly evitable temperature fluctuations at the interfaces of air transport chains. This makes containerised sea transport a worthy choice for Icelandic fresh fish manufacturers depending on the week day and location of processing. However, for maximum remaining freshness period at the time of delivery to the buyer in Europe the results showed that air transport with the new boxes is the more advantageous transport mode relying on shorter transport time and improved thermal protection of the new boxes.

5 ACKNOWLEDGEMENTS

This project is a part of the research project Hermun Kæliferla (Thermal modelling of chilling and transportation of fish) funded by AVS Fund of Ministry of Fisheries in Iceland (project no. R037-08), Technology Development Fund of the Icelandic Centre for Research (project. no. 081304508), University of Iceland Research Fund and the EU-funded Integrated Research Project CHILL-ON (contract FP6-016333-2). The financing of this work is gratefully acknowledged.

6 REFERENCES

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.

Dalgaard P, Mejlholm O, Christiansen TJ, Huss HH. 1997. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. Lett Appl Microbiol 24:373–378.

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.

Gao, HY. 2007. Methods of pre-cooling for fresh cod (*Gadus morhua*) and influences on quality during chilled storage at -1.5 °C. United Nations University – Fisheries Training Programme – Final report.

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.

Lauzon HL, Magnússon H, Sveinsdóttir K, Guðjónsdóttir M, Martinsdóttir E. 2009. The effect of brining, modified atmosphere and superchilling on the shelf life of cod (Gadus morhua) loins. J Food Sci 74(6): M258-M267.

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.

Magnússon H, Lauzon HL, Sveinsdottir K, Margeirsson B, Reynisson E., Runarsson AR, Gudjonsdottir M, Thorarinsdottir KA, Arason S, Martinsdottir E. 2009. The effect of different cooling techniques and temperature fluctuations on the storage life of cod fillets (*Gadus morhua*). Matís Report 23-09, 37 pages.

Mai N, Margeirsson B, Margeirsson S, Bogason S, Sigurgisladottir S, Arason S. Temperature mapping of fresh fish supply chains – air and sea transportation. J Food Process Eng (accepted for publication in March 2010).

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

Margeirsson B, Arason S, Palsson, H. 2009. Thermal performance of corrugated plastic boxes and expanded polystyrene boxes. Matís Report 01-09, 24 pages.

Martinsdóttir E, Sveinsdóttir K, Luten J, Schelvis-Smith R, Hyldig G. 2001. Sensory Evaluation of Fish Freshness. Reference Manual for the Fish Sector. QIM-Eurofish

Martinsdóttir E, Guðbjörnsdóttir B, Lauzon HL, Ólafsdóttir G, Þóroddsson Þ, Tryggvadóttir SV, Arnarson GÖ. 2004. Áhrif roðkælingar á gæði fiskflaka. IFL- Report 03-04. 72 pages (in Icelandic).

Martinsdóttir E, Lauzon HL, Tryggvadóttir SV. 2005. Áhrif roðkælingar á gæði fiskflaka – MAP pökkuð og þídd. IFL-report 10-05, 48 pages (in Icelandic).

Martinsdóttir E, Lauzon HL, Margeirsson B, Sveinsdóttir K, Þorvaldsson L, Magnússon H, Reynisson E, Jónsdóttir AV, Arason S, Eden M. 2010. 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. Matís Report 18-10, 57 pages.

NMKL Method No. 71, 5th ed., 1999: *Salmonella*. Detection in foods.

NMKL Method No. 125, 4th ed., 2005: Thermotolerant coliform bacteria and *Escherichia coli*. Enumeration in food and feed.

NMKL Method No. 136, 4th ed., 2007 (Corrections 2008): *Listeria monocytogenes*. Detection in foods and feeding stuffs and enumeration in foods.

Olafsdottir, G, Lauzon, HL, Martinsdóttir E, Oehlenschläger J, 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. J Food Sci 71(2):97-109.

Reynisson E, Lauzon HL, Magnusson H, Hreggviðsson GÓ, Marteinsson VT. 2008. Rapid quantitative monitoring method for the fish spoilage bacteria *Pseudomonas*.J Env Monit. 10(11):1357-1362

Reynisson E, Lauzon HL, Magnusson H, Jonsdottir R, Olafsdottir G, Marteinsson VT, Hreggvidsson GO. 2009. Bacterial composition and succession during storage of North-Atlantic cod (*Gadus morhua*) at superchilled temperatures. BMC Microbiol 9(1):250.

Reynisson E, Lauzon HL, Thorvaldsson L, Margeirsson B, Runarsson AR, Marteinsson V, Martinsdóttir E. 2010. Effects of different cooling techniques on bacterial succession and other spoilage indicators during storage of whole, gutted haddock (*Melnogrammus aeglifinus*). Eur Food Res Technol 231(2):237-246.

Rha, C. 1975. Thermal properties of foods. In RHA, C. (Ed.) Theory, Determination and control of physical properties of food materials. Dordrecht, Boston, Reidel Publishing.

Shewan JM, Macintosh RG, Tucker CG, Ehrenberg ASC. 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J Sci Food Agric 4:283-298.

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.

Statistics Iceland. 2010. Export of fresh fish by mode of transport 1988-2009. *Export in goods.*http://www.statice.is/?PageID=1261&src=/temp_en/Dialog/varval.asp?ma=UTA02407%26ti=Export+of+fresh+fish+by+mode+of+transport+1988%2D2009%26path=../Database/utanrikisverslun/UtflutningurAR/%26lang=1%26units=Fob [Accessed 28 June 2010]. Reykjavík, Iceland.

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

Tryggvadóttir SV, Ólafsdóttir G. 2000. Multisensor for Fish. Questionnaire on quality attributes and control methods texture and electronic nose to evaluate fish freshness. IFL-Report 04-00. 25 pages.