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The effect of different cooling techniques and temperature fluctuations on the storage life of cod fillets *(Gadus morhua)*

Hannes Magnússon
Hélène L. Lauzon
Kolbrún Sveinsdóttir
Björn Margeirsson
Eyjólfur Reynisson
Árni Rafn Rúnarsson
María Guðjónsdóttir
Kristín Anna Þórarinsdóttir
Sigurjón Arason
Emilía Martinsdóttir

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Höfundar / Authors	*Hannes Magnússon, Hélène L. Lauzon, Kolbrún Sveinsdóttir, Björn Margeirsson, Eyjólfur Reynisson, Árni Rafn Rúnarsson, María Guðjónsdóttir, Kristín Anna Þórarinsdóttir, Sigurjón Arason, Emilía Martinsdóttir*		
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Ágrip á íslensku:

Tilgangur tilraunanna var að kanna tvo kælimiðla um borð í veiðiskipi, að nota mismunandi kælitækni við vinnslu, m.a. svonefnda CBC (combined blast and contact) kælingu og kanna áhrif hitastigssveiflna við geymslu í samanburði við stöðuga geymslu við -1 °C.

Lítill munur var á örveru- og efnamælingum hvort sem notaður var plötuís eða vökvaís fyrir vinnslu en samkvæmt skynmati reyndist hópurinn sem var kældur með vökvaís hafa eins dags lengri ferskleikatíma og geymsluþol. Hitastig var yfirleitt aðeins hærra í hópnum þar sem plötuís var notaður fyrir vinnslu yfir geymslutímann.

Samkvæmt skynmati, örverutalningum og efnamælingum reyndist CBC kæling best til lengingar á ferskleikatíma og geymsluþoli. Hitastig reyndist vera lægra í þeim hópum þar sem CBC kæling var notuð.

Örverufjöldi var svipaður í þeim tveimur hópum þar sem CBC kæling var ekki notuð við vinnsluna (vökvakæling og engin kæling). Þessar niðurstöður voru í samræmi við niðurstöður skynmats. TMA gildi voru aðeins hærri á geymsludögum 12-19 í hópnum sem var vökvakældur. Niðurstöður hitastigsmælinga yfir geymslutímann voru svipaðar.

Svipaður örverufjöldi reyndist vera í hópum sem geymdir voru við stöðugt hitastig (um -1 °C) annars vegar og í hópum þar sem hitastigssveiflum var beitt fyrri hluta geymslutímans hins vegar. Fyrstu 15 daga geymslunnar reyndust TVB-N og TMA gildi vera svipuð í hópunum. Þeir hópar sem geymdir voru við stöðugt hitastig fóru ekki í skynmat.

Örverumælingar sem gerðar voru með hinni fljótvirku aðferð qPCR voru í góðu samræmi við ræktunaraðferðir m.t.t. til *Pseudomonas* spp. og *Photobacterium phosphoreum.*

Lykilorð á íslensku: *Kælitækni, hitasveiflur, þorskflök, ferskleiki, geymsluþol*

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Summary in English:

The purpose of this experiment was to examine two different cooling methods on board fishing vessel, to apply different cooling techniques during processing at fish plant including the CBC (combined blast and contact) cooling and to compare storage of packed cod fillets kept either at steady temperature (-1 °C) or under temperature fluctuations.

No marked difference was seen in microbial and chemical measurements whether plate ice or liquid ice was used prior to filleting but according to sensory analysis, the experimental group where liquid ice was used had one day extension in freshness and shelf life compared to the group with plate ice. Temperature was usually slightly higher in the plate ice group than the liquid ice group during storage.

According to sensory, microbiological and chemical analysis, the CBC cooling clearly resulted in longer freshness period and shelf life extension in comparison to the two groups where this technique was not applied during processing. Temperature was lower in these groups during the storage period.

Similar microbial counts were found between the two experimental groups where CBC was not applied during processing (liquid cooling and no cooling). These results were in agreement with results from sensory analysis. TMA values were however higher on storage days 12 to 19 in the group with liquid cooling. Temperature measurements during storage of these two groups were very similar.

No marked difference was seen in microbial counts between groups that were stored at a constant temperature around -1 °C compared to groups where temperature fluctuations were used during early phases of storage. During the first 15 days of storage, TVB-N and TMA values were very similar for these groups. Sensory analysis was not done on the two groups kept at -1 °C.

The rapid qPCR analysis was generally in good agreement with the cultivation methods for *Pseudomonas* spp. and *Photobacterium phosphoreum.*

English keywords: *Cooling techniques, real temperature simulation, cod fillets, freshness, shelf life*

Table of Contents

1 INTRODUCTION

Rapid cooling after catch and maintenance of low temperature throughout the whole chain from catch to consumer is the prerequisite of high quality and long shelf life of fish products.

During the last few years, Skaginn, Akranes has been developing and designing a new method in the area of fish processing which is now patented. This process is called Combined Blast and Contact (CBC) cooling. The technique involves superchilling of the skin side of fillets by moving them through a freezer tunnel on a teflon coated aluminium conveyor belt which has 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 pre-cooler/fluid-ice which contains approximately 2.5% salt and because of the salt content, the fillets can go through the CBC process without freezing the flesh. This superchilling process facilitates handling of the fillets, in particular skinning and effective cooling with a resulting fillet temperature around -1 °C when packed.

The purpose of this experiment was to examine two different cooling methods on board the fishing vessel, to apply different cooling techniques during processing at fish plant including the CBC cooling and to compare storage of packed cod fillets kept either at steady temperature (-1 °C) or under temperature fluctuations.

2 MATERIAL AND METHODS

2.1 Experimental design

Cod used in the experiment was caught by a long liner SW-of Iceland (Jökuldýpi) on Oct-8-2008. After bleeding and gutting the cod was washed in sea-water cooled with liquid ice on deck. Then the fish was transported to the hold where it was on one hand iced with plate ice and the other liquid ice in tubs.

The cod was landed on Oct-9-2008 and processed at a fish plant near Reykjavík Oct-10-2008. The fish was filleted and fillets with skin-on got different treatment (see below) prior to packaging in 5 kg Styrofoam boxes (8 fillets per box). After packaging the cod was transported to Matís ohf were it was stored either at around -1 °C or under real temperature simulation for up to 22 days from catch (20 days from packaging). The purpose of the real temperature simulation is to simulate temperature fluctuations in the cold chain for exported fresh fish that might take place during storage after processing and during transport to airport, loading, flight, unloading and possible poor storage abroad.

The experimental groups were as follows:

A. Plate ice pre-cooling on-board, liquid cooling and combined blast and contact cooling at plant, storage at -1 °C at Matís.

B. Plate ice pre-cooling on-board, liquid cooling and combined blast and contact cooling at plant, storage under real temperature simulation at Matís.

C. Plate ice pre-cooling on-board, liquid cooling at plant, storage under real temperature simulation at Matís.

D. Plate ice pre-cooling on-board, no cooling (control) at plant, storage under real temperature simulation at Matís.

E. Liquid ice pre-cooling on-board, liquid cooling and combined blast and contact cooling at plant, storage at -1 °C at Matís.

F. Liquid ice pre-cooling on-board, liquid cooling and combined blast and contact cooling at plant, storage under real temperature simulation at Matís.

Following abbreviations of experimental groups will be used hereafter:

A. PI, LC-CBC, -1°C
B. PI, LC-CBC, RTS
C. PI, LC, RTS
D. PI, NC, RTS
E. LI, LC-CBC, -1°C
F. LI, LC-CBC, RTS

2.2 Temperature measurements

Temperature at the time of packaging was measured with a handheld thermometer, type TFX410 (Ebro Electronic, Ingolstadt, GER), with a resolution of 0.1 °C and accuracy of ±0.3 °C. Two types of temperature loggers were used for the temperature measurements. For measurements of the product temperature inside the wholesale fish boxes, loggers of type iButton DS1922L with an accuracy of ±0.5 °C, a resolution of 0.0625 °C and an operating range from -40 to +85 °C were used. Product temperature was measured close to the centre of the fillet stack in each of the one to three boxes of each experimental group, which were investigated. Product temperature was recorded at 5 - 10 minutes intervals and read from the loggers at the end of the experiment.

In order to measure the ambient temperature, TidbiT v2 temperature loggers (Onset Computer Corporation) were used. These have an accuracy of ±0.2 °C, a resolution of 0.02 °C and an operating range from -20 to +70 °C. Two ambient loggers were applied right after packaging and thereby the temperature conditions from packaging during transport to the air climate chambers at Matis were yielded. A total of three and six ambient temperature loggers were used for the steady storage temperature groups and dynamic storage temperature groups, respectively. In both cases the loggers were distributed inside the chamber in order to grasp spatial temperature differences. Temperature was recorded at 3 - 5 minutes intervals and read at the end of the experiment.

2.3 Sensory evaluation

Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (2004), was used to assess cooked samples (MA08sky055-56, 59-61, 63-65, 69) of cod. Eleven panellists all trained according to international standards (ISO 1993); including detection and recognition of tastes and odours, trained in the use of scales and in the 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. 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 and others 2009). The sensory attributes were 30 and are described in Table 2.

Samples weighing ca. 40 g were taken from the loin part of the fillets 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 nine sessions (four samples per session). A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystémes) was used for data recording.

All groups evaluated with sensory evaluation were real temperature simulated (RTS). The following experimental groups were evaluated with sensory evaluation:

B. PI, LC-CBC, RTS
C. PI, LC, RTS
D. PI, NC, RTS
F. LI, LC-CBC, RTS

Table 1. 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, characterict fresh odour
meaty	o-meat	meaty odour, reminds of boiled meat or halibut
vanilla, boiled milk	o-vanilla	vanilla, sweet boiled milk
boiled potatoes	o-potatoes	odour reminds of whole, warm, boiled potatoes
frozen storage	o-frozen	reminds of odour found in refrigerator and/or freezing compartment
table cloth	o-cloth	reminds of damp, unclean cloth (left on kitchen table for 36 h)
TMA	o-TMA	TMA odour, reminds of dried salted fish, amine
sour	o-sour	sour odour, spoilage sour, acetic acid
sulphur	o-sulphur	sulphur, matchstick, boiled kale
Appearance		
light/dark colour	a-dark	Left end: light, white colour. Right end: dark, yellowish, brownish, grey
homogenous/	a-heterog.	Left end: homogenous, even colour.
heterogeneous		Right end: discoloured, heterogeneous, stains
white precipitation	a-prec.	white precipitation in the broth or on the fish
Flavour		
salt	f-salt	salt taste
metallic	f-metallic	metallic flavour
sweet	f-sweet	characteristic sweet flavour of very fresh (boiled) cod
meaty	f-meat	meaty flavour, reminds of boiled meat
frozen storage	f-frozen	reminds of food which has soaked in refrigerator/freezing odour
pungent	f-pungent	pungent flavour, bitter
sour taste	f-sour	sour taste, spoilage sour
TMA	f-TMA	TMA flavour, reminds of dried salted fish, amine
off flavour	f-off	strenght of off flavour (spoilage flavour/off-flavour)
Texture		
flakiness	t-flakes	the fish portion slides into flakes when pressed with the fork
firm/soft	t-soft	Left end: firm. Right end: soft.
		Evaluate how firm or soft the fish is during the first bite
dry/juicy	t-juicy	Left end: dry. Right end: Juicy.
		Evaluated after chewing several times: dry - pulls juice from the mouth
tough/tender	t-tender	Left end: tough. Right end: tender. Evaluated after chewing several times
mushy	t-mushy	mushy texture
meaty	t-meaty	meaty texture, meaty mouth feel, grude muscle fibers
clammy	t-clammy	clammy texture, dry red wine, tannin
rubbery	t-rubbery	rubbery texture, springy

Data analysis. QDA data was corrected for level effects (effects caused by level differences between assessors and replicates) by the method of Thybo and Martens (2000). Principal Component Analysis (PCA) on significant mean level corrected values of sensory attributes and samples was performed, using full cross validation. Analysis of

variance (ANOVA) was carried out on QDA data corrected for level effects 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.

2.4 Microbial measurements

In all counts surface-plating was used. 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. TVC were also done on modified Long and Hammer´s agar (mLH) according to van Spreekens (1974) with the exception that 1% NaCl was used instead of 0.5%. Plates were incubated at 15-17 °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 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. All samples were analysed in duplicate and results presented as an average.

2.5 Quantitative PCR measurements

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 Mx3000p instrument. The PCR for *Pseudomonas* spp. was done using Brilliant SYBR green II mastermix and the Brilliant QPCR mastermix was used for *Photobacterium phosphoreum* (Stratagene, La Jolla, CA, USA). Primers were synthesized and purified with HPLC (MWG, Ebersberg, Germany). The reaction volume was 25 µl with 200 nmol l-1 for primer concentration. The thermal profile was as follows: 95 °C for 10 min followed by 40 cycles at 95 °C for 30 s, 57 °C for 60 s and an extension step at 72 °C for 30 s. After the PCR a dissociation curve was carried out where the instrument went at 2 °C min-1 from 55 °C to 95 °C with continuous fluorescence readings.

The DNA standard used for *Pseudomonas* quantification was previously calibrated against viable cell counts on CFC agar. Calibration of the *Photobacterium phosphoreum* standard was done against the PPDM-Malthus conductance method previously described.

2.6 Chemical analysis

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

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 trichloracetic 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. All chemical analyses were done in duplicate.

2.6.2 pH- measurements

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).

2.6.3 Salt content and water content

The water content of each fillet 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 hours. 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 ed. 17 from 2000 (no. 976.18).

2.7 Water holding capacity (WHC)

The water holding capacity (WHC) of the samples was measured with the centrifugal method described by Eide et al. (1982). Approximately 2 g of minced cod was weighed into each sample glass and centrifuged for 5 minutes with a rotational speed of 3600 rpm. Four replicates were evaluated for each sample. During the centrifugation water was removed from the sample. The water drained through a polyester membrane in the bottom of the sample holder where it was collected. The water holding capacity was then calculated with the equation:

$$WHC(\%) = \frac{Water\quad content(\%) - Weight\quad loss(\%)}{Water\quad content(\%)}$$

where the weight loss is defined as

$$Weight\quad loss(\%) = \frac{Weight\quad loss\quad in\quad centrifuge(g)}{Original\quad sample\quad weight(g)} \times 100$$

2.8 Drip measurements

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 water lost during storage to the original weight of the fish.

3 RESULTS AND DISCUSSION

3.1 Temperature measurements

3.1.1 Ambient temperature from processor to Matis

The ambient temperature measured with two different temperature loggers is depicted in Figure 1. The packaged fresh fish fillets were temperature abused for approximately two hours after packaging. The mean ambient temperature during this period was 11.7 °C.

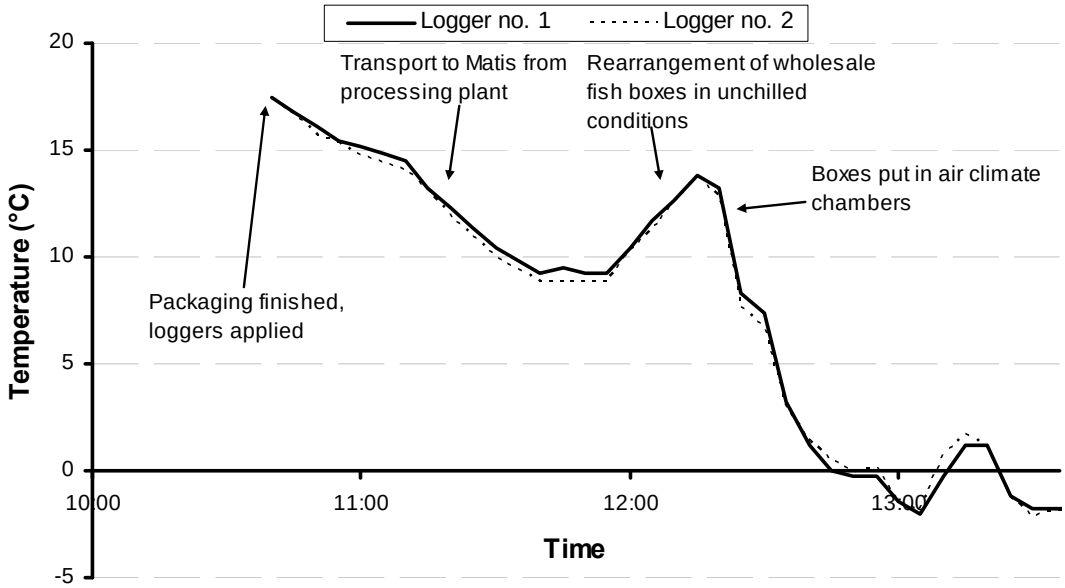

Figure 1. Ambient temperature from the time of packaging at processor until boxes were placed in controlled ambient conditions at Matis.

3.1.2 Steady temperature conditions

The air temperature was very well controlled as can be seen in Figure 2, showing the ambient air temperature at three different locations inside the steady temperature chamber. The mean ambient temperature from the moment the boxes were put inside the steady chamber until the end of the experiment was -1.3 ± 0.2 °C.

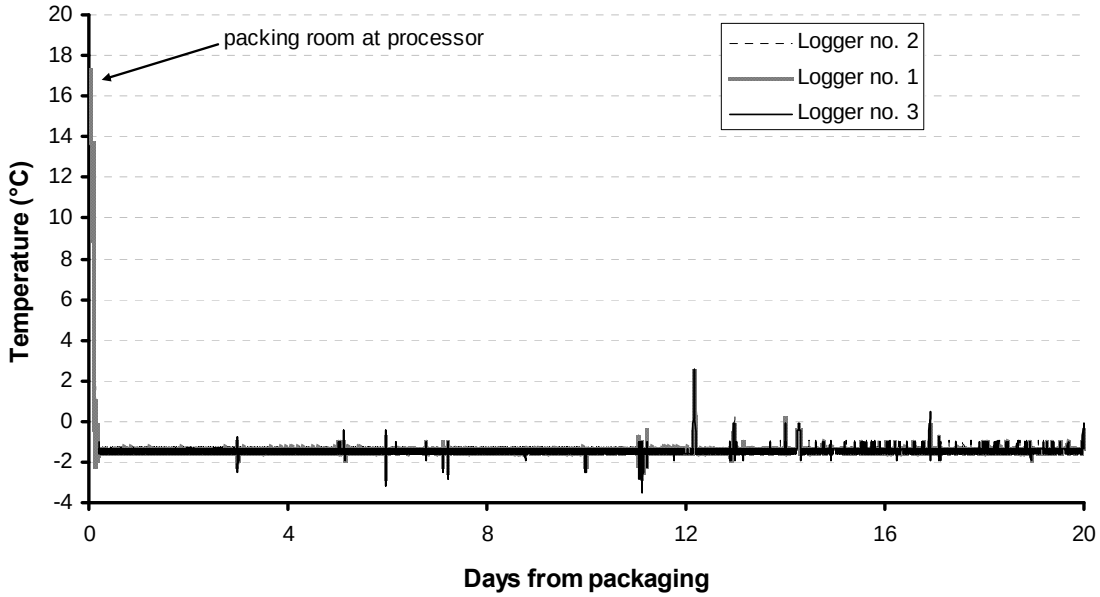

Figure 2. Ambient temperature measured at three different locations during steady storage inside an air climate chamber.

The mean product temperature of groups A and E evolved as shown in Figure 3. The product temperature for these two CBC chilled experimental groups at the time of packaging was -0.7 ± 0.2 °C. As seen in Figure 3, the product temperature was not severely affected by the thermal load from packaging until the boxes were put into the steady ambient conditions in the climate chamber. The mean product temperature over the period from packaging to the end of the experiment was -0.1 ± 0.4 °C and -0.0 ± 0.5 °C for groups A and E, respectively. Interesting is the product temperature increase, which occurs in both groups, from ca. -0.8 °C at the beginning of storage to 0.2 – 0.6 °C at the end of storage despite an ambient temperature of -1.3 °C.

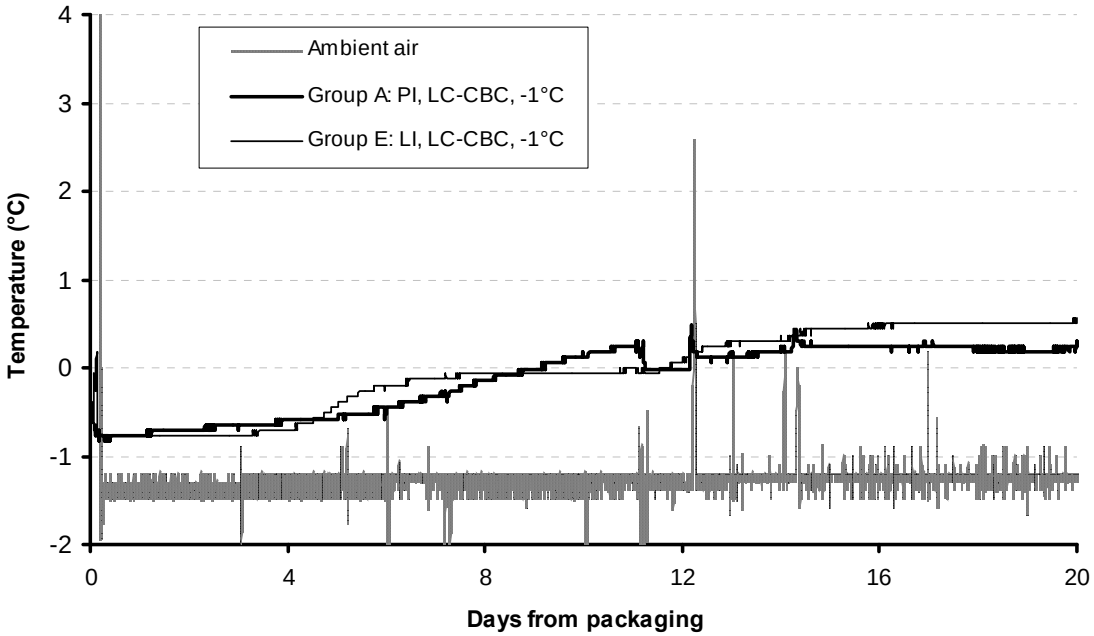

Figure 3. Product temperature for groups A and E during steady temperature storage. Also shown is the mean ambient temperature calculated from three different locations.

3.1.3 Real temperature simulation

The ambient temperature during the real temperature simulation is shown in Figure 4, i.e. the mean temperature at five different locations at the chamber walls. Also shown is the air temperature above the fish boxes in the chamber centre during the latter part of the experiment. The temperature conditions were made dynamic by controlling the mean ambient temperature at 10.2 ± 1.6 °C for 7.5 hours in the beginning and at 9.7 ± 1.1 °C for 16 hours after 2-3 days of storage.

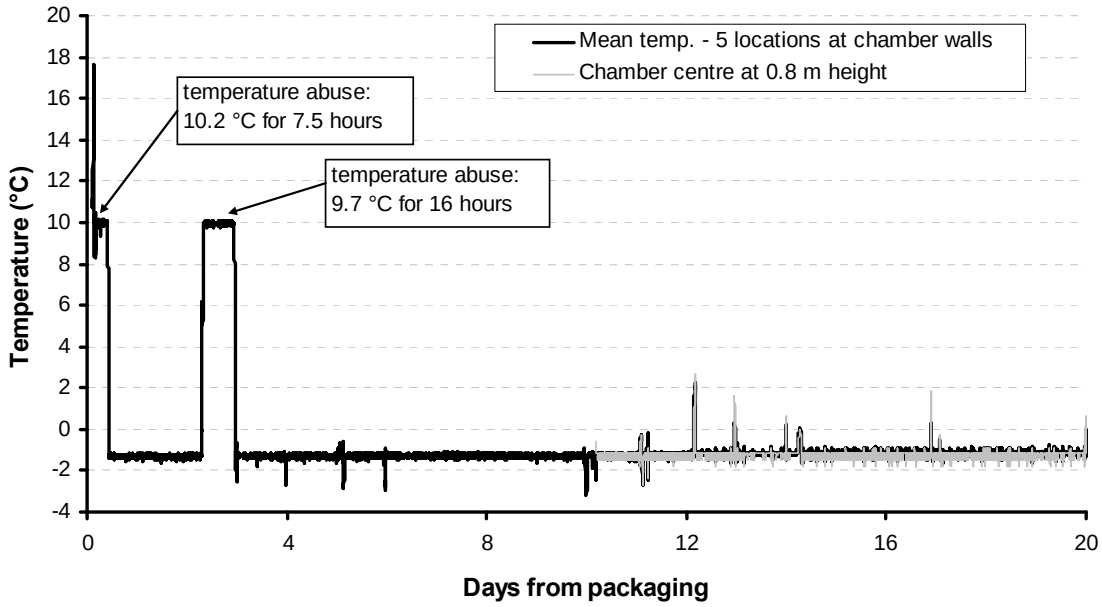

Figure 4. Ambient temperature during simulation of real temperature in a cold chain for fresh fish. The mean temperature at five different locations surrounding the boxes is shown along with the air temperature above the wholesale fish boxes at the climate chamber centre.

The product temperature for the CBC chilled groups (B and F) was -0.7 ± 0.2 °C at the time of packaging but significantly higher for the non-CBC chilled groups, i.e. 1.5 ± 0.5 °C. The fact that the temperature after processing was very similar for groups C and D, i.e. liquid cooling vs. no cooling in processing, implies that the liquid cooling did not work as planned in this trial. The reason was that the cooling liquid used for the liquid cooling process did not contain sufficient amount of ice slurry. The product temperature for the four thermally abused groups (B-C-D-F) is presented in Figure 5. The CBC-cooling proves to be an efficient way to protect the fillets against temperature abuse since the product temperature is almost not affected at all during the first abuse for the two CBC-chilled groups (B and F). The temperature abuse, which occurred from processing until the boxes were at Matís (depicted in Figure 1), obviously raised the product temperature for the two un-CBC-chilled groups C and D. Furthermore, during the latter thermal load, the temperature increase is lower for the two CBC-chilled groups than for the two groups not chilled with CBC. The mean product temperature for the four groups during the whole experiment is given in Table 2 along with the standard deviation.

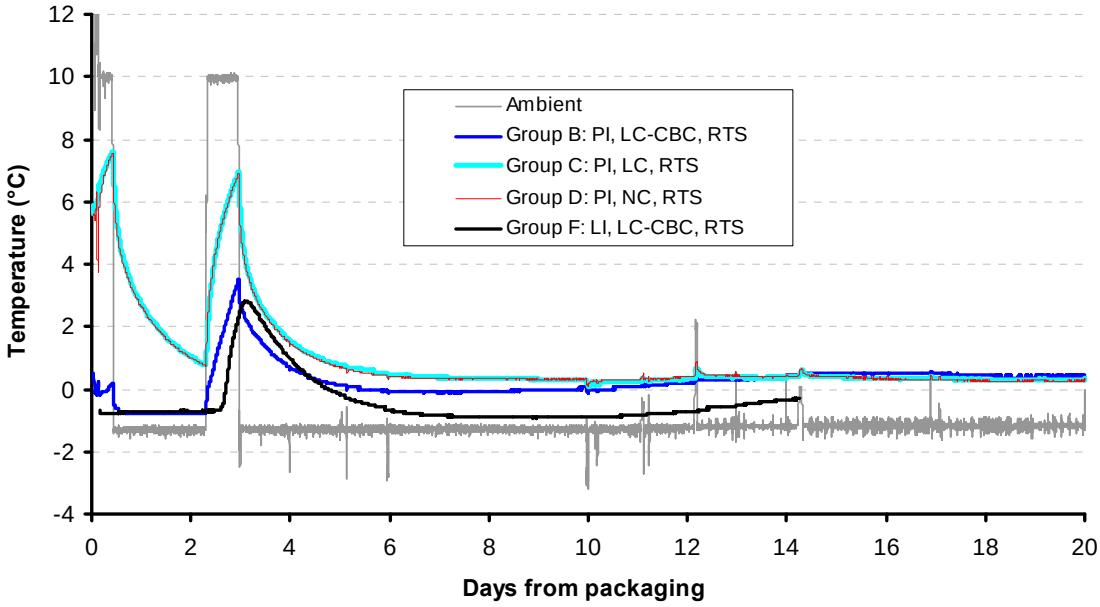

Figure 5. Product temperature in four groups (B-C-D-F) stored at dynamic temperature conditions. Also shown is the mean ambient temperature profile.

Table 2. Product temperature in °C during real temperature simulation.

Group	Mean	St. dev.
B: PI, LC-CBC, RTS	0,2	0,6
C: PI, LC, RTS	0,6	1,6
D: PI, NC, RTS	0,9	1,4
F: LI, LC-CBC, RTS	-0,4	0,8

3.2 Sensory evaluation

Sensory attributes describing significant differences between the samples with storage time were 29 (all except o-potatoes). The PCA (Figure 6) shows an overview of how the significant sensory attributes described the samples, explaining altogether 89% of the sensory variation between the samples in the first and second principal components (PC1 and PC2). The figure shows how the sample groups change with storage time along the first principal component (PC1). At the beginning of storage, the plate ice cooling (PI, LC-CBC-02d) and the liquid ice cooling (LI, LC-CBC-02d) groups were very similar, described with high intensities of odours and flavours characteristic for very fresh cod; sweet, shellfish, vanilla and meat odours, and metallic, sweet and meat flavours. After

five days, these characteristics were slightly less evident, except in the liquid ice cooling group with CBC cooling (LI, LC-CBC), which in addition was more described with light colour (opposite to a-dark). Similar development was noted for these characteristics after eight days of storage, but the difference between the groups along PC1 indicated the groups with liquid cooling (PI, LC) and no cooling (PI, NC) were less described with these characteristics. After 12 days of storage, the group with liquid cooling (PI, LC) and especially the group with no cooling (PI, NC) were clearly more described with sensory attributes describing spoilage, such as TMA and sour odours, off-flavour, sour and TMA flavours. After 15 days of storage, both groups with CBC cooling (LI, LC-CBC-15d and PI, LC-CBC-15d) had similar sensory characteristics as the sample with liquid cooling on day 12 (PI, LC-12d). After 19 days of storage, the samples were mainly described with spoilage attributes.

The vertical axis is the second principal component (PC2) and mainly shows the difference between samples in texture. At the day of processing, the plate ice cooling and liquid ice cooling groups (PI, LC-CBC-02d and LC, LC-CBC-02d) were very similar, but after 5 and 8 days of storage the liquid ice pre-cooling group (LC, LC-CBC) was more described with meaty, clammy and rubbery texture as compared to plate ice pre-cooling groups. The plate ice pre-cooling groups were however more described with tender, mushy, soft and juicy texture as compared to the liquid ice cooling group. These texture differences between the groups diminished with the storage time.

The average sensory results are shown in Table 3 a – 3 d in Appendix 1.

Up to 12 days of storage, the groups were generally described with very sweet and shellfish odours, with evident meet, vanilla and potatoes odours. No frozen storage, table cloth, TMA, sour or sulphur odours were detected in the groups with CBC after cooling, but a hint of table cloth and TMA odours were detected in the PI, LC and PI, NC groups after eight days of storage. On day 12, sweet odour was still evident in the groups with CBC cooling, but hints of table cloth, TMA and sour odours were detected. Odour of table cloth and TMA odour were obvious in the groups with liquid or no cooling after 12 days, but first after 15 days in the groups with CBC after cooling. After 19 days, both groups with CBC cooling were mainly described with odour attributes describing spoilage: table cloth, TMA sour and sulphur.

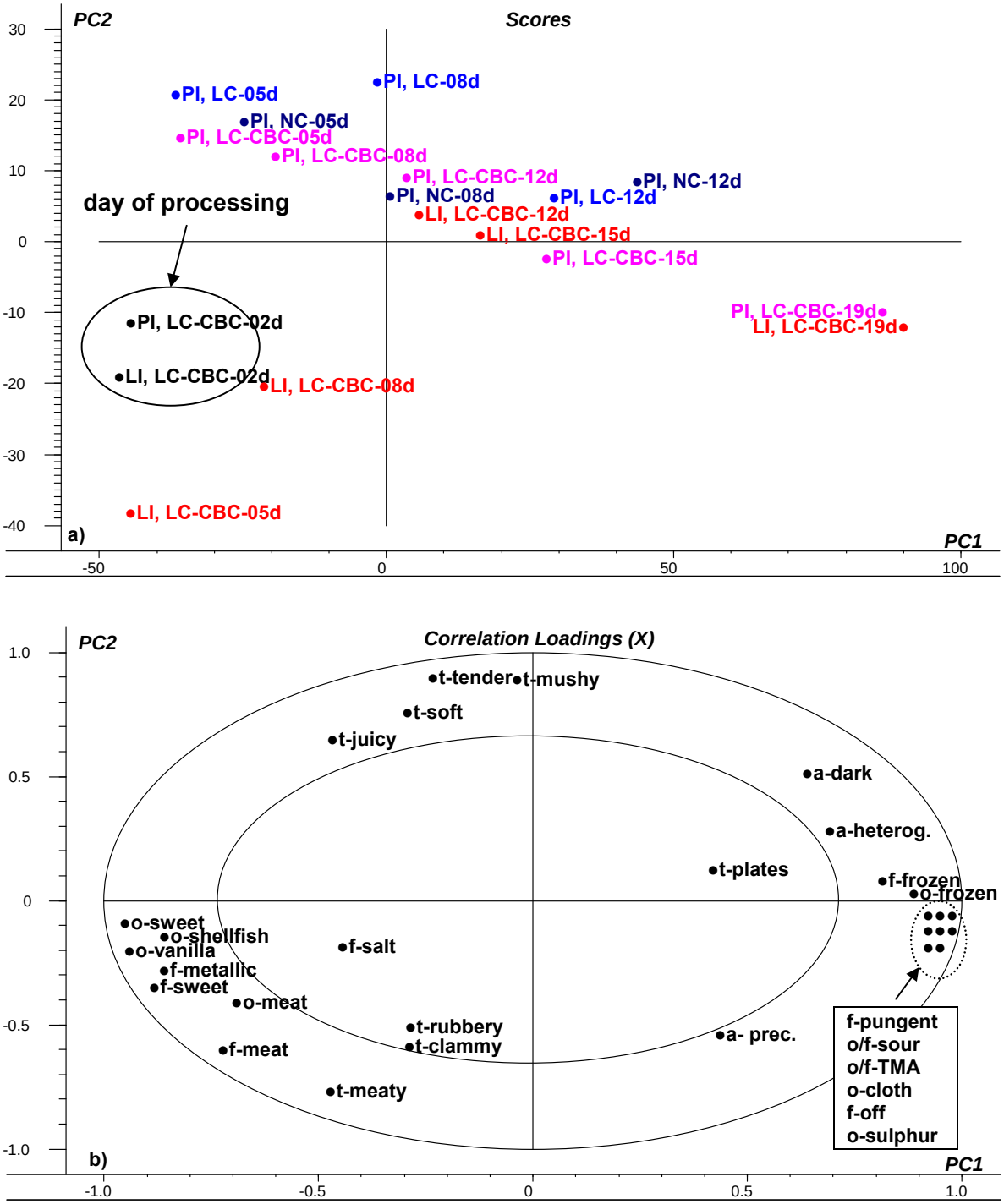

Figure 6. Scores (a) and correlation loadings (b) describing odour (o-), appearance (a-), flavour (f-) and texture (t-) of cod samples during different storage time in days (d). PC1 vs. PC2 (X-expl.: 77% and 12%). Ellipses mark the 50% and 100% explained variance limits.

The groups had rather light and homogenous colour, but the group with liquid ice pre-cooling and CBC cooling (LI, LC-CBC) had generally lighter colour compared to other groups up to day 15.

A hint of salt flavour was detected in the groups with CBC cooling after five days of storage, but salt flavour or frozen storage flavours were generally not detected in the groups. Up to 12 days of storage, the groups were mainly described with metallic and sweet flavours, but meat flavour was also obvious of the group with liquid ice pre-cooling with CBC cooling. Hints of TMA flavour and off-flavour was detected in PI, LC after eight days, but PI, NC had a hint of pungent and TMA flavour and obvious off-flavour after eight days. On day 12, the freshness characteristic flavours were still detectable in groups with CBC cooling, such as metallic flavour, but also hint of pungent, sour, TMA flavours and off-flavour. However, TMA flavour and off-flavour had become obvious in PI, LC and were very high in PI, NC. After 15 days, hints of TMA flavour and off-flavour were detected in LI, LC-CBC, but these characteristics were obvious in PI, LC-CBC on that day. After 19 days, the flavour of PI, LC-CBC and LI, LC-CBC was dominated by sour and TMA flavours and off-flavour.

The groups had generally flaky texture, which did not change much with storage time. At the beginning of storage time, the groups with plate ice and liquid ice pre-cooling did not differ in texture. On day five, the samples were generally more soft, juicy, tender and mushy compared to the first sampling day. The group with liquid ice pre-cooling and CBC cooling (LI, LC-CBC) was less soft, tender and mushy, but more meaty and clammy compared to other groups. The group with plate ice pre-cooling and liquid cooling (PI, LC) was the juiciest. On day eight, a decrease in soft, juicy, tender and mushy texture was noted in most groups, and again, the group with liquid ice pre-cooling and CBC cooling (LI, LC-CBC) was the least soft, juicy, tender and mushy sample, but the most meaty and clammy, and was in addition more rubbery. No significant differences were noted between groups on day 12, 15 or 19, but a general decrease in soft, juicy, tender and mushy texture was noted with storage time.

Figure 7 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. PI, NC

has reached these limits after 5-8 days, PI, LC after 6-7 days, PI, LC-CBC after 6-8 days but LI, LC-CBC after 8-9 days.

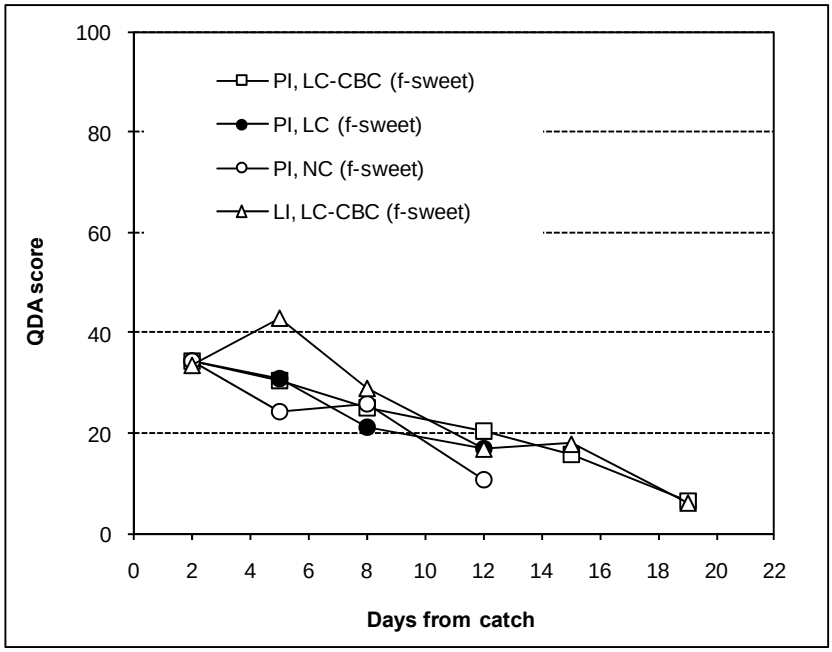

Figure 7. Average QDA scores of odours and flavours related to spoilage for groups approaching/ past shelf life.

Figure 8 shows odour and flavour attributes related to spoilage. 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 (Magnússon and others 2006). According to these criteria, PI, NC and PI, LC was approaching the end of shelf life after eight to nine days due to spoilage related odour and flavour attributes. Both these groups were past shelf life on day 11-12 due high scores of table cloth, sour and TMA odours, off-flavour and TMA flavours. PI, LC-CBC had scores around 20 for table cloth and TMA odours and around or above 20 for off-flavour and TMA flavour on day 15. LI, LC-CBC had slightly lower scores for these attributes on day 15, but had scores above 20 for table cloth odour and off-flavour.

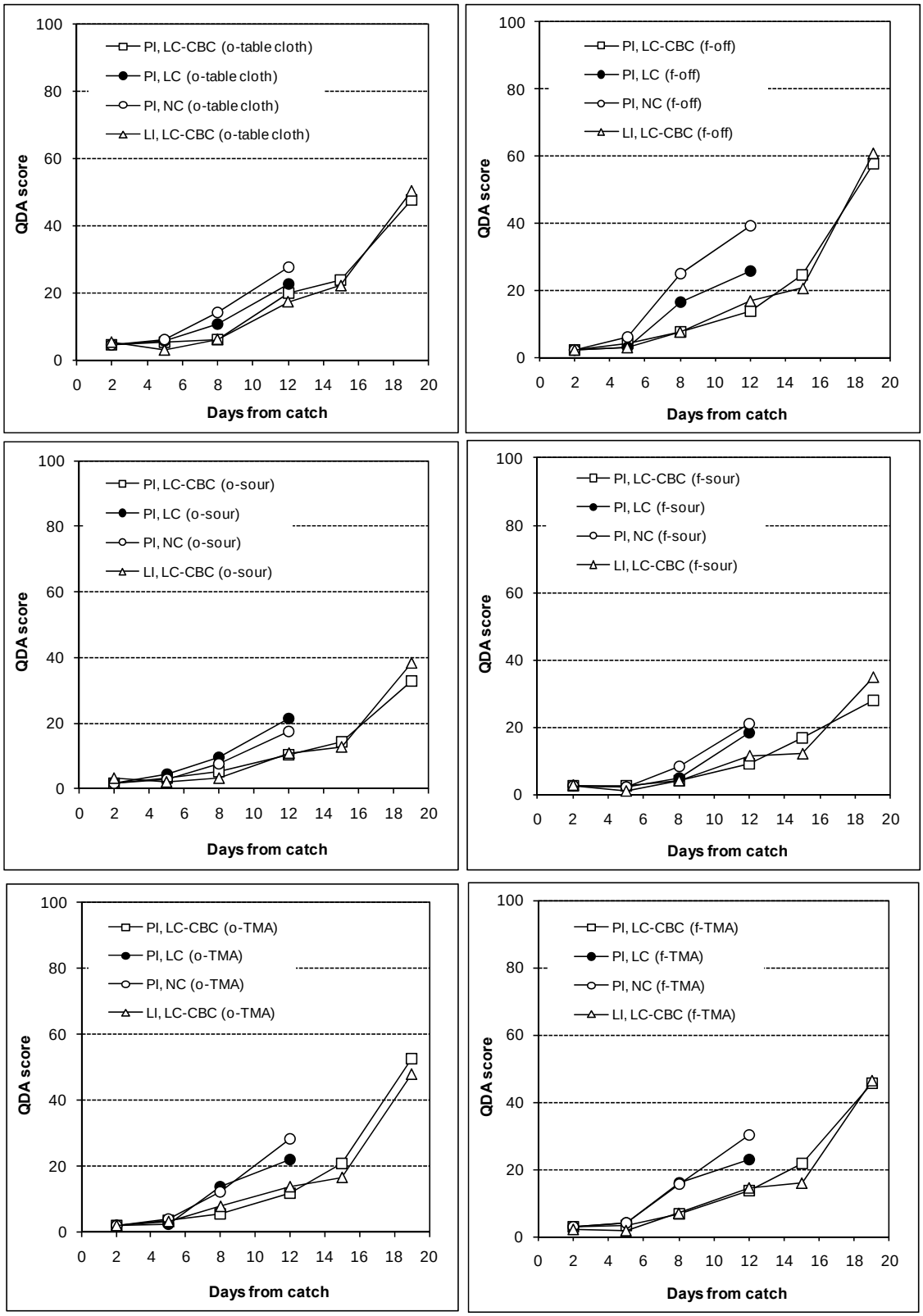

Figure 8. Average QDA scores of odours and flavours related to spoilage for groups approaching/ past shelf life.

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 4.

Table 4. Freshness period and maximum shelf life according to sensory evaluation

Group	freshness period	shelf life
PI, LC-CBC	6-8 days	14-15 days
PI, LC	6-7 days	9-12 days
PI, NC	5-8 days	8-11 days
LI, LC-CBC	8-9 days	15-16 day

3.3 Microbial measurements

Results from microbial counts are shown in Figures 9 to 12. Total viable counts (TVC) on Iron agar for all experimental groups are shown in Figure 9. TVC were also done on mLH agar but results are not shown since high correlation was found between the two media (R^2 =0.98). Initial counts on day 2 were around log 4-5/g. On days 8 and 12 highest counts (>1 log/g on day 8) were obtained in groups PI, LC, RTS and PI, NC, RTS or in those two groups were combined blast and contact cooling (CBC) was not applied. Thereafter, similar counts were generally obtained in all groups, being about log 8-9/g in all groups on day 19 apart from one high point for group PI, LC-CBC, -1°C. Similar growth curves were seen for the other four experimental groups, indicating no marked difference between plate or liquid ice pre-cooling and whether the fillets were kept at around -1 °C or under real temperature simulation (RTS).

Results from counts of H_2S-producing bacteria and pseudomonads are shown in Figures 10 and 11. Growth curves for these two groups were similar apart from higher initial counts of pseudomonads. As for TVC, highest counts were most often seen in experimental groups PI, LC, RTS and PI, NC, RTS were CBC cooling was not applied. This was especially noticeable on day 8.

Growth curves for *Photobacterium phosphoreum* are shown in Figure 12. Lower numbers were generally seen for *P. phosphoreum* than for other bacterial groups. The number of

this bacterium were clearly higher in the experimental groups PI, LC, RTS and PI, NC, RTS on all sampling days up to day 19. As for TVC, similar growth curves for H_2S-producing bacteria, pseudomonads and *P. phosphoreum* were seen in the other four experimental groups, indicating no marked difference between plate or liquid ice pre-cooling or whether the fillets were kept at around -1 °C or under RTS.

It is evident that the use of the combined blast and contact cooling technique during processing can lead to slower microbial growth, especially during the early stages of chilled storage, which in turn can result in extended shelf life of cod fillets.

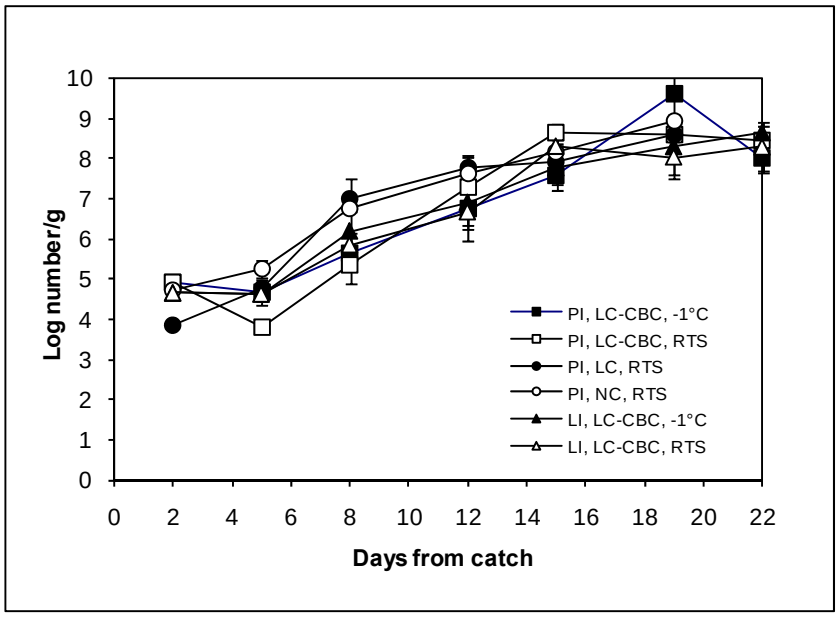

Figure 9. Total viable counts (TVC) on iron agar in cod fillets. Average values of duplicate samples are shown. Error bars show SD. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

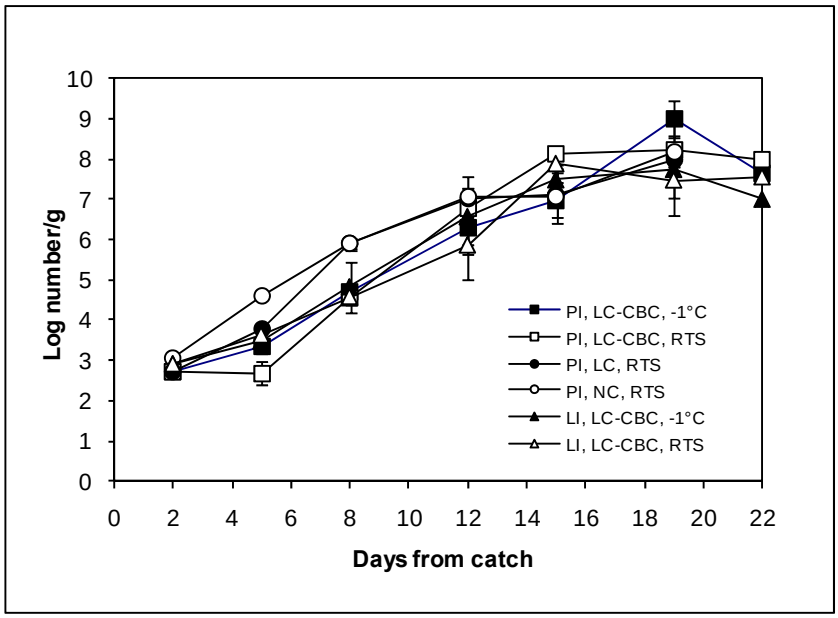

Figure 10. Growth of H_2S-producing bacteria on iron agar in cod fillets. Average values of duplicate samples are shown. Error bars show SD. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

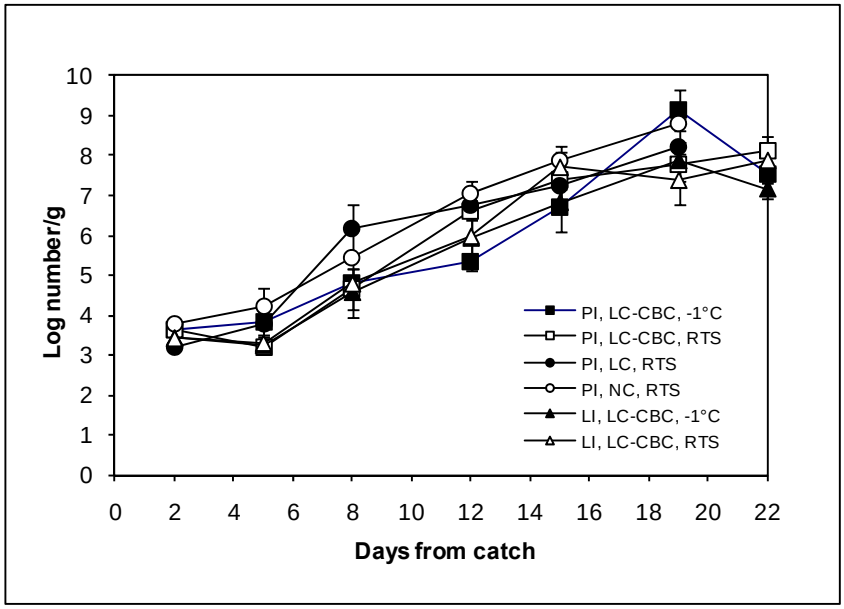

Figure 11. Growth of presumptive pseudomonads in cod fillets. Average values of duplicate samples are shown. Error bars show SD. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

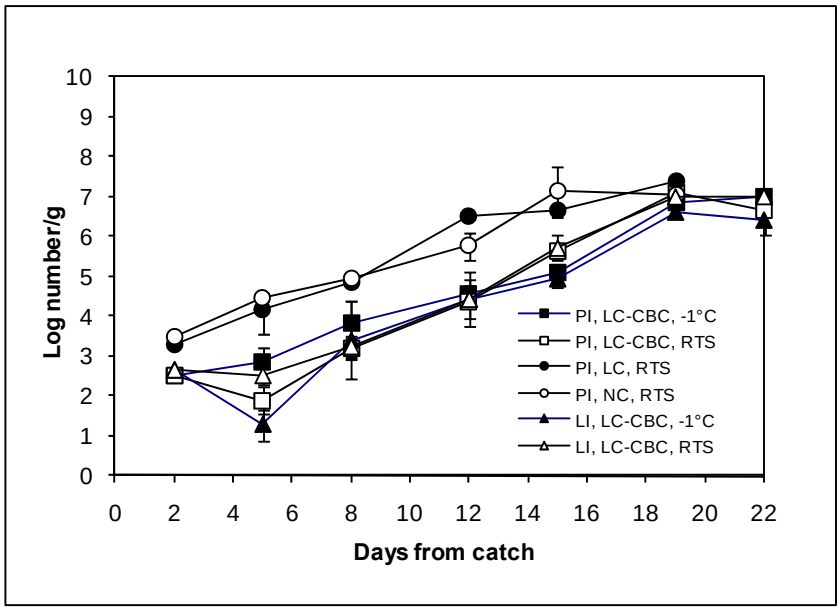

Figure 12. Growth of *Photobacterium phosphoreum* in cod fillets. Average values of duplicate samples are shown. Error bars show SD. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

3.4 Quantitative PCR analysis on spoilage microbes

The qPCR analysis was generally in good agreement with cultivation as shown in a correlation plot where all data points from the storage trial are plotted (Figure 13). The correlation and accuracy between the qPCR and the reference method was slightly better in the *Pseudomonas* assay (slope=0.9901, R^2=0.936) than the *Photobacterium phosphoreum* assay (slope=0.9826, R^2= 0.8347).

The growth trend for pseudomonads was quite similar between all the test groups where the raw material contained 3.3-3.7 Log10 CFU/g and increased gradually in numbers up to 7.3-8.4 Log10 CFU/g as a result of the qPCR analysis. It showed a slight increase of pseudomonads in the non-CBC groups after 15 days of storage and slightly reduced growth in the LI, LC-CBC, RTS groups on the same storage period. These patterns were not as apparent with the cultivation (Figure 14).

For *Photobacterium phosphoreum* the qPCR analysis showed a rapid growth during the first eight days in the non-CBC groups up to 6 log bacteria/g fish and maintained this cell concentration throughout the storage. These groups showed also increased growth in

comparison to other test groups with the Malthus enumeration but at reduced growth rate and did not show cell concentration of 6 log bacteria/g fish until 12-15 days of storage.

Of the CBC groups, the RTS groups showed a slightly faster *Pp* growth after 5 days of storage but up until day 12 all the CBC groups contained similar quantities of *Pp* as shown using qPCR analysis (Figure 15). After that the PI, LC-CBC, RTS showed slightly higher counts but not statistically significantly. Unfortunately, the analysis of sample PI, LC-CBS, -1°C failed on day 10 which could possibly be explained by the DNA extraction procedure which has now been optimised for increased efficiency.

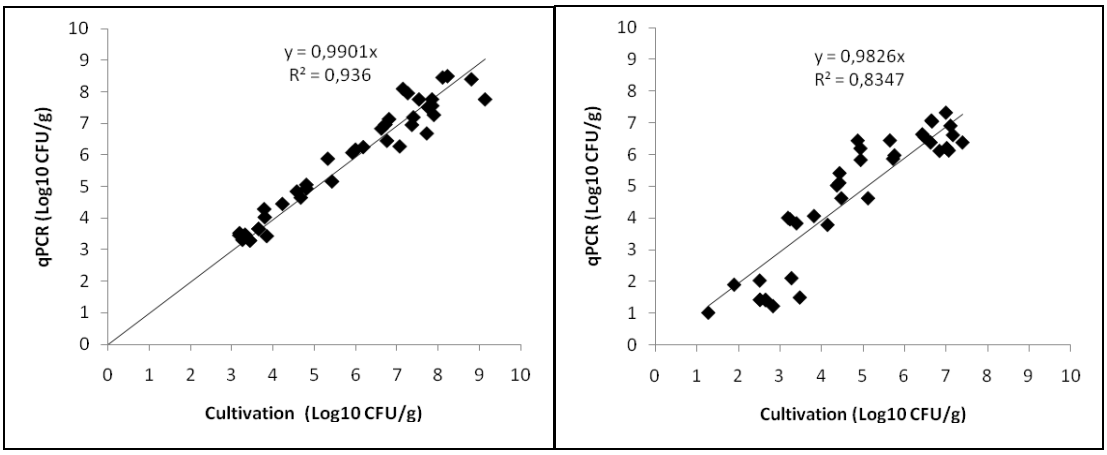

Figure 13. Correlation plot between quantification of *Pseudomonas* spp. (left) and *Photobacterium phosphoreum* (right) using qPCR and cultivation.

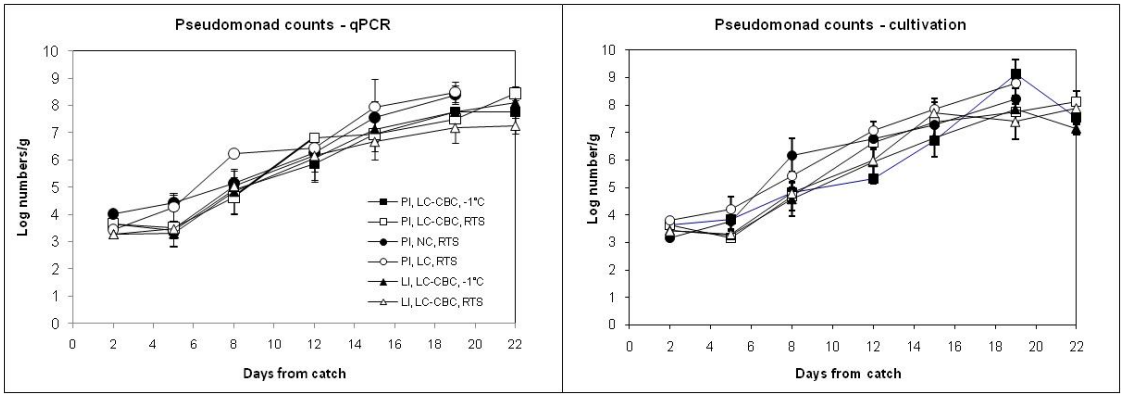

Figure 14. Pseudomonad developments in fish fillets during storage by quantitative PCR analysis (left) and cultivation (right).

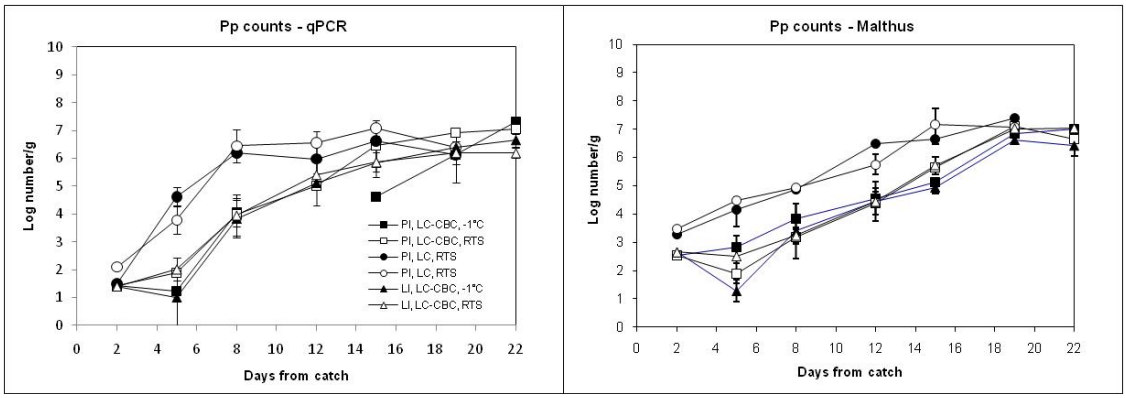

Figure 15. ***Photobacterium phosphoreum*** **developments in fish fillets during storage by quantitative PCR analysis (left) and cultivation (right).**

3.5 Chemical measurements

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

The results from TVB-N and TMA measurements are shown in Figures 16 and 17. Much higher values of TVB-N and TMA were obtained in the two groups where CBC cooling was not applied (PI, LC, RTS and PI, NC, RTS). These results are in good harmony with results from microbial counts, especially of *P. phosphoreum* and H_2S-producing bacteria. *P. phosphoreum* is a very active reducer of trimethylamine oxide (TMAO) to TMA in MA-packed fish. H_2S-producing bacteria, like *Shewanella putrefaciens*, can also produce TMA but to a lesser extent under MA conditions with considerable levels of carbon dioxide (Dalgaard 1995a,b). On days 12 to 19 TMA values were however somewhat higher in group PI, LC, RTS where liquid cooling was applied than in PI, NC, RTS (no cooling). Microbial counts where very similar on these days between the two groups.

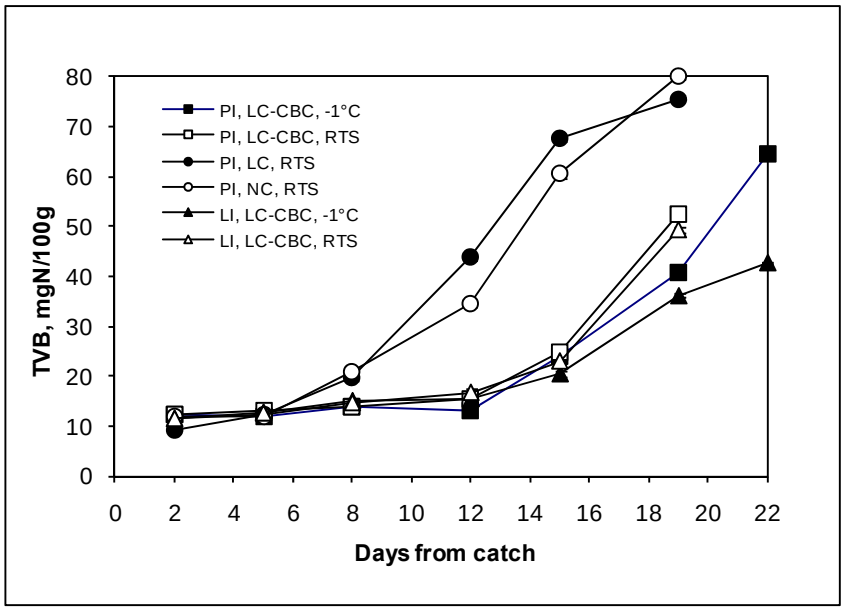

Figure 16. Total Volatile Base Nitrogen (TVB-N) in cod fillets. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

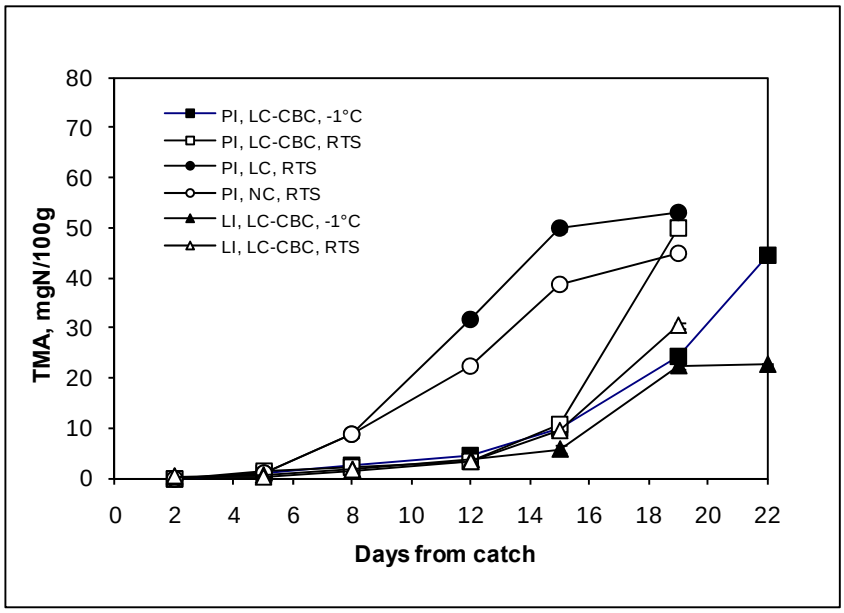

Figure 17. Trimethylamine (TMA) in cod fillets. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

3.5.2 pH – measurements

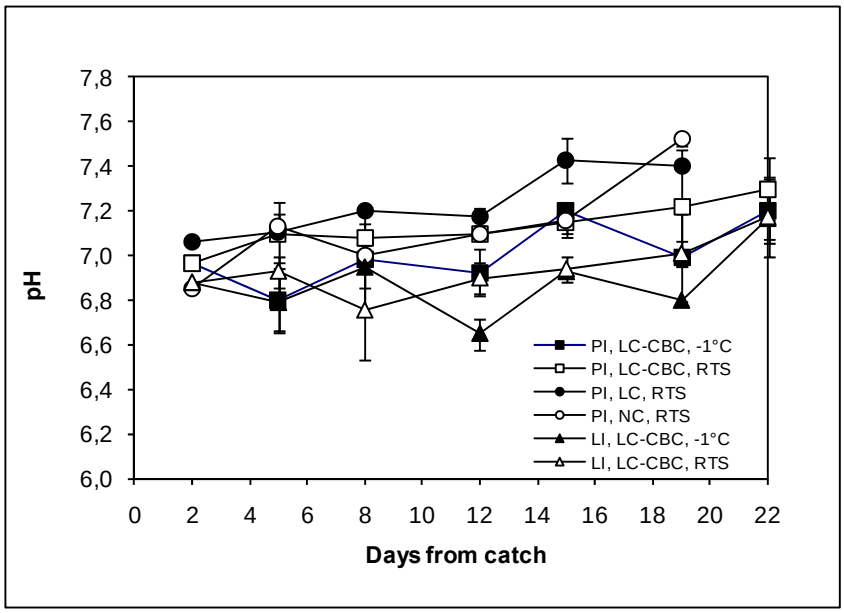

Figure 18. Acidity (pH) in cod fillets. Average values of duplicate samples are shown. Error bars show SD. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

Results from pH measurements are shown in Figure 18. Some increase was observed in most experimental groups over the storage period. Highest values were usually seen in the groups PI, LC, RTS and PI, NC, RTS.

3.5.3 Salt content and water content

Salt content at the day of processing (2 days post catch) was as follows: Groups A/B (PI, LC-CBC) 0.3%, group C (PI, LC) 0.4%, group D (PI, NC) 0.2% and groups E/F (LI, LC-CBC) 0.2%. Liquid cooling resulted in slight increase in salt content of the fish kept in plate ice but changes were not observed in fish kept in liquid ice.

The water content of CBC-cooled fillets was generally slightly lower than in fillets where this technique was not applied (Figure 19). The main difference between these categories was partial freezing of thin layer (1 mm) of the fillet due to contact freezing and cold air blast during the CBC-cooling. This could not be explained by differences in the cell structure according to previous studies on CBC-cooling (Martinsdóttir and others 2004).

No significant effect of other experimental factors; cooling technique onboard or storage time was observed.

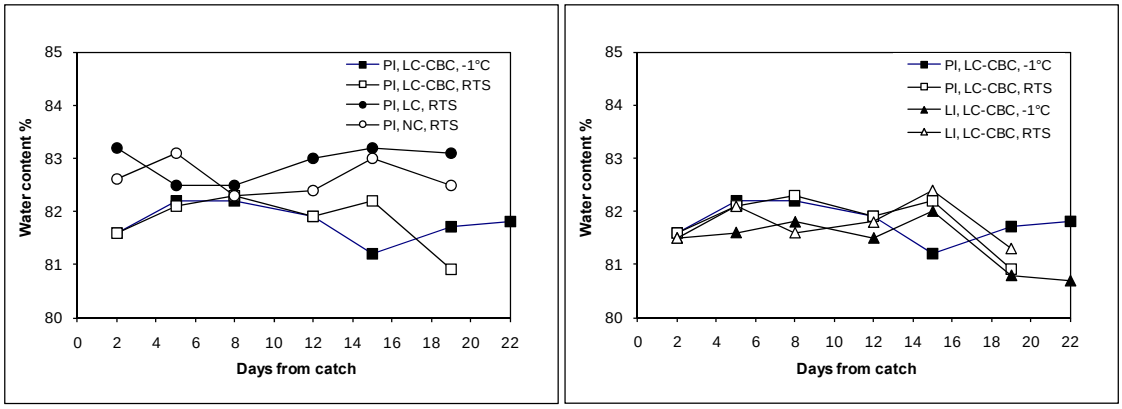

Figure 19. Water content in cod fillets. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

3.6 Water holding capacity (WHC)

The water holding capacity of CBC-cooled fillets was higher on the processing day (at day 2 from catch) but decreased during storage more than in other groups (Figure 20). The fillets processed from fish stored in liquid ice after catch tended to have higher water holding capacity compared to fish stored in plate ice. During the first 2 weeks of storage temperature fluctuations reduced water holding capacity in fish that had been iced with plate ice after catch. The opposite tendency was observed in fish stored in liquid ice.

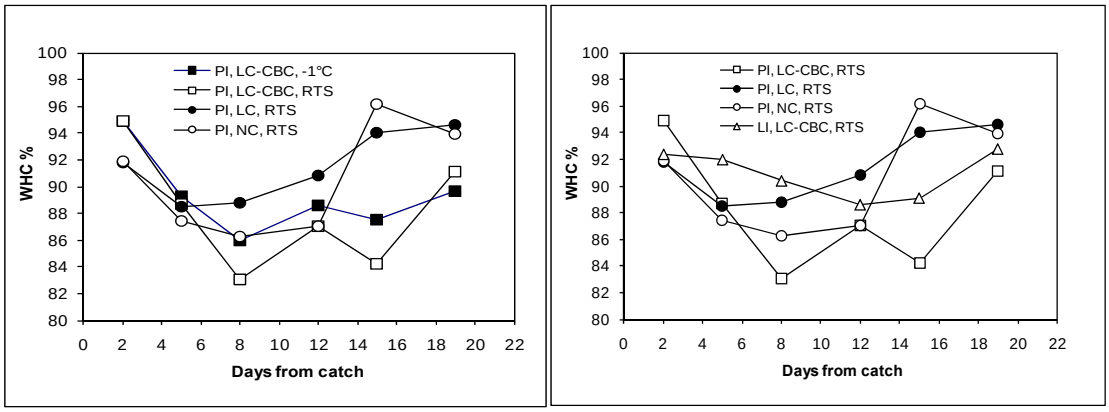

Figure 20. Water Holding Capacity (WHC) in cod fillets. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

The water holding capacity after 5 and 8 days was highest in fillets processed from fish stored in liquid ice (Figure 21). These results supported the sensory analysis, where this group was rated as juicier than the others.

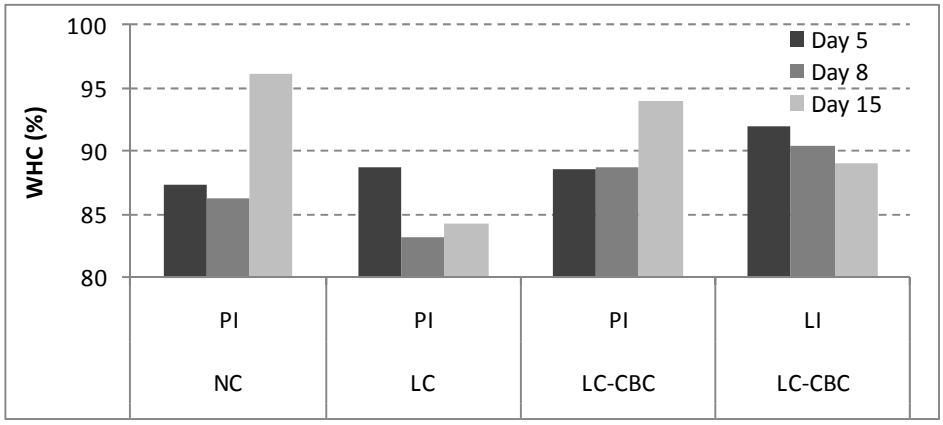

Figure 21. Water Holding Capacity (WHC) in cod fillets, from the same groups as used for sensory analysis. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

3.7 Drip measurements

Drip was only measured after 8 and 15 days from catch. It was low, in the range of 0.3% to 1.8% (Figure 22). The results did not indicate effects of cooling technique on-board on the drip but it was slightly influenced by cooling during processing and storage conditions. Liquid cooling and CBC increased drip and it was higher in fillets kept at temperature fluctuations compared with steady temperature (-1°C). The increased drip with longer storage time was explained by partial degradation of the muscle during storage. The relationship between drip and water holding capacity was poor. Higher values for water holding capacity can be expected when the drip has been high, i.e. when the majority of loosely bound water has leaked out of the muscle.

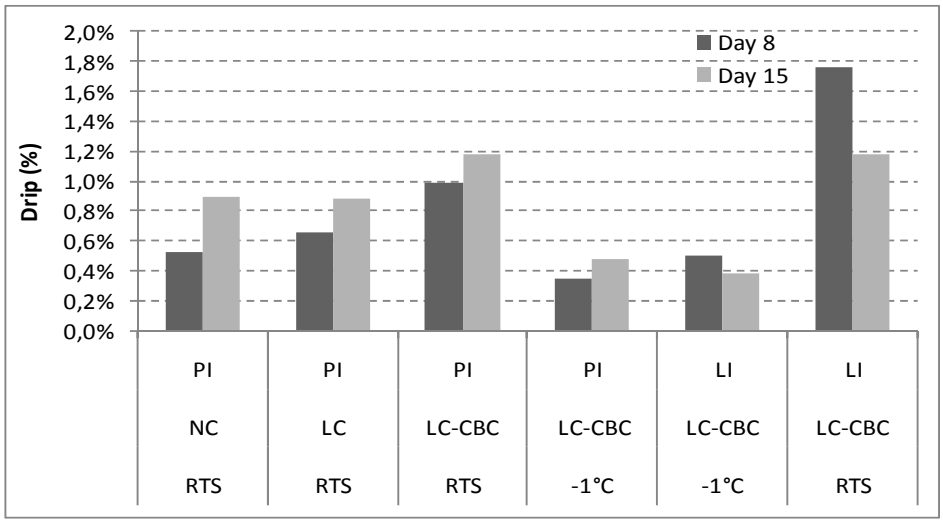

Figure 22. Drip in cod fillets. (PI: Plate ice, LI: Liquid ice, LC: Liquid cooling, NC: No cooling, CBC: Combined blast and contact cooling, RTS: Real temperature simulation).

4 CONCLUSION

No marked difference was seen in microbial and chemical measurements whether plate ice or liquid ice was used prior to filleting but according to sensory analysis, the experimental group where liquid ice was used had one day extension in freshness and shelf life compared to the group with plate ice. Temperature was usually slightly higher in the plate ice group than the liquid ice group during storage.

According to sensory, microbiological and chemical analysis, the combined blast and contact (CBC) cooling clearly resulted in longer freshness period and shelf life extension in comparison with the two groups where this technique was not applied. Temperature was lower in the groups where CBC cooling was applied at processing during the storage period.

Similar microbial counts were found between the two experimental groups where CBC was not applied during processing (liquid cooling and no cooling). These results were in agreement with results from sensory analysis. TMA values were however higher on storage days 12 to 19 in the group with liquid cooling. Temperature measurements during storage of these two groups were very similar.

No marked difference was seen in microbial counts between groups that were stored at a constant temperature around -1 °C compared to groups where temperature fluctuations were used during early phases of storage. During the first 15 days of storage, TVB-N and TMA values were very similar for these groups. Sensory analysis was not done on the two groups kept at -1 °C.

The rapid qPCR analysis was generally in good agreement with the cultivation methods for *Pseudomonas* spp. and *Photobacterium phosphoreum.*

5 ACKNOWLEDGEMENTS

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APPENDIX 1

Table 3 a. Average sensory scores (QDA scale 0-100%) for odour attributes. Different superscript letters show significant differences within a row (between groups within a storage day).

Group	Days from catch	sweet	shellfish	meat	vanilla	potatoes	frozen	cloth	TMA	sour	sulphur
	p-value:	*0,763*	*0,279*	*0,628*	*0,638*	*0,983*	*0,139*	*0,784*	*0,927*	*0,473*	*0,814*
PI, LC-CBC2 (0-point)		56	37	25	36	24	5	5	2	2	2
LI, LC-CBC2 (0-point)		55	43	29	32	24	3	5	2	3	1
	p-value:	*0,192*	*0,601*	*0,382*	*0,492*	*0,256*	*0,742*	*0,360*	*0,946*	*0,889*	*0,994*
PI, LC-CBC	5	47	34	18	32	30	2	5	3	3	1
PI, LC	5	42	36	18	27	24	2	5	2	3	1
PI, NC	5	39	32	19	26	24	2	6	3	2	1
LI, LC-CBC	5	48	38	24	32	31	1	3	3	2	1
	p-value:	*0,789*	*0,772*	*0,500*	*0,088*	*0,974*	*0,953*	*0,079*	*0,062*	*0,243*	*0,297*
PI, LC-CBC	8	35	30	22	31	30	5	6	5	5	4
PI, LC	8	33	32	26	24	31	5	11	14	10	4
PI, NC	8	36	33	22	29	29	6	14	12	8	6
LI, LC-CBC	8	37	34	26	34	31	5	6	8	3	2
	p-value:	*0,204*	*0,874*	*0,992*	*0,624*	*0,725*	*0,363*	*0,145*	***0,001***	***0,008***	***0,013***
PI, LC-CBC	12	28	20	16	26	27	5	20	12 ^{b}	10 ^{b}	8 ^{b}
PI, LC	12	21	19	16	21	29	6	23	22	21 ^{a}	8 ^{b}
PI, NC	12	22	20	15	22	32	7	28	28 ^{a}	17	14 ^{a}
LI, LC-CBC	12	26	21	15	25	28	6	17	14 ^{b}	11 ^{b}	7 ^{b}
	p-value:	*0,617*	*0,243*	*0,433*	*0,417*	*0,812*	*0,910*	*0,779*	*0,368*	*0,687*	*0,855*
PI, LC-CBC	15	24	17	15	20	30	7	24	21	14	9
LI, LC-CBC	15	26	21	18	24	29	7	22	17	13	9
	p-value:	*0,913*	*0,900*	*0,603*	*0,862*	*0,597*	*0,785*	*0,730*	*0,559*	*0,484*	*0,507*
PI, LC-CBC	19	11	14	14	14	25	9	48	53	33	27
LI, LC-CBC	19	10	15	12	13	22	8	50	48	38	31

Table 3 b. Average sensory scores (QDA scale 0-100%) for appearance attributes. Different superscript letters show significant differences within a row (between groups within a storage day).

Group	Days from catch	dark	heterog.	prec.
	p-value:	*0,663*	*0,494*	*0,665*
PI, LC-CBC2 (0-point)		14	15	17
LI, LC-CBC2 (0-point)		16	17	18
	p-value:	***0,016***	*0,079*	***0,000***
PI, LC-CBC	5	25 [a]	21	24 [b]
PI, LC	5	21 [a]	15	19 [b]
PI, NC	5	23 [a]	22	23 [b]
LI, LC-CBC	5	14 [b]	17	41 [a]
	p-value:	***0,000***	***0,000***	*0,220*
PI, LC-CBC	8	23 [b]	23 [b]	23
PI, LC	8	35 [a]	32 [a]	21
PI, NC	8	35 [a]	37 [a]	26
LI, LC-CBC	8	19 [b]	22 [b]	30
	p-value:	***0,038***	*0,655*	***0,034***
PI, LC-CBC	12	24	26	23
PI, LC	12	28	30	30 [a]
PI, NC	12	29	28	20 [b]
LI, LC-CBC	12	21	27	25
	p-value:	*0,352*	*0,825*	*0,660*
PI, LC-CBC	15	29	31	34
LI, LC-CBC	15	26	30	33
	p-value:	*0,220*	***0,027***	*0,098*
PI, LC-CBC	19	26	24 [b]	31
LI, LC-CBC	19	30	31 [a]	37

Table 3 c. Average sensory scores (QDA scale 0-100%) for flavour attributes. Different superscript letters show significant differences within a row (between groups within a storage day).

Group	Days from catch	salt	metallic	sweet	meat	frozen	pungent	sour	TMA	off
	p-value:	*0,778*	*0,457*	*0,883*	*0,993*	*0,621*	*0,803*	*0,927*	*0,809*	*0,965*
PI, LC-CBC	2 (0-point)	8	37	34	30	5	4	3	3	2
LI, LC-CBC	2 (0-point)	9	41	34	30	4	4	3	2	2
	p-value:	***0,026***	*0,104*	***0,014***	***0,033***	*0,432*	*0,757*	*0,984*	*0,819*	*0,851*
PI, LC-CBC	5	12 [a]	27	31 [b]	21	3	2	2	3	4
PI, LC	5	10	32	31	18	2	4	2	4	3
PI, NC	5	6 [b]	30	24 [b]	15 [b]	4	6	2	4	6
LI, LC-CBC	5	13 [a]	40	43 [a]	29 [a]	2	4	1	2	3
	p-value:	*0,127*	*0,858*	*0,321*	*0,051*	***0,026***	*0,072*	*0,535*	***0,034***	***0,001***
PI, LC-CBC	8	10	25	25	17	5 [b]	9	4	7	8
PI, LC	8	9	24	21	17	5 [b]	10	5	16	17
PI, NC	8	8	27	26	19	8 [a]	18	8	15 [a]	25 [a]
LI, LC-CBC	8	11	25	29	24	5 [b]	7	4	7 [b]	8 [b]
	p-value:	*0,430*	*0,076*	***0,031***	*0,734*	*0,075*	*0,159*	***0,015***	***0,000***	***0,000***
PI, LC-CBC	12	9	21	20 [a]	15	5	10	9 [b]	14 [b]	14 [b]
PI, LC	12	10	17	17	15	7	15	18	23	26 [b]
PI, NC	12	7	13	11 [b]	12	8	16	21 [a]	30 [a]	39 [a]
LI, LC-CBC	12	8	20	17	15	5	11	11	15 [b]	17 [b]
	p-value:	*0,632*	*0,689*	*0,600*	*0,809*	*0,915*	*0,081*	*0,233*	*0,190*	*0,502*
PI, LC-CBC	15	10	16	16	16	6	16	17	22	25
LI, LC-CBC	15	9	17	18	17	6	11	12	16	21
	p-value:	*0,135*	*0,589*	*0,841*	*0,515*	*0,732*	*0,676*	*0,395*	*0,990*	*0,827*
PI, LC-CBC	19	8	7	6	9	7	23	28	46	58
LI, LC-CBC	19	6	8	6	13	8	26	35	46	61

Table 3 d. Average sensory scores (QDA scale 0-100%) for texture attributes. Different superscript letters show significant differences within a row (between groups within a storage day).

Group	Days from catch	flakes	soft	juicy	tender	mushy	meaty	clammy	rubbery
	p-value:	*0,779*	*0,673*	*0,353*	*0,238*	*0,410*	*0,155*	*0,225*	*0,178*
PI, LC-CBC2 (0-point)		51	52	59	66	33	33	21	13
LI, LC-CBC2 (0-point)		50	49	54	60	38	40	26	18
	p-value:	*0,106*	***0,022***	***0,003***	***0,000***	***0,024***	***0,000***	***0,005***	*0,194*
PI, LC-CBC	5	55	68 ^{a}	62 ^{b}	77 ^{a}	55 ^{a}	30 ^{b}	14 ^{b}	8
PI, LC	5	61	67 ^{a}	73 ^{a}	80 ^{a}	51 ^{a}	21 ^{b}	15 ^{b}	9
PI, NC	5	51	63	60 ^{b}	77 ^{a}	54 ^{a}	29 ^{b}	17 ^{b}	12
LI, LC-CBC	5	53	53 ^{b}	54 ^{b}	49 ^{b}	35 ^{b}	53 ^{a}	30 ^{a}	17
	p-value:	*0,545*	***0,023***	***0,037***	***0,000***	***0,013***	***0,000***	***0,000***	***0,005***
PI, LC-CBC	8	55	58	62 ^{a}	68 ^{a}	47 ^{a}	27 ^{bc}	19 ^{b}	17 ^{b}
PI, LC	8	54	65 ^{a}	58	69 ^{a}	52 ^{a}	22 ^{c}	17 ^{b}	13 ^{b}
PI, NC	8	57	58	56	63 ^{a}	50 ^{a}	33 ^{ab}	19 ^{b}	17 ^{b}
LI, LC-CBC	8	58	52 ^{b}	51 ^{b}	51 ^{b}	35 ^{b}	40 ^{a}	36 ^{a}	27 ^{a}
	p-value:	*0,196*	*0,666*	*0,799*	*0,969*	*0,619*	*0,811*	*0,750*	*0,461*
PI, LC-CBC	12	60	55	55	63	49	25	24	20
PI, LC	12	56	56	57	65	46	26	25	14
PI, NC	12	53	54	57	63	46	26	21	15
LI, LC-CBC	12	56	52	55	63	43	29	21	15
	p-value:	*0,131*	*0,614*	*0,511*	*0,239*	*0,801*	*0,966*	*0,473*	*0,588*
PI, LC-CBC	15	53	52	51	55	47	32	27	16
LI, LC-CBC	15	57	54	53	60	48	33	24	17
	p-value:	*0,821*	*0,441*	*0,714*	*0,342*	*0,547*	*0,248*	*0,149*	*0,575*
PI, LC-CBC	19	56	42	49	54	30	17	9	9
LI, LC-CBC	19	57	46	47	59	35	22	15	11