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The effect of different cooling techniques on the quality changes and shelf life of whole cod *(Gadus morhua)*

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Report summary

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Ágrip á íslensku:	Tilgangur tilraunarinnar var að kanna áhrif þriggja mismunandi kæliaðferða á geymsluþol heils, slægðs þorsks: (i) Kæling með muldum plötuís, (ii) kæling með vökvaís, (iii) forkæling með vökvaís og síðan kæling með muldum plötuís. Fylgst var með hitastigsferlum með hitanemum í öllum hópum yfir geymslutímann. Sýni voru metin með skynmats-, örveru- og efnamælingum þá 10 daga sem fiskurinn var í geymslu. Niðurstöður örveru- og efnamælinga voru yfirleitt í góðu samræmi við niðurstöður skynmats. Samanburður á tilraunahópum leiddi í ljós að þorskur kældur með vökvaís hafði um tveggja til þriggja daga skemmra geymsluþol en hinir tveir hóparnir. Geymsluþol þorsksins var töluvert styttra en ýmsar fyrri rannsóknir hafa sýnt og þá sérstaklega í hópnum sem var kældur með vökvaís (aðeins 9-10 dagar). Nú liggur fyrir að þorskurinn sem var kældur með vökvaís var vanísaður um borð í veiðiskipinu miðað við hina tvo hópana. Auk þess var kæling við geymslu eftir löndun ekki eins góð og æskileg gæti talist en hitastigið sveiflaðist á milli 2-5 °C. Þetta gæti mögulega skýrt skemmra geymsluþol allra hópa miðað við fyrri rannsóknir.		
Lykilorð á íslensku:	*Kælitækni, heill þorskur, gæði, ferskleiki, geymsluþol*		
Summary in English	The aim of this experiment was to investigate the effect of three different cooling methods on the storage quality of whole, bled gutted cod: (i) Cooled with crushed plate ice, (ii) cooled with liquid ice, (iii) pre-cooled in liquid ice and then cooled with crushed plate ice. The temperature history of each group was studied using temperature loggers. The samples were analysed with sensory, microbiological and chemical methods for up to 10 days from catch. The results from microbial and chemical measurements were generally in good agreement with the results from sensory evaluation. Comparison of the groups showed that the use of liquid ice instead of plate ice resulted in two to three day shorter shelf life than in the other two groups. The shelf life in this study was considerably shorter compared to previous studies with whole cod, especially in the experimental group where liquid ice was used for cooling (only 9-10 days). It is now known that the liquid iced group in this experiment was insufficiently iced on board the fishing vessel compared to the other two groups. Additionally, the ambient temperature in the cold room of the fish plant was relatively high and fluctuated between 2 – 5 °C during the storage period. This could possibly explain the shorter shelf life of all groups compared to some earlier studies.		
English keywords:	*Cooling techniques, whole cod, quality, freshness, shelf life*		

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. Whole cod can be stored in plate ice up to 15 days according to sensory evaluation (Magnússon and Martinsdóttir 1995). However, whole fish is usually stored for a short period before processing (one to ten days in ice) depending on choice of processing method, transport and market.

At Matís, research projects under the EU-funded Integrated Research Project CHILL-ON Chill-add-on funded by the AVS research fund and the Technology Development Fund at the Icelandic Centre for Research (project no. 061358006) have been done on different cooling media during early days of storage. In Matís report 09-23 the fish was on one hand iced with plate ice and the other liquid ice in tubs after bleeding, gutting and washing. The fish was filleted and fillets with skin-on got different treatments prior to packaging and was kept either at -1 °C or under real temperature simulation for up to 22 days from catch (20 days from packaging). 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. In the study by Reynisson and others (2010) a comparison was made of two different types of liquid ice during the first 8 days of storage of haddock. However in that experiment the fish was stored ungutted and uniced on-board the ship until landing (4-6 hours) then gutted and cooled with liquid ice and stored. There the results showed that the bacterial growth behaviour observed for differently cooled fish was actually not supported by their temperature profiles. The use of liquid ice prepared from brine provided faster initial cooling of whole fish but created unfavourable conditions under extended storage where the active spoiler *P. phosphoreum* became dominant.

The aim of this experiment was to investigate the effect of three different cooling methods on the storage quality of whole, bled gutted cod. The following cooling methods on board the fishing vessel were studied: (i) Cooled with crushed plate ice, (ii) cooled with liquid ice, (iii) pre-cooled in liquid ice and then cooled with crushed plate ice. The temperature history of each group was studied using temperature loggers. The samples were analysed with sensory, microbiological and chemical methods for up to 10 days from catch.

2 MATERIAL AND METHODS

2.1 Experimental design

Cod was caught SW of Iceland on the 10th of November 2009 using a Danish seine. The cod was bled, gutted and washed and then put in iced tubs in the hold.

The comparison groups of the experiment can be seen on Table 1. The cod was put directly in its respective cooling medium for groups CPI and LI. In group LC-CPI however, the cod was pre-cooled in liquid ice for 30 minutes before being iced with crushed plate ice.

Fish tubs constructed of polyethylene and polyurethane with Styrofoam insulated walls and covers were used for the packing of fish and ice. Each tub contains 460 L which was sufficient storage capacity for each experiment group. Approximately 300 kg of fish were packed in the appropriate type of ice. The exact amount of fish and ice in each tub were not measured specifically since the conditions to measure the weight of ice and fish while it was being iced in the hold did not allow for it.

Table 1. Experiment setup

Group	Cooling medium	Pre-cooling
CPI	Crushed plate ice	None
LI	Liquid ice	None
LC-CPI	Crushed plate ice	30 min in liquid ice

The catch was landed in a village located SW-Iceland in the evening of 10th of November and transported to a cool storage room of a fish plant nearby the morning after. Samples were taken from each group on days 1-2-3-6-8-10 from catch (Nov 11-Nov 20). On days 1 and 3 each tub was re-iced with approximately 20 kg of crushed plate ice.

2.2 Temperature logging

The fish in each group was packed in five layers and temperature loggers inserted into the fish flesh in two individuals at the bottom layer, middle layer and top layer. This made a total of 6 loggers which were used to measure the temperature in the fish flesh for each group. In addition to those loggers one temperature logger was used to measure ambient temperature outside the tub for each group and another one which measured the temperature in the medium, approximately 10 cm below surface.

The initial temperature of fish was approximately 8 °C. In order to get a fair comparison the initial time t_0 was determined from when the temperature first reached 7.5 °C. The mean of the temperature time series for each layer was then found. Since the number of temperature logs which was retrieved from each layer was not always equal, the total average temperature of each group was found such that

$$\bar{T} = \frac{1}{3}\left(\frac{1}{N_1}\sum_i T_{1,i} + \frac{1}{N_3}\sum_j T_{3,j} + \frac{1}{N_5}\sum_k T_{5,k}\right)$$

were $T_{1,i}$ is the temperature time series for the i-th fish at the bottom layer, $T_{3,j}$ is the temperature time series for the j-th fish in the middle layer and $T_{5,k}$ is the temperature time series for the k-th fish at the top layer. N_1, N_3 and N_5 denote the total number of temperature logs in their respective layers.

Two types of thermometers were used for temperature logging

a. iButton temperature loggers, type DS1922L. (http://www.maxim-ic.com/quick_view2.cfm/qv_pk/4088). 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. The iButton loggers were used in the experiments with the whole fish, positioned as deep in the fish flesh as possible.

b. Onset temperature logger, type UTBI-001. (http://www.onsetcomp.com/products/data-loggers/utbi-001). This logger has an accuracy of ±0.2 and a resolution of 0.02 °C and an operating range of -20 to 70 °C. The diameter is 30 mm and the thickness is 17mm. Two of these temperature loggers were used for each group, one outside the tub for ambient temperature while the second recorded the temperature in the medium.

2.3 Sampling

On each sampling day, 3 fishes from each sampling group were collected from the tubs. Normally, fish under the top layer was pulled out of the tub. The samples were kept chilled in Styrofoam boxes and shipped to the laboratory were analysis was carried out approximately 1-2 hours after sampling.

2.4 Sensory evaluation

To estimate the freshness of the whole raw cod, Quality Index Method (QIM) was used. QIM is a freshness grading system originally developed by the Tasmanian Food Research Unit (TFRU) (Bremner, 1985), adapted for whole cod (Jónsdóttir, 1992; Larsen and others, 1992). Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (2004), and the Torry freshness score sheet based on the scheme developed by Shewan and others (1953) were used to assess cooked samples of cod. Thirteen 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 and experienced in using the QIM, QDA method and Torry freshness score sheet for 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 QDA attributes were defined and described by the sensory panel during other projects (Sveinsdottir and others 2009). The sensory attributes altogether were 30 and are described 10 attributes of odour; sweet, shellfish/algae, meat, vanilla/warm milk, boiled potatoes, frozen storage, table cloth,

TMA, sour and sulphur, three attributes of appearance; colour (light/dark), appearance (even colour/discoloured), white precipitation, nine attributes of flavour; salt, metallic, sweet, meaty, frozen storage, pungent, sour, TMA and off-flavour and eight attributes of texture; flakiness, soft, juicy, tender, mushy, meaty mouth feel, clammy and rubbery.

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 in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95-100°C with air circulation and steam, to a core temperature of 67°C and then served to the panel. Each panellist evaluated duplicates of each sample in a random order in nine sessions (maximum four samples per session). A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystémes) was used for QDA data recording. Guidelines from Martinsdóttir and others (2001) were followed during the sensory evaluation with QIM. Each sampling day, three whole raw cod from each sample group were placed on a white clean table at room temperature, under white fluorescent light. Each fish was coded with a three-digit random numbers.

2.5 Microbial measurements

Three samples were analyzed on each day of sampling. Each sample consisted of muscle taken from one side of three cod (altogether three skinless fillets). First the skin was disinfected with 70% isopropanol and then aseptically cut away and the underlying muscle collected. The muscle from three cod was then minced together and 20 g weighed in 180 g of Maximum Recovery Dilutent (MRD, Oxoid) and blended in a Stomacher® Lab Blender 400 (Seward, UK) for 1 min to obtain 1/10 dilution. The remaining mince was used for pH, water, salt, total volatile bases (TVB-N) and trimethylamine (TMA) measurements.

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 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. In all the above counts surface-plating was used.

Counts of *Photobacterium phosphoreum* were obtained by a quantitative PCR method. Briefly, One ml of the tenfold diluted fish samples in MRD buffer was frozen at -20 °C for later DNA extraction. For the DNA extraction, the diluted samples were centrifuged at 11.000 x g for 7 min to form a pellet. The supernatant was discarded and DNA was recovered from the pellet using the Promega Magnesil KF, Genomic system (MD1460) DNA isolation kit (Promega Corporation, Madison, USA) in combination with KingFisher magnetic beads automatic DNA isolation instrument (Thermo Labsystems, Waltham, USA) according to the manufacturers´ recommendations. All PCR reactions were done using the Mx3005p instrument. The PCR was done using Brilliant QPCR mastermix (Stratagene, La Jolla, CA, USA). Primers were synthesized and purified with HPLC (MWG, Ebersberg, Germany). The DNA standard used for quantification was previously calibrated against the PPDM-Malthus conductance method (Dalgaard and others 1996).

Cooled MRD buffer was used for all dilutions. All samples were analysed in triplicate as stated above and results presented as an average.

2.6 Chemical and physical measurements

2.6.1 *Total Volatile Base Nitrogen and trimethylamine*

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 triplicate.

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 *Water content, salt content and water holding capacity*

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).

The water holding capacity (WHC) was determined by a centrifugation method (Eide and others 1982). Approximately 2 g of the minced fish was weighed accurately and centrifuged (Heraeus Biofuge Stratos, Kendro Laboratory products, USA) at 210 x g for 5 minutes at 0-5°C. WHC (%) was then calculated with the following equations:

$$WHC\ (\%) = \frac{Water\ content\ (\%) - Weight\ loss\ (\%)}{Water\ content\ (\%)}\ x\ 100$$

where the weight loss is defined as:

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

2.7 Data analysis

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

3 RESULTS AND DISCUSSION

3.1 Temperature logging

3.1.1 *Ambient temperature*

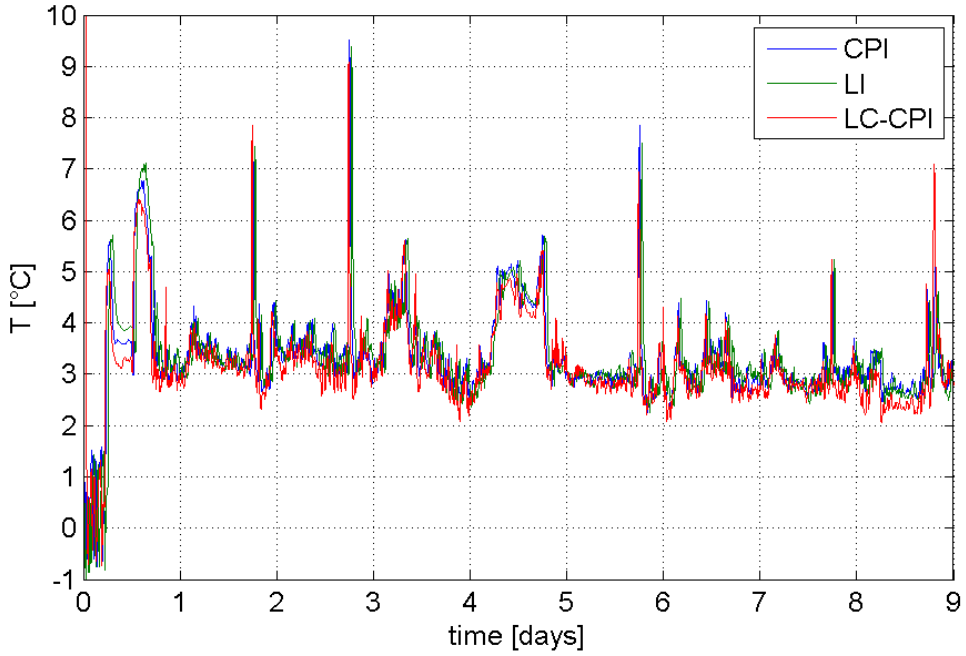


Figure 1: Ambient temperature during storage period

The tubs were stored in the fish plant cold storage room where the temperature fluctuated between 2 - 5 °C. Figure 1 shows the ambient temperature outside each tub. No significant difference can be seen in ambient temperature between groups.

3.1.2 *Product temperature*

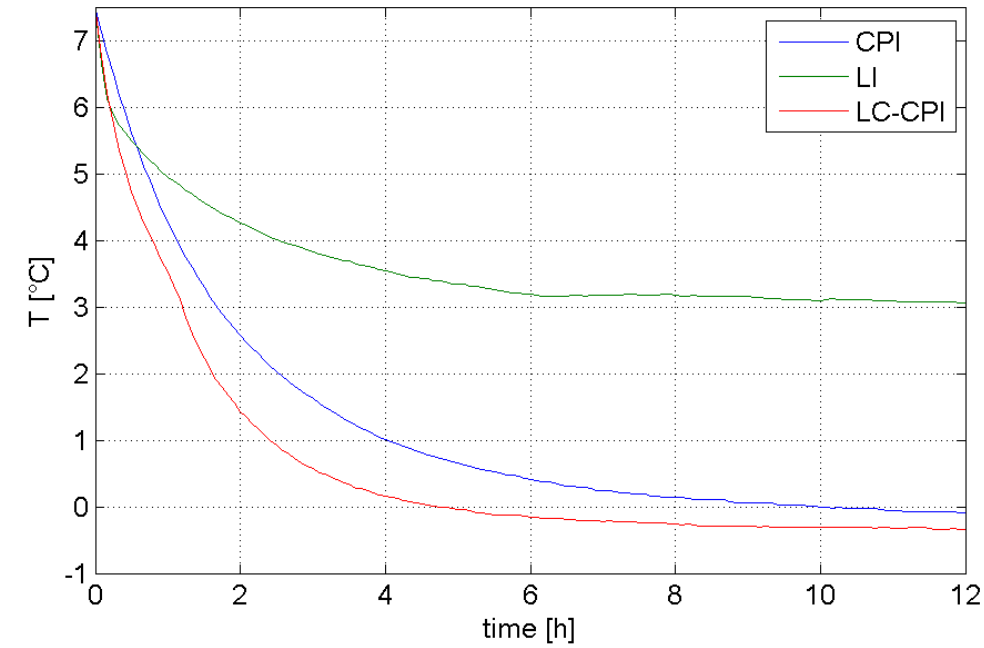


Figure 2: Core temperature of whole cod during the first 12 hours.

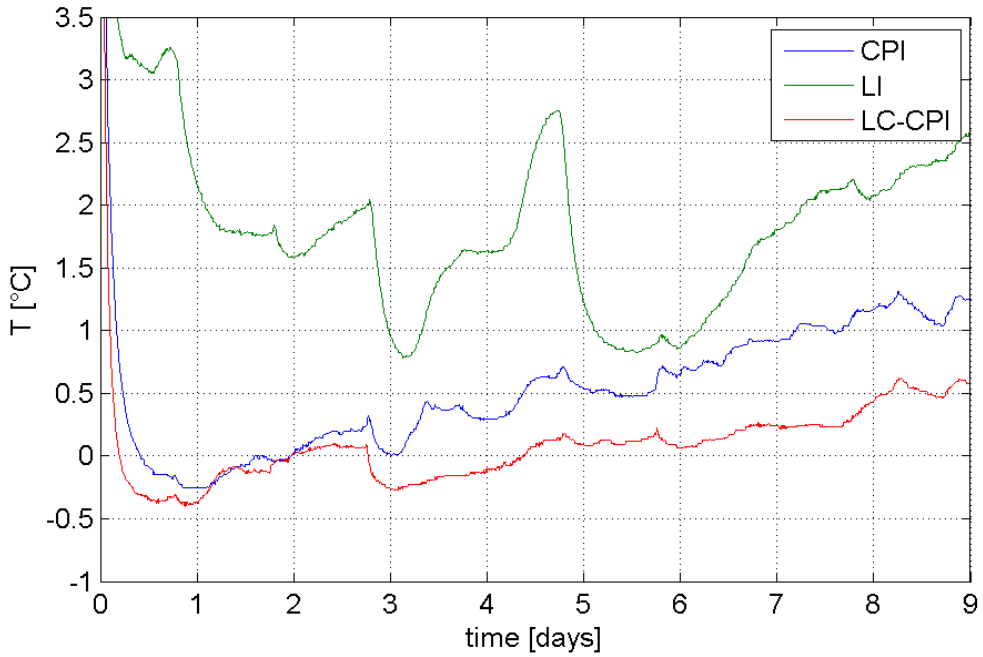


Figure 3: Core temperature of whole cod over the entire storage period.

Figure 2 shows the core temperature of whole cod for the first 12 hours. The most rapid cooling was achieved in group LC-CPI where the catch was pre-cooled in liquid ice and then re-iced with crushed plate ice. The group reached 0 °C from 7.5 °C in 4.75 hours, while group CPI was 10 hours to cool down to the same temperature. The group LI never reached 0 °C and only went down to approximately 3 °C after 12 hours. It is therefore obvious that the icing was insufficient in that group.

Figure 3 shows the core temperature of whole cod over the entire storage period. Both groups CPI and LC-CPI reached minimum temperature after one day, where CPI reached -0.25 °C and LC-CPI reached -0.40 °C.

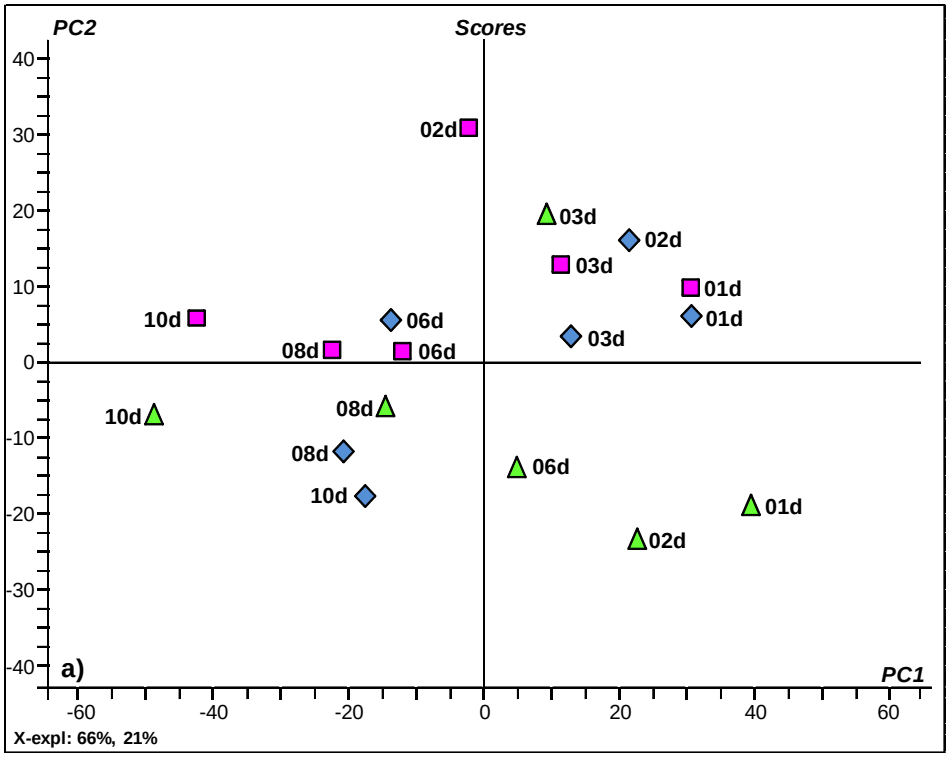
The tubs were re-iced with crushed plate ice after 2.8 days which caused the mean temperature of the LI group to drop from 2.04 °C down to 0.78 °C. The temperature of groups CPI and LC-CPI dropped by 0.30 °C and 0.36 °C respectively but then continued to steadily increase until the end of the storage period with minimal influence from fluctuations in ambient temperature. In experimental group LI, temperature however increased significantly at day four, when the ambient temperature in the cool storage room reached approximately 5 °C. During that period the mean temperature in the LI group increased to 2.75 °C but then dropped again down to 0.82 °C when the ambient temperature decreased to about 3 °C on day 5. From day 6 onwards, a rapid increase in temperature was observed in the LI group until the end of the storage time.

At the end of the storage period groups CPI, LI and LC-CPI had reached 1.25 °C, 2.57 °C and 0.61 °C respectively. This means that the group which was pre-cooled in liquid ice before being packed in crushed plate ice both experienced the most rapid cooling and maintained the temperature better than the comparison groups. The LI group was obviously insufficiently iced from the beginning of the experiment. High ambient temperature in the cold room during storage is the most likely reason for relatively high temperature values at the end of storage in all groups.

3.2 Sensory evaluation

Figure 4 shows how the cooked samples were characterized by the sensory attributes. Altogether 87% of the sensory variation was explained in the first two principal

components. The main variation (66%, PC1) between the samples was due to differences explained by storage time. Sensory attributes characteristic for cod at the beginning of storage, such as sweet and metallic flavour, sweet and shellfish odours are located to the right in the upper part of Figure 4b describing samples after 1-3 days of storage (Figure 4a). As storage time progressed, these sensory attributes become less evident but sour and TMA odours and flavours become somewhat more characteristic. At the end of the storage time, table cloth and potato odours appear to be more characteristic (lower left part of Figure 5b), especially for the LI and LC-CPI samples. The sample groups differed with regard to texture (21%, PC2), mainly at the beginning of storage. The LI samples were more rubbery and meaty, but less tender, soft and juicy compared to LC-CPI and PI samples. Tables A-C in appendix show how in more detail how the sample groups were characterized by sensory attributes.



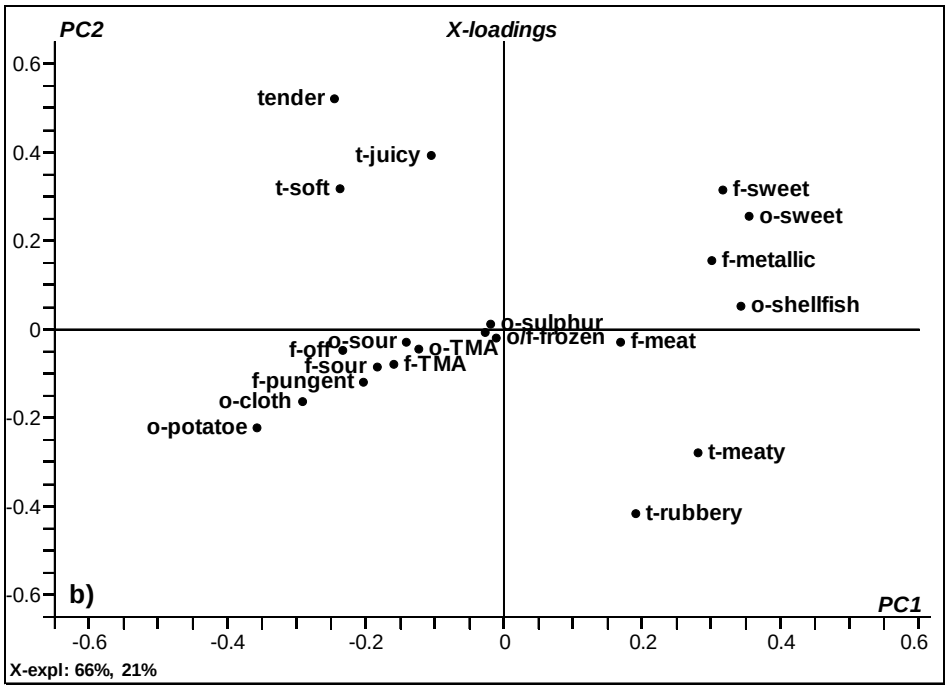


Figure 4. PCA describing sensory quality, odour (o-), appearance (a-), flavour (f-) and texture (t-) of the sample groups with storage time (d). Symbols: Liquid ice; Plate ice; LC-plate ice (LC: Liquid ice pre-cooling). PC1 VS PC2 (X-expl.: 66% and 21%). a) scores, b) X-loadings.

Figure 5 shows how the Torry freshness scores changes with storage time. A Torry score around seven indicates the fish has lost most of its freshness odour and flavour characteristics, and has a rather neutral odour and flavour (Shewan and others 1953). These limits were obtained after c.a. seven days for all groups. When the average Torry score is around 5.5 most of the sensory panellists detect spoilage attributes, and these limits have been used as the limits for consumption at Matís (see e.g. Olafsdottir and others 2006). According to this, the maximum shelf life of the LI was nine days, LC-CPI 10 days but the PI group had not reached the end of shelf life at the end of the experiment at 10 days from catch.

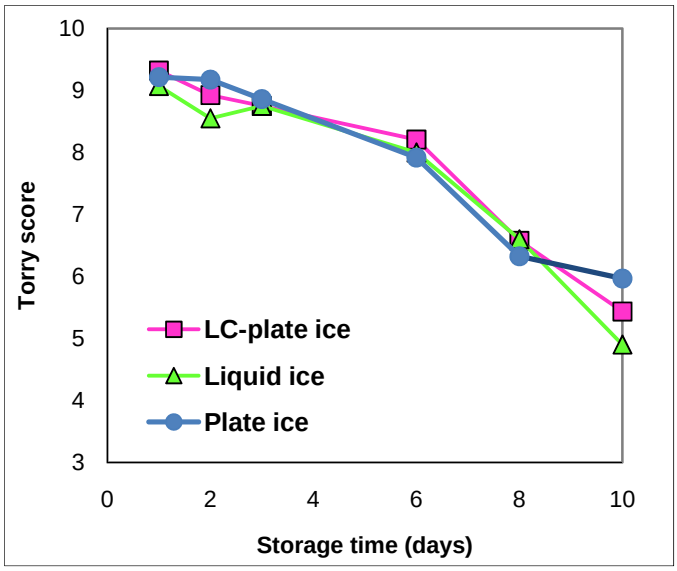


Figure 5. Average Torry freshness scores (LC: Liquid ice pre-cooling).

Figures 6 - 11 show how odour and flavour attributes related to spoilage change with storage time. End of shelf life is usually determined when sensory attributes related to spoilage become evident. When the average QDA score for those attributes is above the value 20 (on the scale 0 to 100) most panellists detect them (Bonilla and others 2005; Magnússon and others 2006). In the PI group, the spoilage related attributes, such as TMA and sour odours, TMA flavour and off-flavour were not detected through the storage time, but hints of table cloth odour was detected 8 and 10 days from catch. Table cloth odour was evident and hints of pungent and sour flavours and off-flavour were detected in the LC-CPI group on the last sampling day (10 days after catch). On the last sampling day, the table cloth odour and off-flavour in the LI group were obvious and hints of TMA and sour odours and flavours were detected. According to this, that the PI group had not reached the end of shelf life, LC-CPI was close to the limits of acceptable sensory quality on day 10 from catch, but the LI group already had reached the end of shelf life on day 10 from catch.

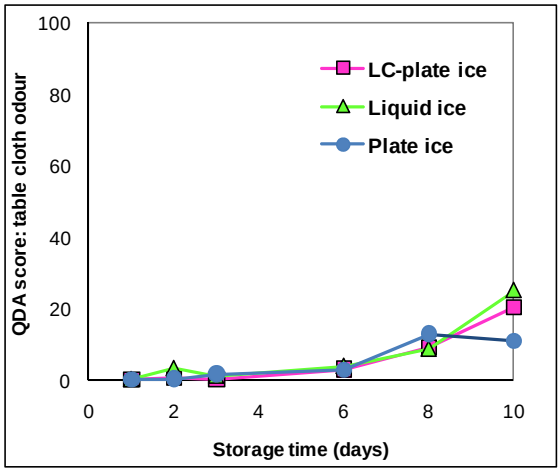


Figure 6. Average QDA scores of table cloth odour (LC: Liquid ice pre-cooling)

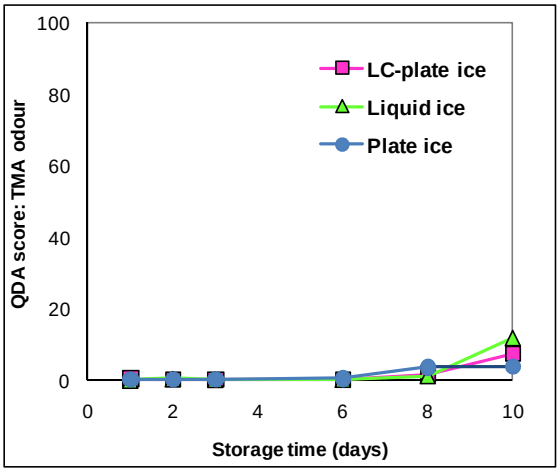


Figure 7. Average QDA scores of TMA odour (LC: Liquid ice pre-cooling)

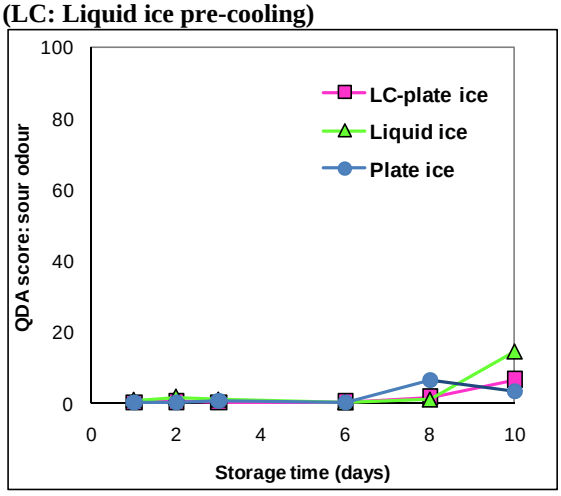


Figure 8. Average QDA scores of sour odour (LC: Liquid ice pre-cooling)

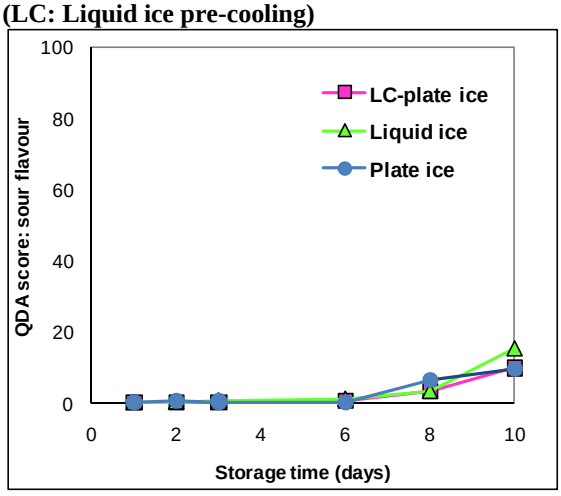


Figure 9. Average QDA scores of sour flavour (LC: Liquid ice pre-cooling)

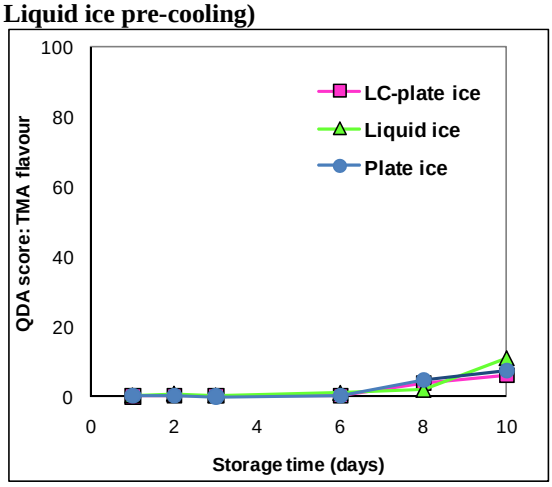


Figure 10. Average QDA scores of TMA flavour (LC: Liquid ice pre-cooling)

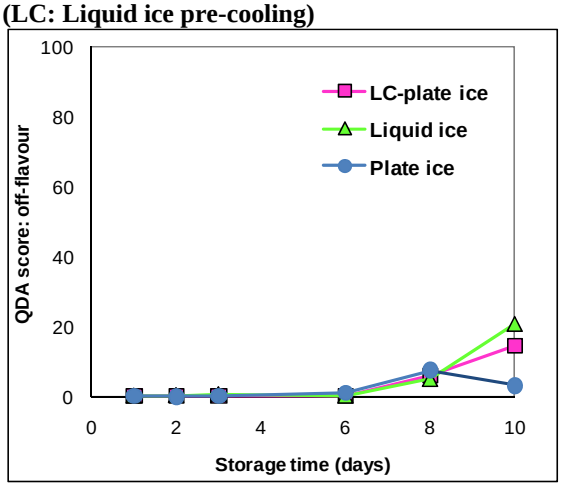


Figure 11. Average QDA scores of off-flavour (LC: Liquid ice pre-cooling)

Figure 12 shows how the Quality Index (QI) scores changed with storage time. The QI increases linearly with the storage time (R^2= 0.98 for all three groups). At the end of the storage trial, the QI was around 16 for the PI and LC-CPI groups, but 19 for the LI group, which was significantly higher ($p<0.05$). Based on the results of very well-controlled storage studies of whole, fresh (gutted) cod stored in ice under good manufacturing conditions on board the vessel (proper gutting, washing and use of fish/ice ratio), the maximum shelf life of whole cod has been found to be up to 15 days (Martinsdóttir and others, 2001). A QI score of 18 corresponds to the end of shelf life according to sensory evaluation of cooked cod samples. To estimate the remaining storage time the regression line based on the linear relationship between the QI and storage time in ice in Martinsdóttir and others (2001) is used. Based on this, the LI group was already past the limits of acceptable sensory quality on day 10 from catch (by 0-1 day), while the other two groups still had 1-2 days remaining.

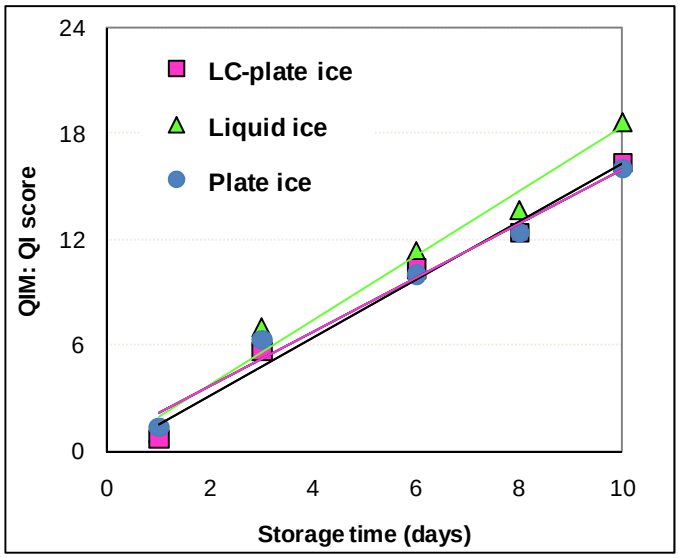


Figure 12. Average QI scores. Trend lines; y = 1,817x + 0,1579, R^2 = 0,977 (Liquid ice); y = 1,5301x + 0,6316, R^2 = 0,9822 (Plate ice); y = 1,6504x-0,1754, R^2 = 0,9844 (LC-plate ice)

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 2.

Table 2. Freshness period (days) and maximum shelf life (days) according to sensory evaluation. (LC: Liquid ice pre-cooling)

Group	Freshness period (days)	Shelf life (days)
LC-plate ice	7	11-12 (estimate)
Plate ice	7	11-12 (estimate)
Liquid ice	7	9-10

Comparison of the groups showed that the use of only liquid ice instead of plate ice resulted in two to three days shorter shelf life. However the temperature recording showed an insufficiently icing on board the fishing vessel of this group (liquid ice). The estimated shelf life in this study is considerably shorter as compared to previous studies with whole cod, especially in the experimental group where liquid ice was used for cooling (only 9-10 days). Additionally, the ambient temperature in the cold room of the fish plant was relatively high and fluctuated between 2 – 5 °C during the storage period. At the end of storage on day 10, the fish temperature was about 2.5 °C in the LI group, just above 1 °C in the CPI group and about 0.5 °C in the LC-plate ice group. That could possibly explain the shorter shelf life of all groups compared to some earlier studies.

3.3 Microbial measurements

Results from microbial counts are shown in Figures 13 and 14. Total viable counts (TVC) and counts of *Photobacteriun phosphoreum* are shown in Figure 13. Highest numbers of TVC and *P. phosphoreum* were generally found during most of the storage time in the LI group. This was especially noticeable on days 6 and 8 of storage. Microbial numbers were similar in the other two groups (LC-CPI and PI) during the storage period. The results showed that in some occasion's higher values were obtained for *P. phosphoreum* than TVC which can be explained by the different methodologies used. The standard used in the real-time PCR analysis was previously calibrated against Malthus conductance method but the relationship between cell numbers and DNA copy numbers can fluctuate during the growth phase of the bacterium.

At the end of storage on day 10 from catch, TVC were about 1.600.000/g in the LI group and about 300.000/g in the other two groups. At that time, counts of *P. phosphoreum* were 250-270.000/g in the LI and LC-CPI groups and about 130.000/g in the PI group. This indicates that this is a dominating species at the end of storage in all test groups, although in less relative ratio in the LI group.

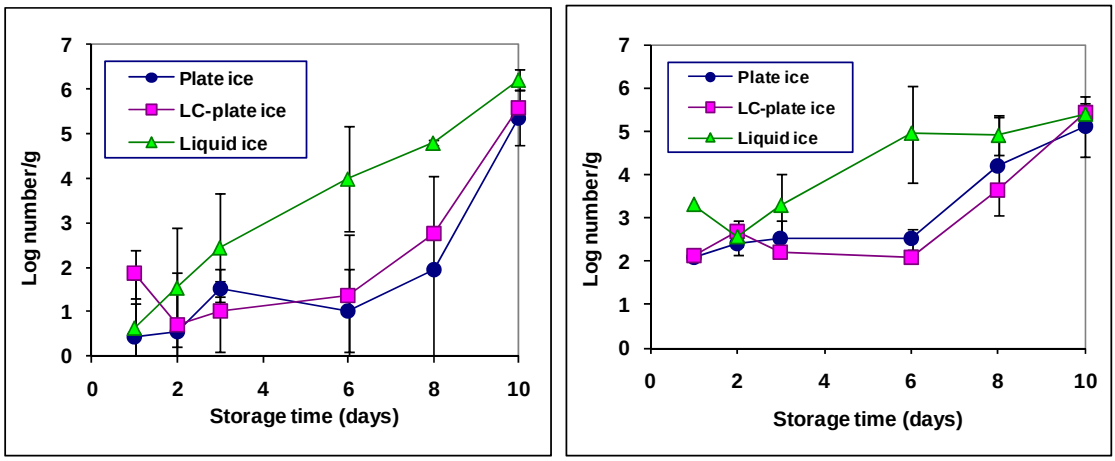


Figure 13. Total viable counts (TVC) on IA (left) and *Photobacterium phosphoreum* (right) in cod mince (ave ± stdev, n=3). (LC: Liquid ice pre-cooling).

Results from counts of H_2S-producing bacteria and presumptive pseudomonads are shown in Figure 14. Both these groups were either undetectable or in very low numbers in all experimental groups (<100/g) apart from day 10 of storage. Highest counts were most often found in the LI group, though not on day 10. The number of these bacteria in all groups at the end of storage on day 10 was in the range of 2.500-32.000/g.

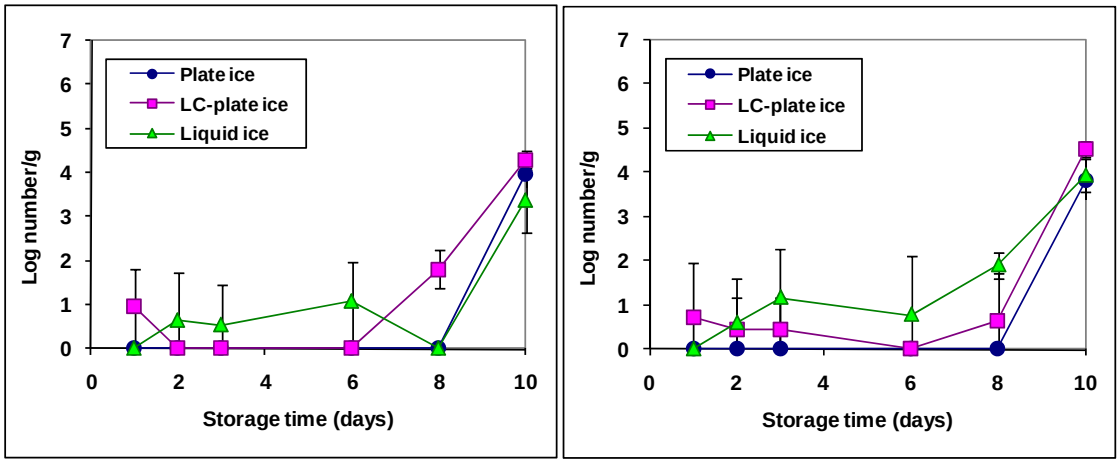


Figure 14. Growth of H_2S-producing bacteria (left) and presumptive pseudomonads (right) in cod mince (ave ± stdev, n=3). (LC: Liquid ice pre-cooling).

The microbial parameters measured indicate that the *P. phosphoreum* is the main bacterial spoiler. Nevertheless, bacterial genera such as *Psychrobacter* has been shown to be present in high relative quantities after 8 days storage of whole gutted haddock on plate ice while *Flavobacterium* was the dominating bacteria when liquid ice with icing on top was used as cooling medium (Reynisson and others, 2010). Either of these bacteria are likely to be in high numbers during late storage in the present study.

3.4 Chemical and physical measurements

Results from TVB-N and TMA measurements are shown in Figure 15. Hardly any increase was noticed in these measurements over the storage period apart from in the LI group on day 10. At that time, TVB-N was 25.5mgN/100g in the LI, 17.2 in the LC-CPI and 14.9 in the PI group. TMA reached 11mgN/100g on day 10 in the liquid ice group but was hardly measureable in the other two groups.

3.4.1 *Total Volatile Base Nitrogen and trimethylamine*

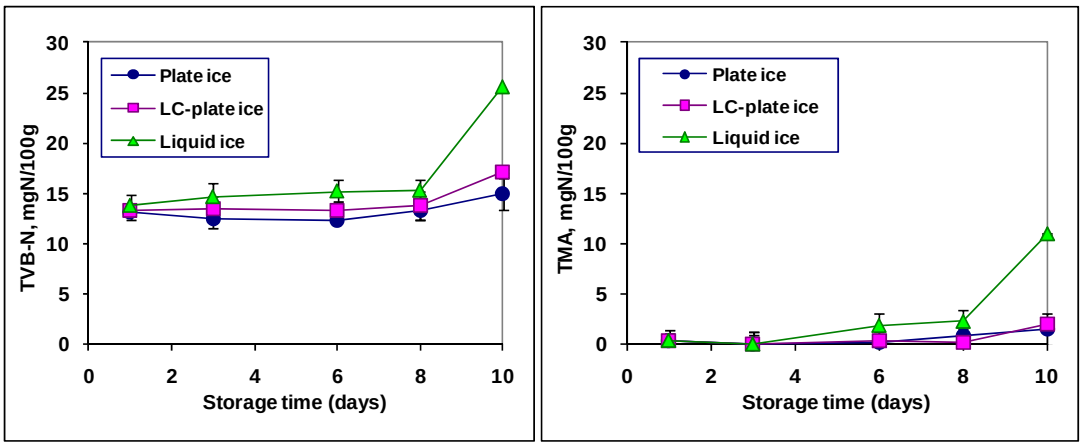


Figure 15. Total Volatile Base Nitrogen (TVB-N-left) and trimethylamine (TMA-right) in cod mince (ave ± stdev, n=3). (LC: Liquid ice pre-cooling).

The increase in both TVB-N and TMA in the later stages of the storage period may be largely attributed to growth of the spoilage bacterium *P. phosphoreum* but this bacterium is a very active reducer of trimethylamine oxide (TMAO) to TMA. This applies especially to the LI group which contained also the highest numbers of *P. phosphoreum*.

It is noted that when TMA values began to increase over 1 mg N/100g which was at day 6 in the LI group and day 10 in the other two groups, the *P. phosphoreum* values were around 100.000 CFU/g in all cases (Figures 13 and 15). Similar trends and relatively low TVB-N and TMA values were also observed in a study by Reynisson and others (2010) on whole gutted haddock stored in liquid or plate ice medium. The ratio of TMA within the volatile base measurement (TVB-N) is usually high in whole cod.

The results from microbial and chemical measurements were generally in good agreement with the results from sensory evaluation presented earlier (see e.g. Table 2).

3.4.2 *pH-measurements*

Results from pH measurements are shown in Figure 16. During the storage time the pH was in the narrow range of 6.4-6.8 in all experimental groups. At the end of storage, the pH was 6.6 in the Liquid ice group but 6.4 in the other two groups.

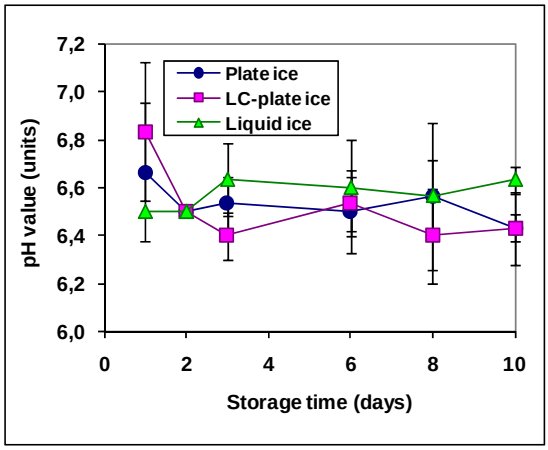


Figure 16. pH values in cod mince (ave ± stdev, n=3). (LC: Liquid ice pre-cooling).

3.4.3 *Water content, salt content and water holding capacity*

Results from measurements of % water content and % water holding capacity (WHC) are shown in Figure 17. Water content was generally highest in the LI group as expected. WHC was in all cases lowest in the PI group apart from day 8 where slightly lower values were found in the LI group. These results might indicate that putting the fish directly after catch in tubs with crushed plate ice might have some damaging effect on the cell structure of the muscle which could result in lower WHC.

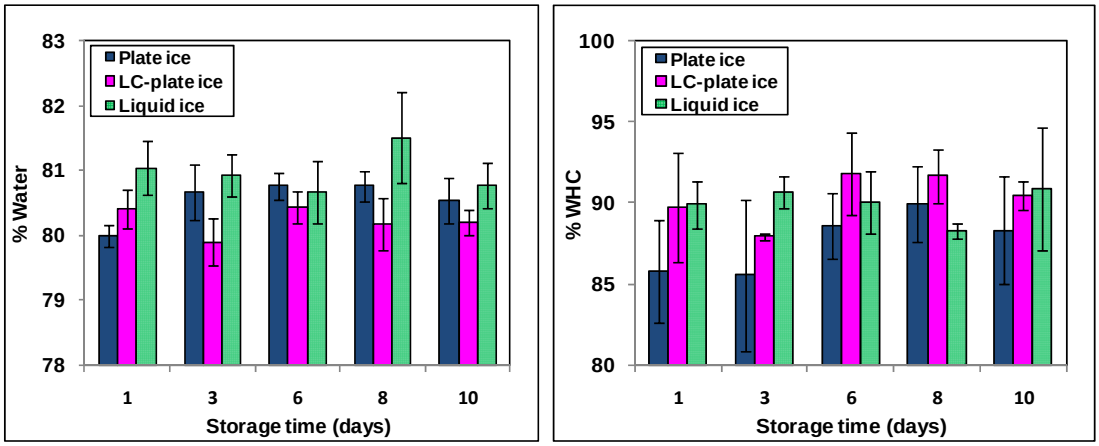


Figure 17. Water content (left) and Water Holding Capacity (WHC-right) in cod mince (ave ± stdev, n=3). (LC: Liquid ice pre-cooling).

Salt was in most cases not in measureable amount. Highest salt content measured was 0.3%.

4 CONCLUSION

The results from microbial and chemical measurements were generally in good agreement with the results from sensory evaluation. The temperature recording showed an insufficient icing on board the fishing vessel of the liquid ice group as the temperature was never lower than 0°C. Insufficient cooling with only liquid ice on-board and during storage resulted in a at least two days shorter shelf life than cooling with crushed plate ice and liquid ice followed by crushed plated ice.

The group which was pre-cooled in liquid ice before being packed in crushed plate ice both experienced the most rapid cooling and maintained the temperature better than the comparison groups. This difference was however not well reflected in the microbial developments although both plate ice storage groups showed generally lower bacterial numbers than fish stored solely in liquid ice. High ambient temperature (fluctuated between 2 – 5 °C) in the cold room during storage is the most likely reason for relatively high temperature values at the end of storage in all groups and could explain the shorter shelf life of all groups compared to earlier studies.

This study empathises well the importance of proper and sufficient icing and maintenance of low environmental temperature of the raw material right from catch to processing.

5 ACKNOWLEDGEMENTS

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7 APPENDIX

Table A. Average sensory scores (QDA) for odour.

QDA-odour	o-sweet	o-shellfish	o-meat	o-vanilla	o-potatoes	o-frozen	o-cloth	o-TMA	o-sour	o-sulphur
p-value	***0,000***	***0,000***	0,713	0,827	***0,000***	***0,000***	***0,000***	***0,000***	***0,000***	***0,000***
LC-plate ice-01d	58 [a]	47 [ab]	25	33	14 [bcd]	0 [bc]	0 [c]	1 [c]	1 [d]	0 [b]
LC-plate ice-02d	44	35	24	26	14 [c]	0 [c]	1 [c]	0 [c]	1 [d]	0 [b]
LC-plate ice-03d	43	36	21	27	13 [c]	1 [bc]	0 [c]	1 [c]	0 [d]	0 [b]
LC-plate ice-06d	30 [ce]	36	24	19	26	1 [c]	3 [bc]	1 [c]	1 [d]	1 [b]
LC-plate ice-08d	29 [ce]	24 [ce]	17	27	30	1 [bc]	9 [bc]	2 [c]	2 [d]	0 [b]
LC-plate ice-10d	29 [ce]	26 [ce]	20	31	39 [a]	2 [a]	20 [a]	8 [b]	7 [b]	2 [a]
Liquid ice-01d	52 [ac]	50 [a]	27	27	18 [bcd]	1 [bc]	1 [c]	0 [c]	1 [d]	0 [b]
Liquid ice-02d	39	42 [ac]	26	22	15 [bcd]	1 [c]	4 [bc]	1 [c]	2 [bd]	0 [b]
Liquid ice-03d	40	37	24	24	15 [bcd]	0 [bc]	1 [c]	0 [c]	1 [d]	0 [b]
Liquid ice-06d	34 [ce]	37	20	24	19 [bcd]	0 [c]	4 [bc]	1 [c]	0 [d]	1 [b]
Liquid ice-08d	29 [ce]	29 [bcd]	18	27	34 [ab]	1 [bc]	9 [bc]	2 [c]	1 [d]	1 [b]
Liquid ice-10d	25 [de]	21 [de]	18	28	39 [a]	2 [a]	25 [a]	12 [a]	15 [a]	3 [a]
Plate ice-01d	57 [ab]	52 [a]	24	28	14 [bcd]	1 [bc]	1 [c]	0 [c]	1 [d]	0 [b]
Plate ice-02d	52 [ac]	43 [ac]	26	28	10 [d]	0 [bc]	1 [c]	0 [c]	0 [d]	0 [b]
Plate ice-03d	44	34	22	31	13 [c]	0 [c]	2 [c]	0 [c]	1 [d]	0 [b]
Plate ice-06d	35 [bcd]	33	25	20	27	1 [bc]	3 [bc]	1 [c]	0 [d]	0 [b]
Plate ice-08d	30 [ce]	27 [bcd]	19	25	33 [ac]	1 [bc]	13 [b]	4 [c]	7 [bc]	0 [b]
Plate ice-10d	33 [ce]	27 [bcd]	17	32	33 [ac]	2 [ab]	11 [bc]	4 [c]	4 [bd]	0 [b]

Table B. Average sensory scores (QDA) for flavour.

QDA-flavour	f-salt	f-metallic	f-sweet	f-meat	f-frozen	f-pungent	f-sour	f-TMA	f-off
p-value	0,869	***0,002***	***0,000***	***0,019***	***0,000***	***0,000***	***0,000***	***0,000***	***0,000***
LC-plate ice-01d	5	44 [a]	42 [a]	28	0 [b]	1 [b]	1 [ce]	0 [c]	0 [c]
LC-plate ice-02d	6	33	36 [ac]	17	1 [b]	1 [b]	1 [ce]	0 [c]	0 [c]
LC-plate ice-03d	7	32	35 [ac]	28	0 [b]	3 [b]	0 [ce]	0 [c]	0 [c]
LC-plate ice-06d	8	27	25	18	1 [b]	4 [b]	1 [de]	0 [c]	0 [c]
LC-plate ice-08d	6	28	21 [bcd]	19	3	11	4 [ce]	4 [bc]	6 [c]
LC-plate ice-10d	6	24	17 [ce]	16	2	12	10 [b]	6 [b]	15 [b]
Liquid ice-01d	4	44 [a]	40 [ab]	29	1 [b]	1 [b]	0 [ce]	0 [c]	0 [c]
Liquid ice-02d	6	35	31 [ac]	29	0 [b]	3 [b]	1 [ce]	1 [c]	1 [c]
Liquid ice-03d	7	34	39 [ab]	28	1 [b]	4 [b]	1 [de]	0 [c]	1 [c]
Liquid ice-06d	7	22	24	18	0 [b]	6	1 [de]	1 [c]	0 [c]
Liquid ice-08d	7	29	23	18	2	9	4 [ce]	2 [c]	5 [c]
Liquid ice-10d	6	16 [b]	11 [de]	14	2	17 [a]	16 [a]	11 [a]	21 [a]
Plate ice-01d	4	44 [a]	42 [a]	23	0 [b]	1 [b]	0 [ce]	0 [c]	0 [c]
Plate ice-02d	6	40	35 [ac]	23	0 [b]	1 [b]	1 [ce]	0 [c]	0 [c]
Plate ice-03d	6	37	33 [ac]	26	1 [b]	4 [b]	1 [ce]	0 [c]	0 [c]
Plate ice-06d	7	29	22	19	2	5 [b]	1 [ce]	0 [c]	1 [c]
Plate ice-08d	9	28	18 [ce]	20	3	12	7 [bc]	5 [bc]	8 [c]
Plate ice-10d	5	24	18 [ce]	17	4 [a]	11	10 [b]	8 [b]	3 [c]

Table C. Average sensory scores (QDA) for appearance and texture.

QDA-appearance, texture	a-dark	a-discol	a-prec	t-flaky	t-soft	t-juicy	t-tender	t-mushy	t-meaty	t-clammy	t-rubbery
p-value	0,197	0,414	0,320	0,997	***0,000***	***0,007***	***0,000***	0,075	***0,000***	0,862	***0,000***
LC-plate ice-01d	15	21	29	56	42	50	41	25	45	20	23
LC-plate ice-02d	21	28	21	53	58 [a]	56	61 [a]	45	29 [ce]	16	13 [b]
LC-plate ice-03d	20	23	33	56	46	50	45	27	35 [ce]	23	19 [b]
LC-plate ice-06d	27	29	33	61	38	49	49 [ad]	27	31 [ce]	21	21
LC-plate ice-08d	18	24	31	52	50 [ab]	48	49 [ad]	39	33 [ce]	20	18 [b]
LC-plate ice-10d	27	31	34	51	57 [a]	53	57 [ab]	36	28 [ce]	16	14 [b]
Liquid ice-01d	16	22	35	54	27 [c]	35	25 [efg]	17	60 [a]	22	36
Liquid ice-02d	18	22	27	51	34 [bc]	36	29 [dg]	22	55 [ab]	34	41 [a]
Liquid ice-03d	20	21	32	49	49 [ab]	55	48 [ad]	30	36 [ce]	24	18 [b]
Liquid ice-06d	19	21	28	56	41	37	35 [cdf]	28	39 [bcd]	25	33
Liquid ice-08d	19	29	33	54	48	48	44	35	38 [bcd]	24	19 [b]
Liquid ice-10d	22	29	28	54	57 [a]	46	56 [ac]	32	26 [de]	18	15 [b]
Plate ice-01d	16	21	28	53	41	46	43	28	42 [bcd]	16	27
Plate ice-02d	18	27	22	53	44	52	48 [ad]	26	47 [ac]	26	19 [b]
Plate ice-03d	23	26	30	56	42	47	41	28	41 [bcd]	23	18 [b]
Plate ice-06d	26	30	25	62	43	53	49 [ad]	33	32 [ce]	19	18 [b]
Plate ice-08d	20	23	35	55	45	45	43	34	41 [bcd]	24	20 [b]
Plate ice-10d	17	25	30	52	37	43	36 [bcde]	31	35 [ce]	26	23