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Comparison of cooling techniques - Their efficiency during cooling and storage of whole, gutted haddock, and their effect on microbial and chemical spoilage indicators

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Report summary

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Titill / Title	**Comparison of cooling techniques – Their efficiency during cooling their effect on microbial and chemical spoilage indicators**		
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Ágrip á íslensku:

Markmið tilraunanna var að rannsaka áhrif mismunandi ístegunda við niðurkælingu og geymslu á heilum, slægðum fiski á hita- og skemmdarferla. Þrjár ístegundir voru notaðar: hefðbundinn mulinn plötuís („flöguís“) (nefndur PI hér) auk tveggja gerða ískrapa (vökvaíss) framleiddum í þar til gerðum ískrapavélum (nefndar LIA og LIB hér) með mismunandi salt- og íshlutfall ískrapa.

Niðurstöður hitamælinga sýndu fram á mun hraðari niðurkælingu með ískrapa en hefðbundnum flöguís. Þá reyndist niðurkæling nokkru hraðari með annarri tegund ískrapa (LIB) en hinnar (LIA) því hiti ýsu kæld í LIB fór úr 7.5 °C niður fyrir 0 °C á 20 – 30 mín miðað við um 55 – 60 mín í LIA. Samsvarandi tími fyrir hefðbundinn flöguís var um 260 mín. Munurinn á kælitíma í LIA og LIB má að hluta til skýra með 10% þyngri fiskum í LIA hópnum.
Niðurkæling heillar ýsu úr 10 °C og 20 °C gaf sambærilegar niðurstöður og niðurkæling úr 7.5 °C. Kælitími úr 10 °C niður í 4 °C var 24 mín fyrir LIB hópinn og 36 mín fyrir LIA hópinn. Sambærilegur kælitími úr 20 °C í 4 °C var 46 mín fyrir LIB samanborið við 55 mín fyrir LIA.

Niðurstöður örverumælinga með ræktanlegum aðferðum sýndu að lítill vöxtur sérhæfðra skemmdarörvera (SSÖ) á ýsuroði átti sér stað snemma á geymslutímanum, óháð kælingaraðferð. Með frekari geymslu var örveruvöxtur svipaður milli kælihópanna með ísyfirlag efst í kerinu. Sambærilega örveruvaxtarþróun var að sjá í holdi þar til á 8. degi mældist marktækur hærri fjöldi *Photobacterium phosphoreum* og H_2S-myndandi baktería í LIB-kældum fiski. Það er athyglisvert að nefna að þau mismunandi hitastigsprófíl sem mældust meðal kælihópanna endurspegluðu ekki örveruvaxtarþróun sem átti sér stað. Raunar virtist skemmdargeta SSÖ ekki vera minni við köldustu aðstæðum þegar geymslutíminn leið, því marktækt hærra magn TVB-N og TMA mældist í fiskum sem fengu ískrapa meðferð samanborið við hefðbundna ísgeymslu. Hugsanlegt er að þau skilyrði sem skapast við þessar vatnsmeiri og saltaðar aðstæður við notkun ískrapa eru óæskileg og leiða til hraðara skemmdarferlis en gerist við ísaðar aðstæður.

Lykilorð á íslensku: *Kæling, ístegundir, ískrapi, ýsa, sérhæfðar skemmdarörverur*

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Summary in English:

The aim of study was to investigate the effects of different ice media during cooling and storage of whole, gutted whitefish on temperature control and spoilage indicators. The thermodynamic, microbial and chemical properties of whole, gutted haddock were examined with respect to the cooling medium in which it was stored. Three basic types of cooling medium were used: traditional crushed plate ice (PI+PI) and two types of commercially available liquid (slurry) ice, here denoted as LIA and LIB. The ice types were furthermore divided into five groups with different salinity and ice concentration.

Microbiological analysis by cultivation methods revealed that growth of some specific spoilage organisms (SSO) on fish skin was delayed at early storage, independently of the cooling methods. With further storage, little or no difference in counts was seen among traditionally iced fish and those cooled in liquid ice for 2 h before draining and top layer icing. Even less difference was observed in the flesh microbiota developing until significant growth increase in *Photobacterium phosphoreum* and H_2S-producing bacteria was seen on day 8 in LIB cooled fish. Interestingly the differences obtained in the temperature profiles of fish cooled differently were not supported by different bacterial growth behaviour. In fact, SSO spoilage potential was not reduced in the coolest treatments as time progressed, as demonstrated on day 8 by the significantly higher TVB-N and TMA content of fish cooled in liquid ice compared to traditional icing. Conditions created by liquid ice environment (salt uptake of flesh) may have been unfavourable, causing an even faster fish deterioration process with increasing storage time compared to traditional ice storage.

Evaluation of the thermodynamic properties showed that LIB gave slightly faster cooling than LIA. For haddock stored in LIB the flesh reached 0 °C in 20-30 min, but it took 57 min in LIA and around 260 min in crushed plate ice (PI). The difference in the cooling rate of LIA and LIB might, apart from the physical properties of the ice, partially be explained by the fish weight, being on average 10% more in the LIA group.

The additional cooling rate experiments where whole, gutted haddock was cooled down from 20 °C and 10 °C gave similar results. When cooled down from 20 °C the haddock reached 4 °C in 46 min when chilled in LIB while the same process in LIA required 55 min. Similar difference was seen when the material was cooled down from 10 °C, where fish chilled in LIB reached 4 °C in 24 min and fish chilled in LIA reached 4 °C in 36 min.

English keywords: *Cooling, ice media, liquid ice, haddock, specific spoilage organisms*

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Introduction

Effective cooling prolongs the shelf life of food and fish in particular. In recent years, improvements in cooling technologies have led to the development and commercialisation of various slurry ice machines where mixtures of water, ice, salt and gases have been used to increase cooling rate and optimise temperature during transportation. The overall aim is to reduce bacterial growth, extend shelf life and thereby increase product quality (Bellas and Tassou 2005). Several different slurry ice production technologies and a number of slurry ice brands exist (e.g., Liquid Ice, Flow Ice, Optim-Ice, Fluid Ice and Bubble Slurry Ice). The thermal and rheological properties along with other characteristics of ice slurries have been thoroughly described (Egolf *et al*. 2005). Information on how these new techniques affect the spoilage microbiota present on the skin and flesh of the fish is limited at present. Examples of well-documented fish spoilage organism are members of the *Pseudomonas* genus and *Shewanella putrefaciens* which are frequently used as bacterial indicators for spoilage and are present in different food types (Gram and Huss 1996, McMeekin 1975, von Holy and Holzapfel 1988). *Photobacterium phosphoreum* has not been investigated as intensively but there are scientific evidences that it is the main spoilage organism when environmental conditions are favourable such as in chilled modified atmosphere packed fish (Lauzon *et al.* 2009, Dalgaard *et al*. 1997, Emborg *et al*. 2002). Other workers have reported its presence in aerobically spoiled fish (van Spreekens 1974, Esaiassen *et al*. 2004) and its importance in the spoilage of air-stored gadoid fish products was recently reported (Olafsdottir *et al*. 2006a,b). Knowledge on how the spoilage microbiota will respond to different cooling methods can help to better understand the spoilage process and design cooling methods that can prolong shelf life.

The aim of study was to investigate the effects of different ice media during cooling and storage of whole, gutted whitefish on temperature control and spoilage indicators. The thermodynamic, microbial and chemical properties of whole, gutted haddock were examined with respect to the cooling medium in which it was stored. Three basic types of cooling medium were used: traditional crushed plate ice (PI+PI) and two types of commercially available liquid (slurry) ice, here denoted as LIA and LIB. The ice types were furthermore divided into five groups with different salinity and ice concentration.

These experiments were carried out as a part of the research project EU-funded Integrated Research Project CHILL-ON (contract FP6-016333-2).

Materials and Methods

Main experimental setup (13th-21st of October 2008)

Five groups were used in the experiments carried out during the 13th-21st of October.

1. **PI+PI**: 100 kg of fish is packed in 45 kg of crushed plate ice (PI) in a drained tub and stored for 7-8 days.
2. **LIB**: 100 kg of fish is packed in 100 kg of liquid ice B (LIB) with ice ratio 47% (x_{salt} = 2.1±0.1%, T=-2.4 °C). Chilled for 2 h with tub undrained and not re-iced. Stored in a drained tub.
3. **LIB+DRAIN**: 100 kg of fish is packed in 100 kg of liquid ice B (LIB) with ice ratio 47% (x_{salt} = 2.1±0.1%, T=-2.2 °C). Drained gently (tub stoppers partly open) from the beginning and not re-iced.
4. **LIB+PI**: 100 kg of fish is packed in 100 kg of liquid ice B (LIB) with ice ratio 34% (x_{salt} = 2.1±0.1%, T=-2.2 °C). Chilled for 2 h with tub undrained then re-iced by adding 12 kg of crushed plate ice (PI) on top of the top layers. Stored in a drained tub.
5. **LIA+PI**: 100 kg of fish is packed in 100 kg of liquid ice A (LIA) with ice ratio 34% (x_{salt} = 2.9±0.1%, T=-2.2 °C)**.** Chilled for 2 h with tub undrained then re-iced by adding 12 kg of crushed plate ice (PI) on top of the top layers. Stored in a drained tub.

Temperature logging

The fish in each group was packed in five layers and temperature loggers inserted into the fish flesh in four individuals weighting approximately 600-1300g at the bottom, middle and top layer. This made a total of 12 loggers which were used to monitor the temperature in the fish flesh for each group every 5 min. In addition to those loggers one temperature logger was used to record 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 the 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

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

where $T_{1,i}$ 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.

Sampling of whole, gutted fish

At initial sampling (d0), four fish were removed aseptically from the fish tubs, pooling 2 fish per sample. During subsequent sampling (d2-4-8), four fish were removed aseptically from the top layer of the differently treated tubs, pooling two fish per sample for each treatment examined. Fish were transferred to the laboratory in styrofoam boxes and analysed within 30-60 min.

Microbiological analyses

Microbiological counts were evaluated for both the skin and flesh of haddock. Two pieces of skin were aseptically cut from one side of each fish (2 fish = 1 sample), giving a total area of 30 cm^2, and mixed with 60 mL of cooled Maximum Recovery Diluent (MRD, Oxoid) for 1 min in a stomacher (Stomacher Lab Blender 400, A.-J. Seward Laboratories, London, UK). Successive tenfold dilutions were done as required. The other side of each fish was aseptically skinned, pieces of flesh removed, minced, diluted tenfold in cooled MRD and stomached for 1 min. Spread-plating of aliquots was done on several media and all plates incubated aerobically. Total viable psychrotrophic counts (TVC) and counts of H_2S-producing bacteria were evaluated on iron agar (IA) as described by Gram *et al*. (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. TVC were also done on modified Long and Hammer´s agar (LH) according to van Spreekens (1974) with the exception that 1% NaCl was used instead of 0.5%. Plates were incubated at 17 °C for 5 d. 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 above mentioned methods, surface-plating was used. Counts of *Photobacterium phosphoreum* were estimated by using the PPDM-Malthus conductance method (Dalgaard *et al.* 1996), as described by Lauzon (2003). All samples were analysed in duplicate and counts presented as an average of colony-forming units (CFU).

Chemical analyses

The minced flesh samples prepared for microbiological analyses were used. 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 mince with 7.5% aqueous trichloroacetic acid (TCA) solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulphuric acid solution. TMA was measured in TCA extract by adding 20 mL of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine. The pH measurements were performed in 5 g of mince moistened with 5 mL of deionised water. Salt content was measured with the Volhard Titrino method according to AOAC ed. 17 from 2000 (no. 976.18). All chemical analyses were done in duplicate.

Statistical analysis

Statistical analysis of microbial and chemical data was carried out with NCSS 2000 software (UT, USA) using analysis of variance methods (Kruskal–Wallis one-way ANOVA on ranks for salt content and one-way ANOVA for all other variables). Comparison of data with respect to treatments was performed using the Kruskal–Wallis multiple comparison z-value test and Fisher's LSD multiple comparison test, respectively. The threshold for significance was 0.05.

Additional cooling rate experiment (27th-29th of October)

During the 27th-29th of October, cooling rate experiments were carried out for different types of liquid ice. In all cases 100 kg of gutted haddock were packed in 50 kg of ice. It was assumed that even though some spoilage would have begun in the raw material used in the main experiment, it would still keep its thermodynamic properties. The same raw material was therefore used as in the main experiment. Two batches of gutted haddock were heated up to 20 °C and another two up to 10 °C

using hot water. The same methodology was used for temperature logging as in the main experiment, except that 6 loggers were used in each layer for the groups which were cooled down from 10 °C. Having inserted all the temperature loggers and packed the raw material in ice, a lid was put on each tub which was stored in HB Grandi's cool storage room for approximately 16 h.

Table 1. Experimental groups for cooling rate experiment

Group #	x_{ice} [%]	T_0 [°C]	ice type	T_{ice} [°C]
1	24	20	LIB	-2.3
2	24	20	LIA	-2.4
3	24	10	LIB	-2.3
4	24	10	LIA	-2.3

Equipment

Raw material

Haddock was caught using a longline on October 13, 2008, in Hvalfjörður by Svalur BA120, stored ungutted and uniced in 2 tubs according to the needs of the experiment. Upon landing (about 4-6 h from catch), the fish was brought to the market storage room, and then gutted and rinsed. The fish was 6-9 h old from catch when received in the storage room in HB Grandi (Iceland) and its temperature was around 8 °C.

Containers

Fish tubs constructed of polyethylene and polyurethane with styrofoam insulated walls and cover, were used to store the fish in different cooling treatments. Each 310 L tub had a sufficient storage capacity for 100 kg of fish and ice.

Temperature logging

Two types of temperature recording devices were used for temperature monitoring:

a. iButton temperature loggers, type DS1922L: This logger has an accuracy of ±0.5 °C, a resolution of 0.0625 °C and an operating range of -40 to +85 °C. The diameter is 17 mm and the thickness is 5 mm. The iButton loggers were used in the whole fish, positioned as deep in the fish flesh as possible. http://www.maxim-ic.com/quick_view2.cfm/qv_pk/4088

b. Onset temperature logger, type UTBI-001: This logger has an accuracy of ±0.2 °C, a resolution of 0.02 °C and an operating range of -20 to +70 °C. The diameter is 30 mm and the thickness is 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. http://www.onsetcomp.com/products/data-loggers/utbi-001

Ice makers

Two commercially available ice makers were used for the experiment. The LIB ice was produced on site while the LIA ice had to be transported ca. 6 km in an insulated tub to the experiment facilities.

Results

Main experiment (13th-21st of October)

Figure 1 shows the temperature development of gutted haddock during the first 6 h of the experiment. The groups where LIB was used seem to give slightly faster cooling. In those groups 0 °C was reached in 20-30 min while the same process took around 57 min for LIA. Crushed plate ice (PI+PI) showed the slowest cooling rate where 0 °C was reached after 4 h and 20 min. The cooling rate must however be examined with respect to the average weight of the groups displayed in **Error! Reference source not found.**. The difference in cooling rate (Figure 2) between LIA and LIB might partly be explained by the fact that the fish in the LIA group had on average 10% more weight than other groups. Another reason might be the delayed use of type A of liquid ice, as a period of about 2 h had elapsed between production and use in the experiment.

Figure 3 shows how well temperature was maintained during the storage period. It is evident that excessive amount of ice was used in all cases since the maximum temperature difference during the storage period of 8 days was around 1 °C. The temperature of PI+PI and LIA groups increased by 0.3 °C during the storage period. The temperature in LIB increases by 1 °C during the same period.

On Figure 4 the average temperature of the layers is displayed along with the ambient temperature outside the tub for each group. The temperature in HB Grandi cool storage room fluctuated between 2-5 °C. No significant difference can be seen in ambient temperature between groups.

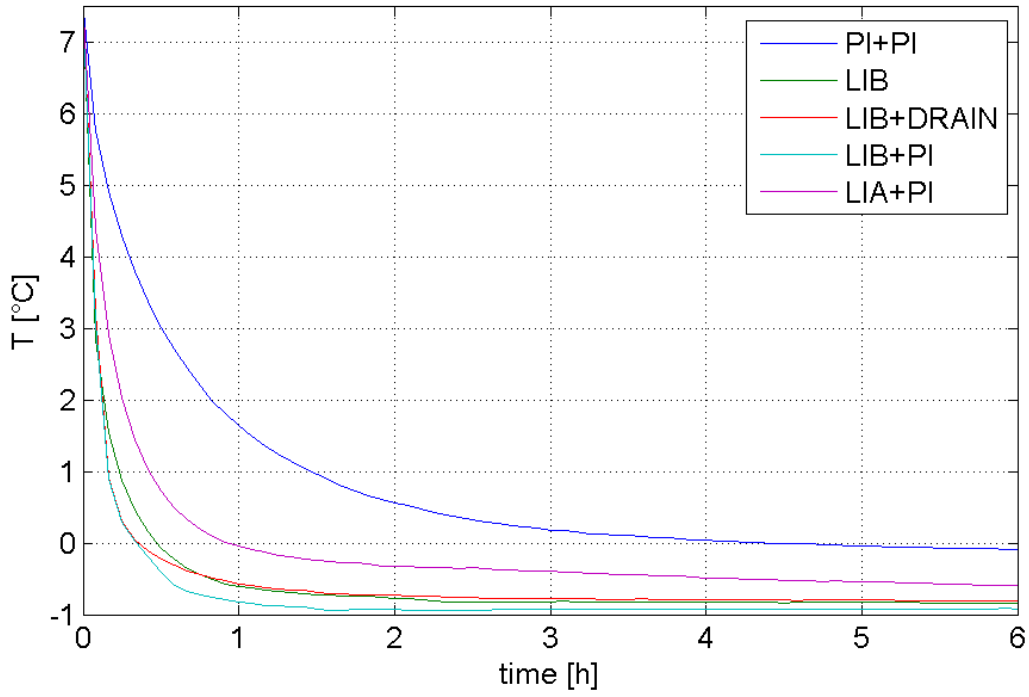

Figure 1: Cooling during the first 6 h – mean temperature of all layers (T0=7.5 °C)

Table 2. Average weight of the fish where temperature was logged and percent deviation based on a standard fish of 952 g

	PI+PI	LIB	LIB+DRAIN	LIB+PI	LIA+PI
Ave. weight [g]	897	915	1000	904	1045
Deviation	-5.8%	-4.0%	5.1%	-5.1%	9.8%

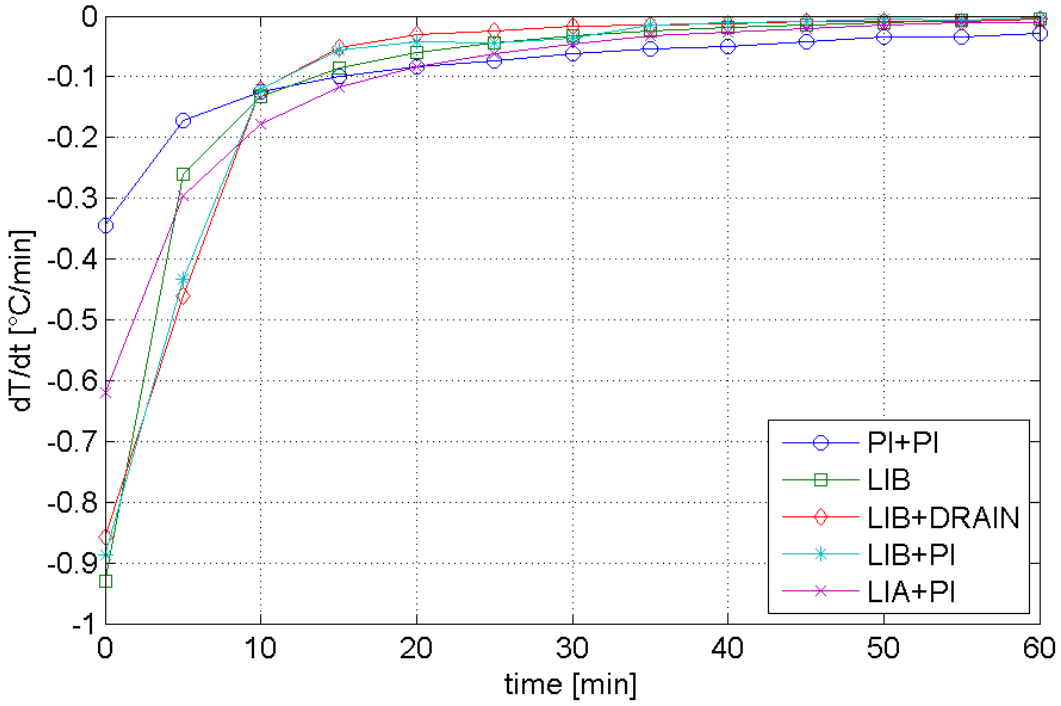

Figure 2: Cooling rate during the first hour – mean temperature of all layers (T0=7.5 °C)

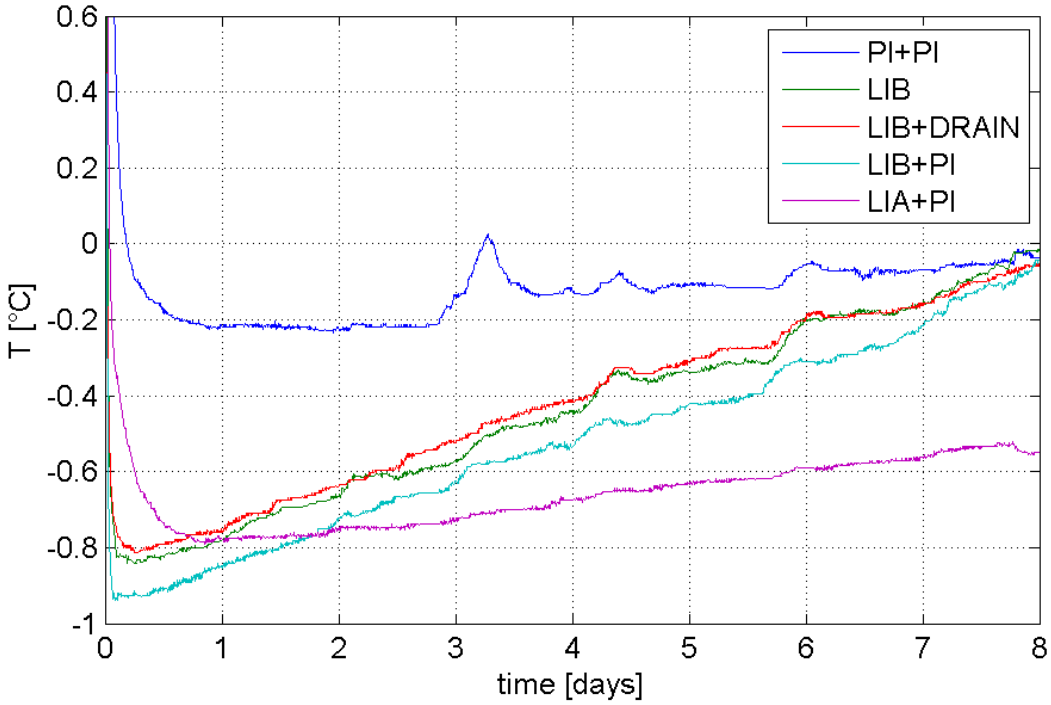

Figure 3: Maintenance of temperature - mean temperature of all layers (T0=7.5 °C)

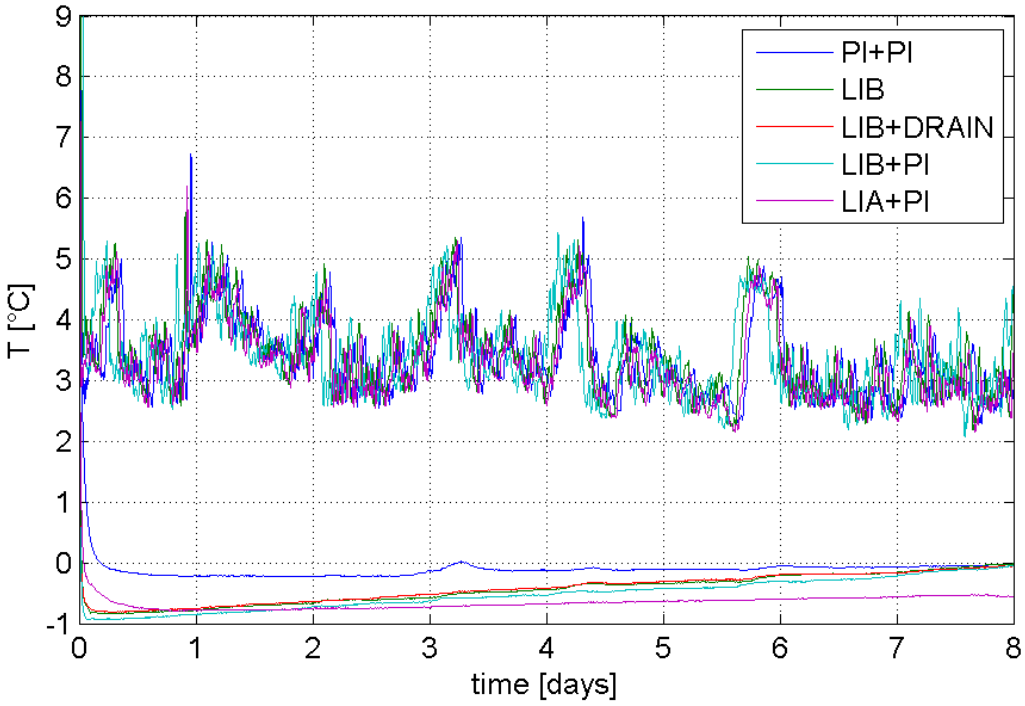

Figure 4: Fish temperature maintenance (curves below 0 °C) and fluctuating ambient temperature of tubs between 2-5 °C

Effects of cooling methods on microbiological load of haddock skin

Skin counts were assessed initially on the raw material used, resulting in an overall psychrotrophic load (TVC) of 3.1 ± 0.1 log CFU/cm^2. *Photobacterium phosphoreum* (Pp = 2.9 ± 0.3 log CFU/cm^2) was found to dominate among the specific spoilage organisms (SSO) assessed (H_2S-producers, 2.2 ± 0.3 log CFU/cm^2; pseudomonads, 0.8 ± 0.3 log CFU/cm^2). The growth behaviour of these SSOs was influenced by the cooling methods, as shown in Figure 5.

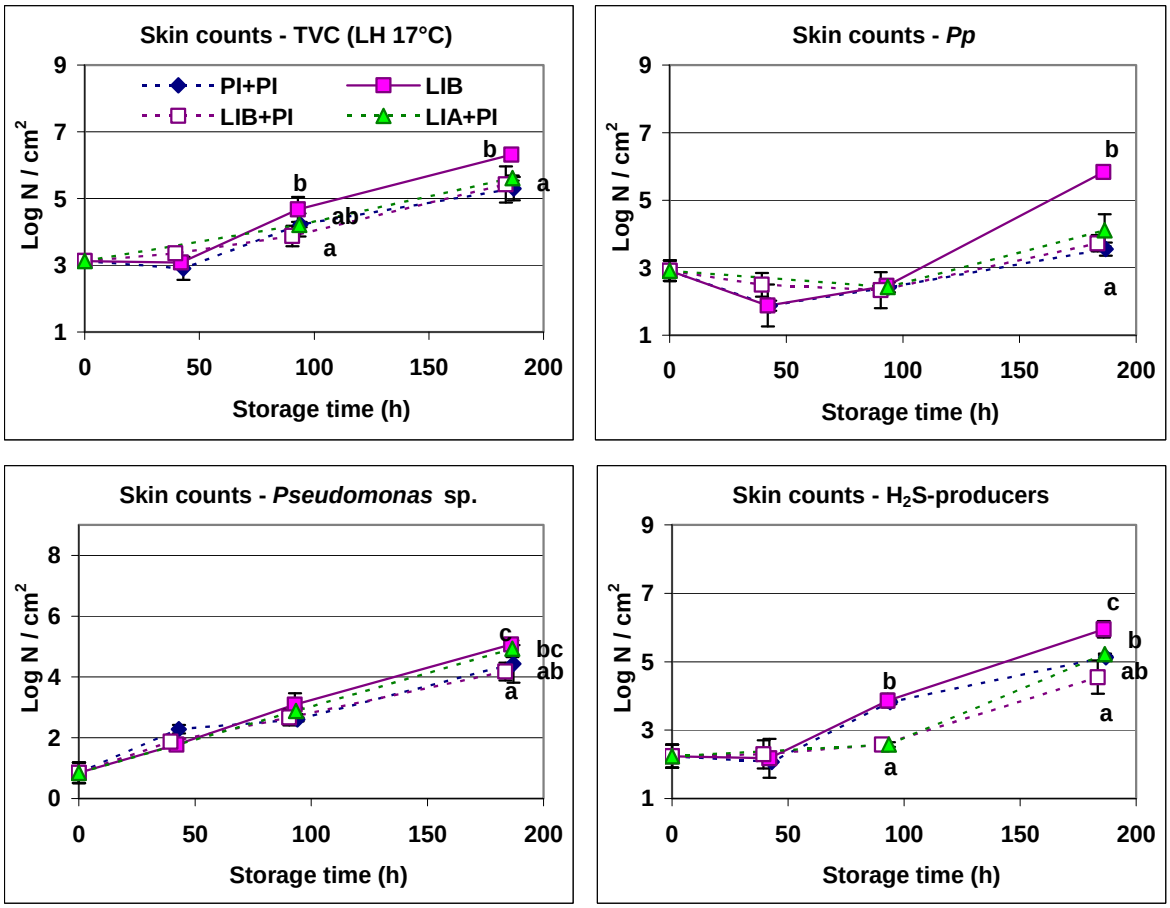

Figure 5: Development of microbiota on haddock skin as influenced by the cooling methods. Different letters indicate significant count differences among cooling methods on respective sampling day ($p < 0.05$).

The least effect was observed during the first 4 days of storage for pseudomonads which grew steadily, independently of the cooling methods used. However after 8 days of storage, pseudomonad counts were significantly ($p < 0.05$) lower on fish cooled with LIB+PI than LIB alone or LIA+PI. Ice storage performed as well as LIB+PI in slowing down pseudomonads. Pp growth was delayed for at least 4 days by all cooling methods, after which a significant growth increase was seen on fish stored in LIB alone. A similar but shorter delay was seen for H_2S-producers, followed by significant growth in LIB cooled fish. Counts of H_2S-producers were significantly lower on LIB+PI fish than LIA+PI fish, but no significant difference was observed with iced fish. The psychrotrophic microbiota developed significantly faster on LIB stored fish than the other treatments. Overall, it can be said that time of

storage is an important factor in the efficiency assessment of different cooling methods. No significant differences in skin microbial load were observed among the cooling methods after 2 days of storage, while after 4 days H_2S-producers grew significantly faster on LIB stored fish as well as fish stored in crushed plate ice. After 8 days, SSOs evaluated had reached significantly higher levels on LIB stored fish, while no or little difference was seen among other cooling methods. This was observed despite the large temperature variations seen in Fig. 1, with crushed plate ice showing the warmest profile and LIA the coolest one.

Effects of cooling methods on microbiological load of haddock flesh

Flesh count of raw material was not assessed before the start of the experiment, as generally known to be sterile in newly caught fish. Psychrotrophic flesh counts were slightly influenced by the cooling methods on day 2, but no significant differences were seen in TVC and pseudomonad counts as time progressed (Figure 6).

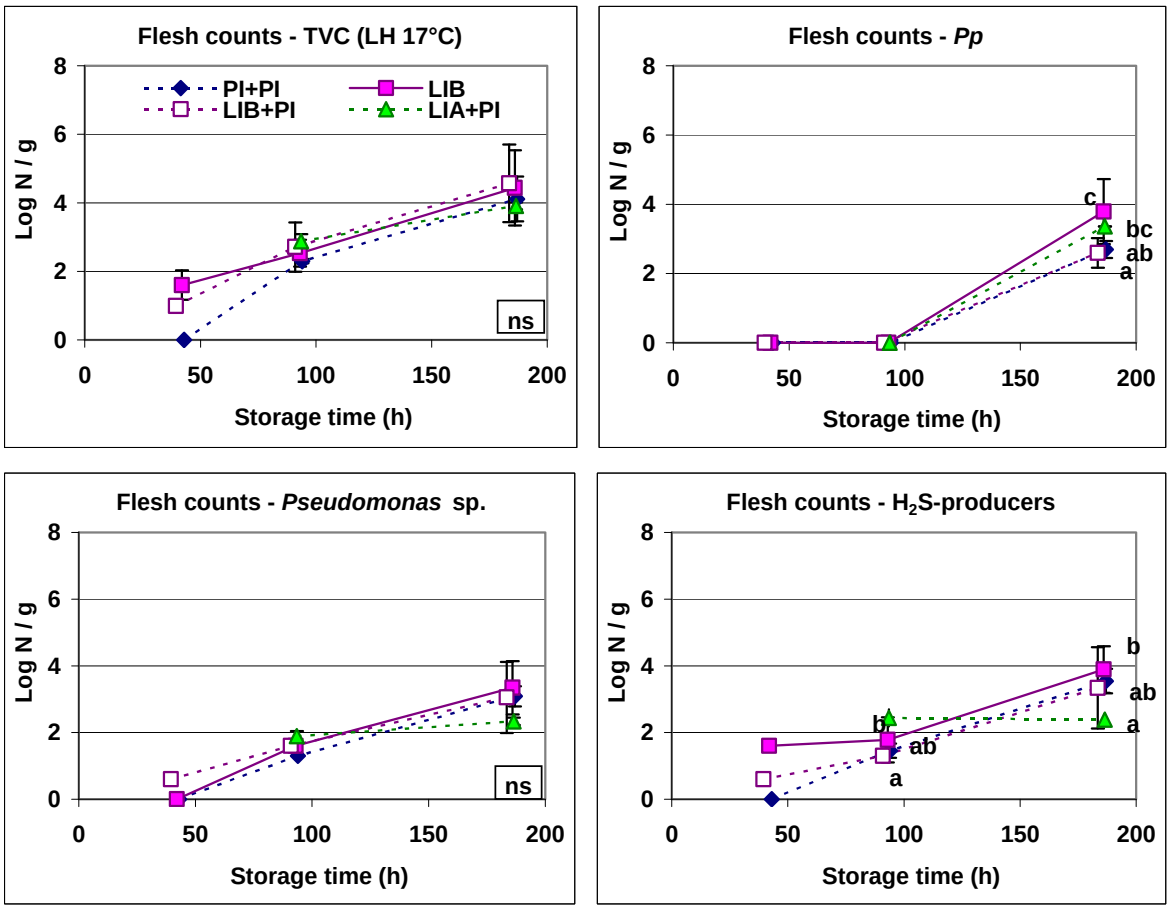

Figure 6: Development of microbiota in haddock flesh as influenced by the cooling methods. Different letters indicate significant count differences among cooling methods on respective sampling day ($p < 0.05$); ns, not significant.

Pp growth was delayed by all cooling methods for at least 4 days, which agrees with the results for skin counts. After 8 days of storage, slowest growth was seen in LIB+PI as well as PI+PI stored fish and fastest growth in LIB stored fish. At that time point, H_2S-producers reached lowest level (2.4 log CFU/g) in LIA+PI stored fish, but highest (3.3-3.9 log CFU/g) in the other groups. Pseudomonads and H_2S-producers are known to be less influenced by superchilling than *Photobacterium phosphoreum* (Olafsdottir *et al*. 2006b). Based on the temperature profile recorded, the 0.5 °C temperature gradient observed between LIB+PI or LIA+PI and ice stored fish, mostly at the superchilling zone, does not explain the similar Pp growth seen in these treatments. Therefore it is suggested that the more salty and wet environment created by the liquid ice may have contributed to the transition of Pp from the skin to the flesh and influenced its development despite this superchilled condition. Finally, it can be pointed out that the effects caused by the different cooling methods on the flesh spoilage microbiota were apparently lesser than those reported for the skin microbiota.

Effects of cooling methods on chemical changes in haddock flesh

Measurements of pH throughout the storage time varied very little (Figure 7). Salt uptake was evaluated in the flesh of fish stored under liquid ice treatments (LIB, LIB+PI, LIA+PI). LIB ice contained 2.1 ± 0.1% NaCl, while 2.9 ± 0.1% was found in LIA+PI. Initial NaCl content on the fish flesh was 0.15 ± 0.07%. After 2 and 4 days of storage, a slight salt uptake was noticed, but after 8 days a significant ($p < 0.05$) increase was found in all liquid ice treatments compared to the NaCl content of the raw material.

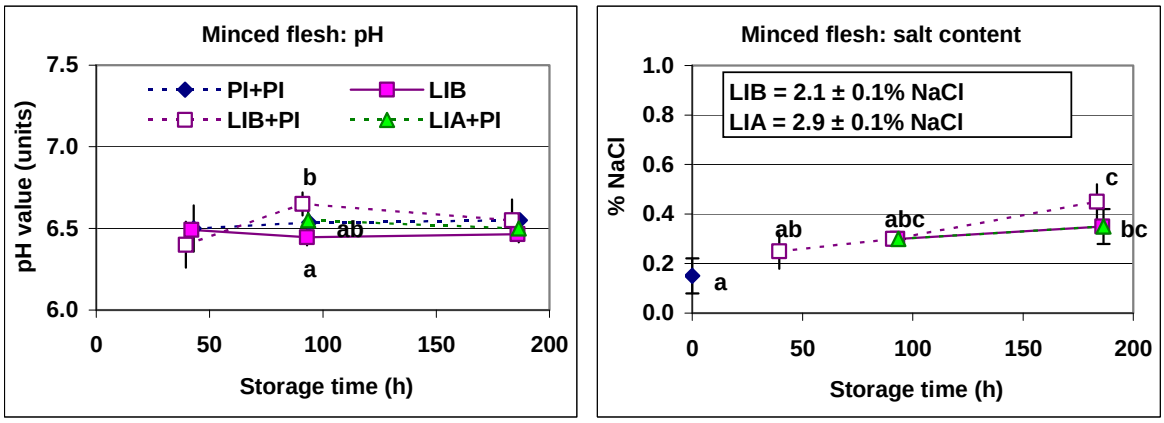

Figure 7: Changes in pH and salt content of haddock flesh as influenced by the cooling methods. Different letters indicate significant differences among cooling methods ($p < 0.05$).

Measurements of TVB-N and TMA were done after 8 days of storage. Their increase usually reflects the production of bacterial spoilage metabolites. In iced gadoid fish, rejection levels are 30-35 mg N (TVB) and 10-15 mg N (TMA) in 100 g of flesh. Initial TVB-N content in the raw material amounted to 11.9 ± 2.8 mg N/100 g. No increase was observed in iced fish after 8 days of storage, in agreement with the undetected TMA. In the liquid ice treatments that were iced on top (LIB+PI and LIA+PI), a significant increase in TVB-N and TMA was found on day 8 (Figure 8). When LIB was used alone, the

highest levels were found. This difference between the liquid ice treatments (with or without icing) can be related to the better temperature control provided by the additional top crushed plate ice layer as storage time progressed (Fig.1). In fact, TMA is readily produced as temperature increases. LIB fish had reached 0.5 °C on day 8 in the top layer (data not shown) and their TMA content as well as P ratio (TMA/TVB-N) were the highest. Considering the faster accumulation of volatile basic compounds observed in these liquid ice treatments than in iced fish despite the higher temperature profile of the latter, it can be speculated that the conditions created by the liquid ice environment became more favourable to the bacterial production of these spoilage metabolites. In fact, this can probably be linked to growth of Pp which is an efficient TMA producer (Dalgaard 1995a, b).

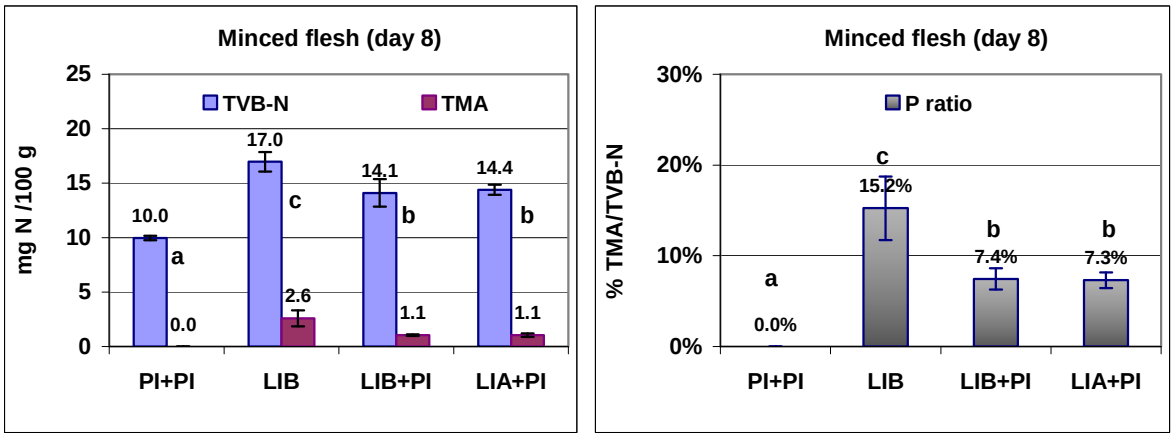

Figure 8: Changes in pH and salt content of haddock flesh as influenced by the cooling methods. Different letters indicate significant differences among cooling methods ($p < 0.05$).

Additional cooling rate experiment (27th-29th of October)

Figure 10 shows the cooling of gutted haddock during the first hour of the experiment. Two reference temperatures are used for both ice types, 10 °C and 20 °C. When cooled from 10 °C the fish packed in LIB took 24 min to reach 4 °C while it took 36 min for fish packed in LIA. When cooled from 20 °C the fish packed in LIB took 46 min to reach 4 °C while it took 55 min for the fish packed in LIA.

The main objective of this experiment was to examine the initial cooling rate of the material rather than the maintenance of temperature. Since the amount of ice which was used was limited none of the groups reached temperatures below 0 °C. This can be seen from Figure 9 where the temperature of all groups stabilises around 2 °C. As can be seen from Table 3 there was little variation of weight between groups. Figure 12 shows that the ambient temperature was around 2-4 °C during most of the storage period and is comparable for all groups.

Table 3. Average weight of the fish where temperature was logged and percent deviation based on a standard fish of 1010 g

	LIA – 20 °C	LIB – 20 °C	LIA – 10 °C	LIB – 10 °C
Ave. weight [g]	1029	985	1010	1016
Deviation	1.9%	-2.5%	0.0%	0.6%

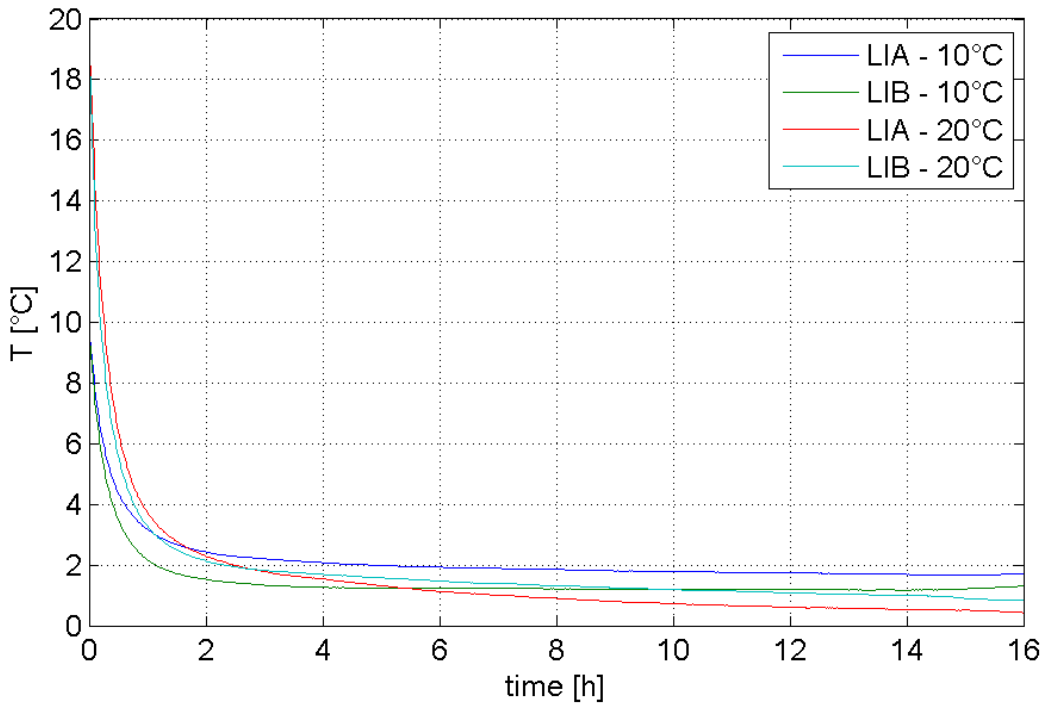

Figure 9: Cooling of haddock from 10 and 20 °C – mean temperature of all layers

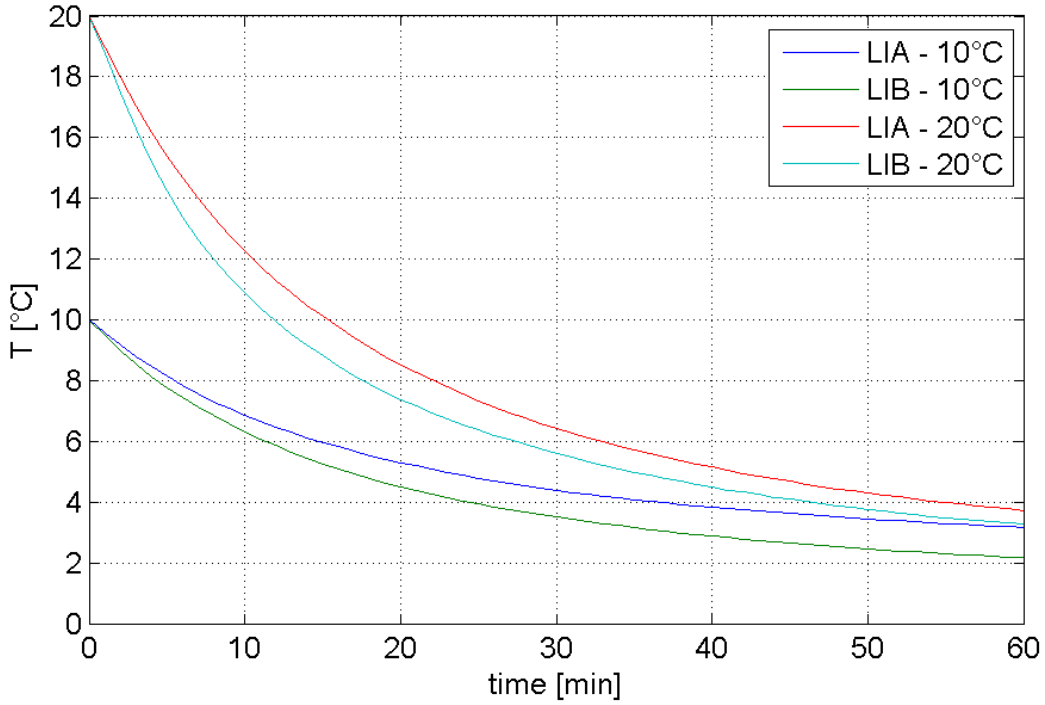

Figure 10: Cooling of haddock from 10 and 20 °C during the first hour – mean temperature of all layers

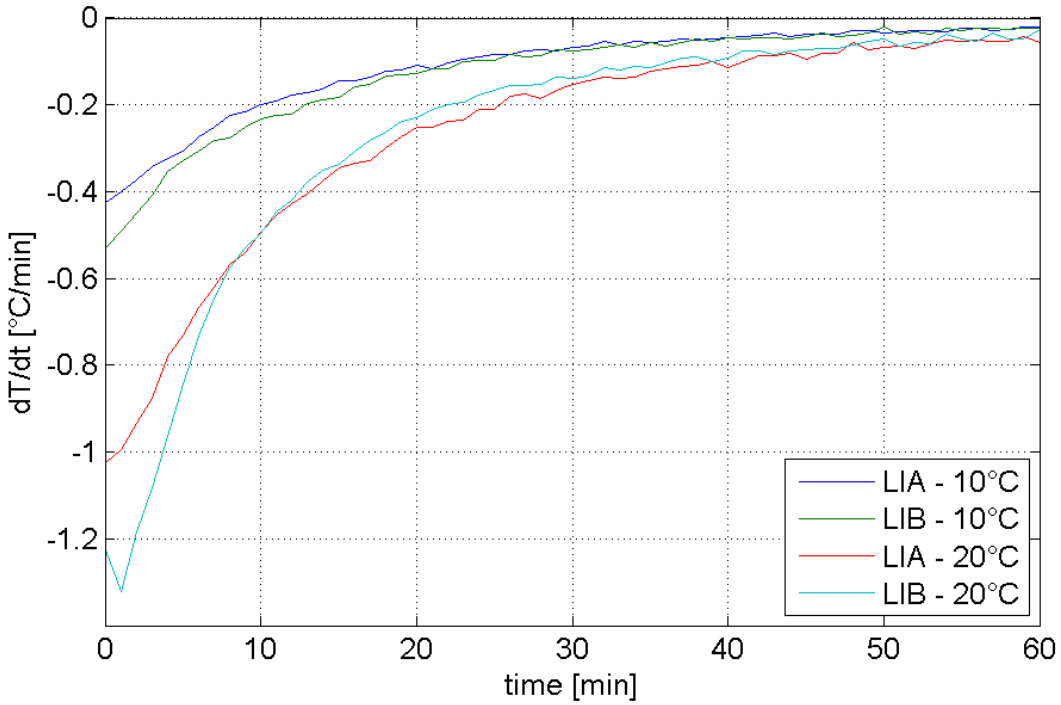

Figure 11: Cooling rate of 10 °C and 20 °C haddock during the first hour – mean of all layers

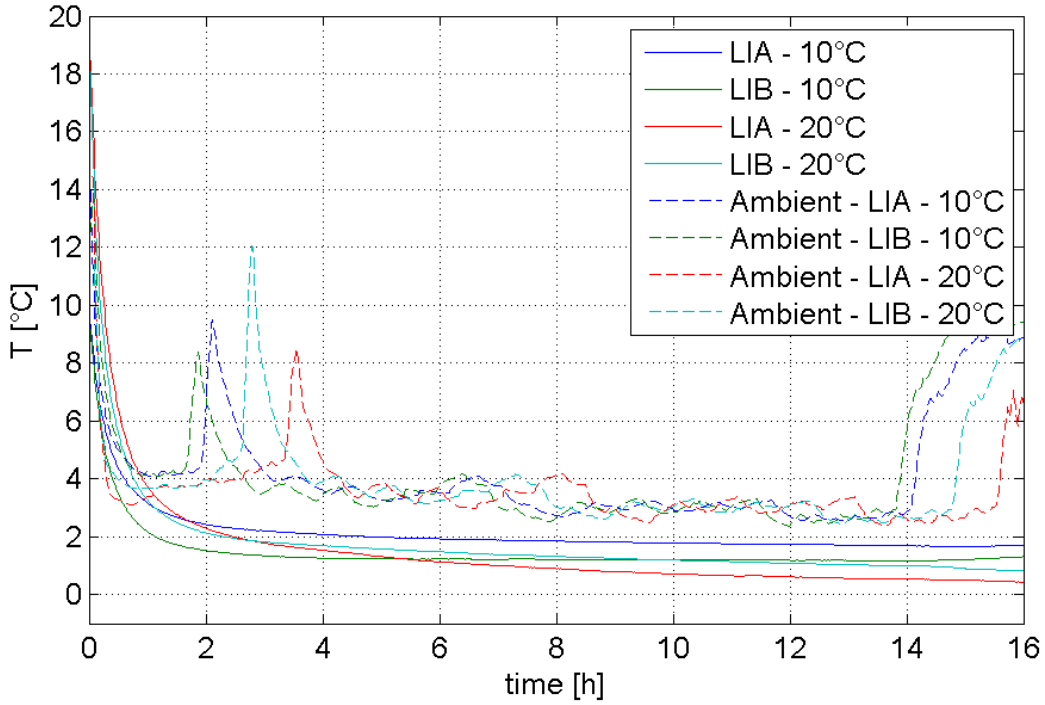

Figure 12: Cooling of whole, gutted haddock and monitoring of ambient temperature

Conclusion

Main experiment (13th-21st of October)

The conditions used in this experiment differed slightly from optimal conditions usually preferred for handling and storage of whole fish. However, this was done to achieve standardised fish in tubs and of similar temperature. Skin and flesh counts of specific spoilage organisms were evaluated separately to obtain a better understanding of the microbial contamination process occurring in whole fish treated differently. Overall, it was found that growth of some SSO on fish skin was delayed at early storage, independently of the cooling methods. With further storage, little or no count difference was seen among traditionally iced fish and those cooled in liquid ice for 2 h before draining and top layer icing. Even less difference was observed in the flesh microbiota developing until significant growth increase in *Photobacterium phosphoreum* and H_2S-producing bacteria was observed on day 8 in LIB cooled fish. Interestingly, the differences obtained in the temperature profiles of fish cooled differently were not supported by different bacterial growth behaviour. In fact, SSO spoilage potential was not reduced as time progressed, as demonstrated on day 8 by the significantly higher TVB-N and TMA content of fish cooled in liquid ice compared to traditional icing. Conditions created by liquid ice environment (salt uptake of flesh) may have been unfavourable, causing an even faster fish deterioration process with increasing storage time compared to traditional ice storage.

LIB gave slightly faster cooling, where 0 °C was reached in 20-30 min, while the same process took 57 min for LIA. This difference might partially be explained by the fact that the individuals used for temperature logging in the LIA group had on average 10% more weight than the other groups. The greatest difference in cooling rate was however observed between the liquid ice groups and crushed plate ice, where the latter took 4.3 h to reach 0 °C. The LIA group seemed to maintain temperature slightly better than the other liquid ice treatments. During a storage period of 8 days the LIB groups increased in temperature by ca. 1 °C, while LIA increased by approximately 0.3 °C.

It is noteworthy that a parallel study using cultivation-independent method (16S rRNA clone analysis) evaluated the effect of cooling treatments on the bacterial community of haddock initially and at the end of storage (Reynisson *et al*., 2010). It was shown that a significant divergence of bacterial developments resulted, explained by the environmental conditions caused by the cooling treatments applied to the fish. The use of liquid ice is in many cases the most suitable cooling medium for whole fish to achieve faster cooling. One drawback of liquid ice is its stimulating growth effect on *P. phosphoreum* during extended storage of marine coldwater fish as a consequence of increased salt concentrations although draining the tubs during initial cooling might minimise this effect.

Results from experiments aiming to examine two different cooling methods on board fishing vessel (Magnússon *et al.* 2009), to apply different cooling techniques during processing including 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 showed that no marked difference was seen in sensory, microbial and chemical measurements in the raw material at processing whether plate ice or liquid ice was used on board. According to sensory analysis towards the end of storage of processed products, the experimental group prepared from liquid ice stored fish had one day extension in freshness and shelf life compared to the group originating from plate ice. However, this difference in shelf life is likely to be due to the temperature difference (0.6 °C) observed among these products during storage.

Further, a recent trial involving whole, gutted cod differently cooled and stored on board was conducted in November 2009 following the quality deterioration of the fish for 10 days (Magnússon *et al*. 2010). Comparison of direct icing versus 30-min liquid cooling prior to re-icing showed that, despite a more rapid cooling rate for liquid cooling treatment (reaching 0 °C in about 5 h compared to 10 h for iced fish), no difference was observed in freshness preservation while slightly longer shelf life was seen for iced fish. The shelf life in this study was considerably shorter compared to previous studies with whole cod, which may be explained by the fluctuating (2 to 5 °C) ambient temperature in the cold room where the tubs were stored.

Additional cooling rate experiment (27th-29th of October)

LIB gave slightly faster cooling than LIA. When cooled from 20 °C, LIB reached 4 °C in 46 min while LIA took 55 min for the same process. The same trend was observed when cooled from 10 °C, where LIB and LIA reached 4 °C in 24 min and 36 min, respectively.

In this experiment the amount of pure ice which was used was limited, such that the raw material never reached the temperature of the liquid ice. The groups seemed to reach equilibrium at 1-2 °C.

The difference in weight of the individuals which were temperature mapped was very small (mean for each group was maximum 2.5% from the overall mean 1010 g).

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