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Effect of modified atmosphere packaging (MAP) and superchilling on the shelf life of fresh cod (*Gadus morhua*) loins of different degrees of freshness at packaging

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Ágrip á íslensku:

Tilgangur þessarar tilraunar var að meta áhrif loftskiptra umbúða (MAP) og ofurkælingar á gæðabreytingar og geymsluþol þorskbita af misfersku hráefni sem var unnið og pakkað eftir 2 og 7 daga frá veiði. Tilraunin var gerð í samvinnu við Samherja, Dalvík og Norðlenska, Akureyri í október og nóvember 2007. Fiskurinn var geymdur heill í ís fram að pökkun við -0.2 ± 0.1°C (2 dagar frá veiði) og -0.2 ± 0.2°C (7 dagar frá veiði). Hnakkastykki voru skorin í tvennt og þeim var síðan pakkað (350-550 g) í loftskiptar umbúðir. Samsetning gasblöndunnar var eftirfarandi: 50% CO_2, 5% O_2 og 45% N_2. Pakkaðir þorskbitar voru geymdir í kæligeymslu við -0.6 ± 1.4°C og sýni tekin yfir 3ja vikna geymslutíma og metin með skynmati, örveru- og efnamælingum. Aldur hráefnis við pökkun hafði greinileg áhrif á skynmat bitanna. Pökkun eftir 2 daga leiddi til lengingar á ferskleikaeinkennum framan af geymslu. Auk þess komu skemmdareinkenni mun síðar fram en í bitum sem pakkað var 7 daga frá veiði. Geymsluþol bita eftir pökkun á 7. degi má gróflega áætla 4-8 dagar en a.m.k. 19 dagar í bitum pökkuðum á 2. degi. Þetta stutta geymsluþol bita frá 7. degi má skýra með þróun örveruflórunnar og myndun rokgjarnra skemmdarefna ásamt hitastigsferli á heilum fiski fyrir pökkun. Áhrif mismunandi pökkununardags hafði veruleg áhrif á örveruflóruna. Þannig var heildarörverufjöldi mun minni í bitum sem pakkað var eftir 2 daga heldur en á 7. degi (log 3.7 vs 5.4/g). Þennan mun má að miklu leyti rekja til mismikils fjölda *Photobacterium phosphoreum* (Pp) í holdi rétt eftir pökkun, en hann greindist ekki við fyrri pökkun á 3. tilraunadegi (undir log 1.3/g) og á 8. degi var fjöldinn aðeins log 2.4/g. Á þeim degi var fjöldi Pp 1000x meiri í bitum pökkuðum á 7. degi og voru þeir ríkjandi út geymslutímann í þessum hópi. Á 8. degi var fjöldi annarra skemmdarörvera (H_2S-myndandi gerla og pseudomonads) nokkru hærri (Δ log 0.6-0.7/g) í þessum hópi miðað við hópinn sem pakkað var á 2. degi. Þessar niðurstöður staðfesta að *P. phosphoreum* sé ein af aðalskemmdarörverum í gaspökkuðum þorskbitum en einnig í kældum, heilum þorski. Niðurstöður TVB-N and TMA mælinga voru í góðu samræmi við örverumælingar en þó sérstaklega Pp. Low Field Nuclear Magnetic Resonance (LF-NMR) tækni var notuð til að mæla "relaxation times" í sýnum yfir geymslutímann. Marktækt hærri "relaxation times" mældust í bitum sem pakkað var eftir 7 daga frá veiði en í bitum sem pakkað var 2 daga frá veiði. Það gefur til kynna meiri bindingu vatnssameinda við umhverfið í 7 daga bitunum. Þetta er í samræmi við almennt hærri vatnsheldni og vatnsinnihald í þeim sýnum yfir geymslutímann. Í heildina sýna niðurstöður mikilvægi þess að nota sem ferskast hráefni til MA-pökkunar og tryggja þannig meiri gæði og lengra geymsluþol sem ætti að skila sér í hærra verði vörunnar.

Lykilorð á íslensku: *Ofurkæling, MAP, geymsluþol, örverur, skynmat, þorskur*

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Summary in English:

The aim of this study was to evaluate the effect of modified atmosphere packaging (MAP) and superchilling on the shelf life and quality changes of fresh loins prepared from Atlantic cod (*Gadus morhua*) of different freshness, i.e. processed 2 or 7 days post catch. The study was performed in cooperation with Samherji (Dalvík, Iceland) and Norðlenska (Akureyri) in October and November 2007. The average fish temperature during storage prior to processing on days 2 and 7 was -0.2 ± 0.1°C and -0.2 ± 0.2°C, respectively. Cod loins (350-550 g) were packed in trays under modified atmosphere (50% CO_2/ 5% O_2/ 45% N_2), stored at -0.6 ± 1.4°C and sampled regularly over a three-week period for sensory, microbiological and chemical analyses.

The results show that the raw material freshness clearly influenced the sensory characteristics of packed loins. Processing 2 days post catch resulted in more prominent freshness sensory characteristics the first days of storage. In addition, sensory indicators of spoilage became evident much later compared to MA-packed fillets from raw material processed 5 days later. The expected shelf life of the MA-packed cod loins could be roughly calculated as 4-8 days when processed 7 days post catch, but at least 19 days when the cod was processed 2 days post catch. This reduced shelf life of MAP products processed at a later stage was also explained by the temperature profile of the whole fish prior to processing, microbial development and volatile amine production observed. In fact, the day of packaging had a major effect on the microflora development, with lower total viable counts (TVC) in loins processed earlier in relation to time from catch (log 3.7 vs 5.4/g). This difference could be linked to large variations in levels of *Photobacterium phosphoreum* (Pp) in the flesh at processing times, being below detection (log 1.3/g) 2 days post catch but found to increase to log 2.4/g in early processed loins 6 days later, in contrast to 1000-fold higher Pp levels in loins processed later. Pp was found to quickly dominate the microflora of loins processed 7 days post catch. Similarly, slightly higher levels (Δ log 0.6-0.7/g) of other spoilage bacteria, H_2S-producing bacteria and pseudomonads, were found 8 days post catch in loins processed later. These results confirm that *P. phosphoreum* is one of the main spoilage organisms in cod, unprocessed as MA-processed. TVB-N and TMA production corresponded well to the microbial development, especially counts of *P. phosphoreum.* Low Field Nuclear Magnetic Resonance (LF-NMR) was used to measure the relaxation times of the samples during storage. The samples packed 7 days after catch showed significantly higher relaxation times than samples packed 2 days after catch. This indicates stronger bindings of the water molecules to their environment in samples packed at a later stage. This is in agreement with the generally higher water holding capacity and water content in the samples during storage. Finally, the results demonstrated that delaying processing of raw material is undesirable if it is intended to be MA-packed and sold as more valuable products.

English keywords: *Superchilling, MAP, shelf life, microorganisms, sensory analysis, cod*

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1 INTRODUCTION

This project is a part of the research project Chill-add-on (Samþætting kælirannsókna – Kælibót) funded by the AVS research fund under the Ministry of Fisheries (project no. R 061-06) and the Technology Development Fund at the Icelandic Centre for Research (project no. 061358006). The project is related to the project Chill-on, which is funded by the European Union.

In recent years the quantity of exported Icelandic fresh fish products and their price have increased rapidly. To meet this change in market demands, the fish processing industry is calling for more research and knowledge on the properties of fresh fish products during processing and storage and on how processing can be improved. That is a prerequisite to improve the economic status of the field and to increase the professional status of Icelandic fish producers. Research in how storage life of fresh fish products can be prolonged leads to changes in the production, transport and storage of products, which in turn can lead to increased quality and safety and thus higher profits for the Icelandic fish products.

The aim of this study was to evaluate the effect of modified atmosphere packaging (MAP) and superchilling on the shelf life of fresh cod loins prepared from Atlantic cod (*Gadus morhua*) of different freshness, i.e. processed 2 or 7 days post catch. The study was performed in cooperation with Samherji in Dalvík, Iceland and Norðlenska in Akureyri in October and November 2007.

2 MATERIAL AND METHODS

2.1 Experimental design

Atlantic cod (*Gadus morhua*) caught on October 29th 2007 North-west of Iceland was placed evenly in three 460 L plastic tubs and chilled with flake ice after bleeding, gutting and rinsing. The sea temperature at catch was in the interval from 0°C to 0.6 °C and the ambient air temperature was 3.4 °C at the time of catch. Six fish in one of the tubs were tagged and temperature loggers (iButton) were placed inside the fish flesh of these fish. Other six temperature loggers (Stowaways) were tied to the fish to monitor the surrounding temperature. Three of the fish in each tub were placed at a lower position in the tub, while the other three were placed at an upper position. Two temperature loggers were then tied to each tub to monitor the ambient air temperature from catch till processing.

The fish was landed in the morning two days after catch and then stored in the processing plant cooling storage at Samherji in Dalvík, Iceland, until processed. The processing and packaging of the fish was performed at two occasions, i.e. 2 and 7 days after catch.

Figure 1: Whole, bled and gutted cod (*Gadus morhua*) iced in 460 L plastic tubs (left). Temperature loggers for ambient temperature measurements tied to tubs (right).

At processing the fish was headed, filleted, deskinned and trimmed. Only the cod loins were used for the experiment. The cod loins were cut in half and placed in 3 kg styrofoam boxes lined with a plastic bag along with two cooling mats. These boxes were then transported to Norðlenska in Akureyri, Iceland (40 min drive) where the cod loins were re-packed in modified atmosphere (MA). Three cod loin pieces (350-550 g) were placed in each tray (FÆRCH plastic, K71-51W – 71051413; material: FPP; volume: 985 mL) lined with an absorbent pad and sealed with a film (Cryovac EOP, 240 mm) following gas flushing. The gas composition was set to 50% CO_2, 5% O_2 and 45% N_2 and measured in 4-5 empty trays at packaging to ensure a correct gas mixture. Temperature loggers (iButton) were placed in chosen trays under the cod loins to monitor the temperature through transport and storage. The trays were placed in paper cartons and transported to Matís in Reykjavik in a cooled truck from Eimskip Flytjandi. Temperature loggers were placed in the paper cartons to monitor the temperature in the surroundings during transport and storage. Upon arrival to Matís the samples were placed in a cooling chamber set to -1.5 °C. Samples were collected regularly over a three-week sampling period.

Figure 2: Deskinned and trimmed cod loins in a 3 kg styrofoam box (left) and trays to be modified atmosphere packed (MAP) (right).

2.2 Temperature measurements

Two types of temperature loggers were used for the measurements. For measurements of temperature inside the fish muscle, loggers of the type iButton DS1922L

(http://www.maxim-ic.com/quick_view2.cfm/qv_pk/4088) with a accuracy of ±0.5 °C, a resolution of 0.0625 °C and an operating range from -40 to +85 °C were used.
For measurements of surrounding temperature, Onset Stowaway-IS loggers (http://www.onsetcomp.com/Products/water_logger_guide.html) were used. These have an accuracy of ±0.5 °C, a resolution of 0.35 °C and an operating range from -40 to +50 °C. In all cases, temperature was recorded at 8 min intervals and read at the end of the experiment.

Figure 3: Temperature loggers used in the study. iButton loggers with 17 mm diameter and 6 mm thickness (left) and Onset Stowaway loggers with 38 mm diameter and 20 mm thickness (right).

The temperature through processing was measured with an Ebro thermometer (measurement range: -50 to 300 °C, resolution: 0.1 °C, precision: ±0.3 °C) at three points in the processing line. The first point was before filleting and skinning, the second after skinning and the last one right before packing. The number of measurements at each point ranged from 20 to 40.

2.3 Gas measurements

Gas composition measurements were performed with a PBI Dansensor (CheckMate 9900) gas detector. Septums were put on the film to enable measurements. A needle, connected to the gas detector with a tube, was stung through the septum, a gas sample collected twice consecutively and the latter measurement recorded for each pack was evaluated. Nine packs were taken for measurements at each sampling day. The gas composition was measured in all nine trays before distribution to the laboratories for further analysis.

2.4 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, according to the formula:

$$Drip \quad [\%] = \frac{w_{packaging} - w_{sampling}}{w_{packaging}} * 100$$

where $w_{packaging}$ is the fish weight at packaging and $w_{sampling}$ is the fish weight at sampling.

2.5 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 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 (mLH) according to van Spreekens (1974) with the exception that 1% NaCl was used instead of 0.5%. Plates were incubated at 15°C for 4-5 d. Bacteria forming black colonies on IA produce H_2S from sodium thiosulphate and/or cysteine. Nitrite-Actidione-Polymyxin (NAP) agar was used for counts of lactic acid bacteria (LAB). The medium was prepared according to Davidson and Cronin (1973). Plates were incubated at 22°C for 4-5 d and LAB count confirmed by testing for the presence of catalase-negative colonies. 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 4-5 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.

2.6 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 and Brilliant SYBR green II mastermix (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.

2.7 Sensory analysis

Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (1985), was used to assess cooked samples (MA07sky058-060, MA07sky 063-066) of two sample groups of cod during storage time (Table 1). 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 (Bonilla et al., 2007; Wang et al., 2008). The sensory attributes were 30 and are described in Table 2.

Samples weighing ca. 40 g were cut from the loins and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 6 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95-100°C with air circulation and steam, and then served to the panel. Each panellist evaluated duplicates of each sample in a random order in each session.

A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording.

2.7.1 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). 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.

Table 1: Definition of sample groups evaluated by sensory evaluation

Sample group	Packaging time from catch (days)	Total storage time from catch (days)
MAP-PD2	2	3 8 10 15 18 21
MAP-PD7	7	8 10 15

Table 2: Sensory vocabulary for cooked samples of cod (*Gadus morhua*)

Sensory attribute	Description of attribute
Odour	
sweet	Sweet odour
shellfish, algae	Characteristic, fresh
meat	Reminds of boiled meat or halibut
vanilla/warm milk	Vanilla, warm milk
boiled potatoes	Odour reminds of boiled potatoes
frozen storage	Refrigerator, freezer storage odour
table cloth	Reminds of a table cloth (damp cloth to clean kitchen table, left for 36 h)
TMA	TMA odour, reminds of dried salted fish, amine
sour	Sour odour, sour milk, spoilage sour, acetic acid
sulphur	Sulphur, matchstick
Appearance	
colour	Left end: light, white colour. Right end: dark, yellowish, brownish, grey
appearance	Left end: homogenous, even colour. Right end: discoloured, heterogeneous, stains
white precipitation	White precipitation on the fish surface
Flavour	
salt	Salty taste on tongue
metallic	Characteristic metallic flavour of fresh cod
sweet	Sweet flavour
meat	Reminds of boiled meat, meat-sour
frozen storage	Refrigerator, freezer storage flavour
pungent	Pungent taste on tongue
sour	Sour taste, spoilage sour
TMA	TMA flavour, reminds of dried salted fish, amine
off-flavour	Intensity of off-flavour
Texture	
flakiness	The fish portion slides into flakes when pressed with the fork
soft	Left end: firm. Right end: soft. Evaluate how firm or soft the fish is during the first bite
juicy	Left end: dry. Right end: Juicy. Evaluated after chewing several times: dry - draws juice from the mouth
tender	Left end: tough. Right end: tender. Evaluated after chewing several times
mushy	mushy, porridge
meaty mouthfeel	reminds of meat texture, rough fibers
clammy	Clammy texture, tannin (dry redwine)
rubbery	Rubbery texture, chewing gum

2.8 Chemical analysis

2.8.1 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.

2.8.2 pH- measurements

The pH measurements were performed with a pH electrode (SE 104 Mettler Toledo GmbH, Greifensee, die Schweiz) connected to a Knick pH meter (Portames 913 pH, Knick, Berlin, Germany). The electrode was immersed in the minced samples at 20 ± 2 °C.

2.8.3 Water content, salt content and water holding capacity (WHC)

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) 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

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

2.9 Nuclear Magnetic Resonance

For Low field Nuclear Magnetic Resonance (NMR) measurements a low-field Bruker Minispec mq 20, bench top NMR-analyzer was used (Bruker Optics GmbH, Am Silberstreifen D-76287 Rheinstetten, Germany). The NMR analyzer works at a frequency of 20 MHz and the magnetic field at the strength 0.47 T. Test tubes of 10 mm width

were used for the measurements. All samples were allowed to reach thermal equilibrium before measurements by keeping them in a Julabo FP50-MC cooling bath (Julabo Labortechnik GmbH, D-77960 Seelbach, Germany) set to 2°C. The transverse relaxation time, T_2, was measured with a Carr-Purcell- Meiboom-Gill (CPMG) pulse sequence at 2 °C with an interpulse spacing of 250 µs, a Receiver Gain of 10 s, Gain 70 dB, number of scans 16 and number of fitted points 8100. The data was fitted with a bi-exponential curve.

The longitudinal relaxation time, T_1, was measured with an Inversion Recovery (IR) pulse sequence at 2 °C. The gained data was fitted with a mono-exponential curve. The number of measuring points was 30, the duration factor used was 1.322 and the number of scans was 4. Other settings were adjusted as for the transverse relaxation time measurements.

2.9.1 Data analysis

All data were measured with the Bruker Minispec software. Software functions and pulse sequences were chosen to give normalized results. Exponential fits of the relaxation time data were performed in the software. The transverse relaxation times were estimated by using a bi-exponential fit for the interpulse spacings of $250\,\mu s$, while the longitudinal relaxation time was estimated with a mono-exponential fit. Statistical analysis and plotting of figures was performed in Microsoft Excel. Four replicates were prepared from the minced samples. The measured relaxation times are mean values calculated from the results from these samples. Stated error margins are the standard deviation calculated from the measurements.

3 RESULTS AND DISCUSSION

3.1 Temperature mapping

3.1.1 Temperature from catch to main processing

Results from the temperature mapping from catch to the main processing plant in Dalvík are shown in Figure 4-Figure 6.

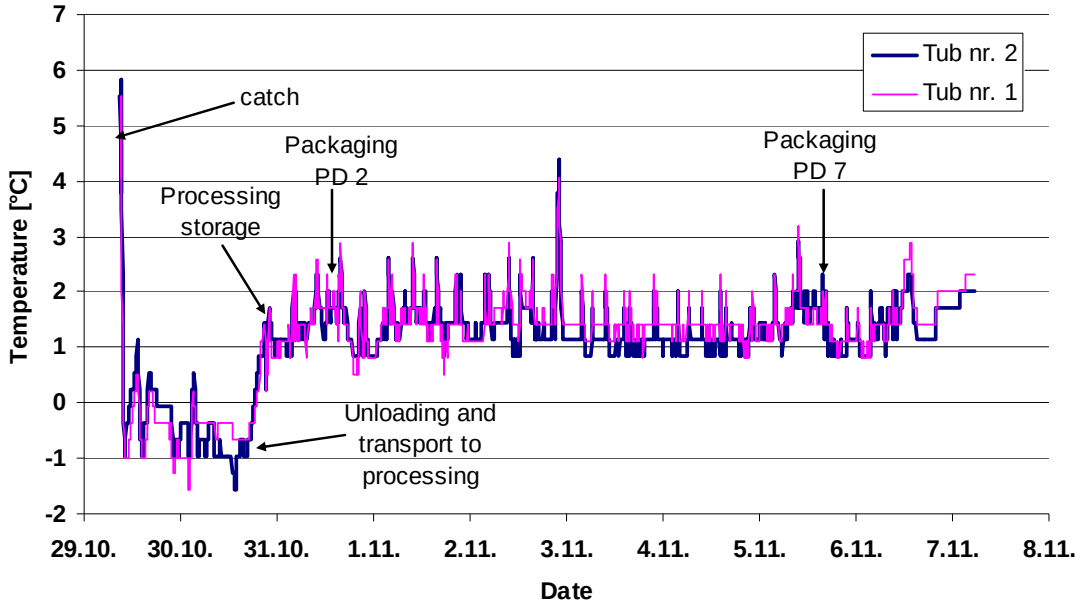

Figure 4: Ambient air temperature from catch to processing.

According to Figure 4 the chilled storage on board the vessel fulfilled the requirement of an ambient temperature below 2 °C; since the maximum temperature during the storage in the ship was around 1 °C, with a minimum of -1.5 °C and on average approximately -0.5 °C. The ambient air temperatures increased during unloading and transport to the processing plant in Dalvík, where the fish tubs were stored in the processing plant storage until the fish was processed, two versus seven days after catch. The average ambient temperature during the storage at the processing plant was 1.4 ± 0.4 °C, while the average temperature in the iced tubs ranged from -0.18 to -0.74 °C, depending on the position of the temperature loggers, with the highest temperatures at the top of the tub and the lowest temperatures at the bottom.

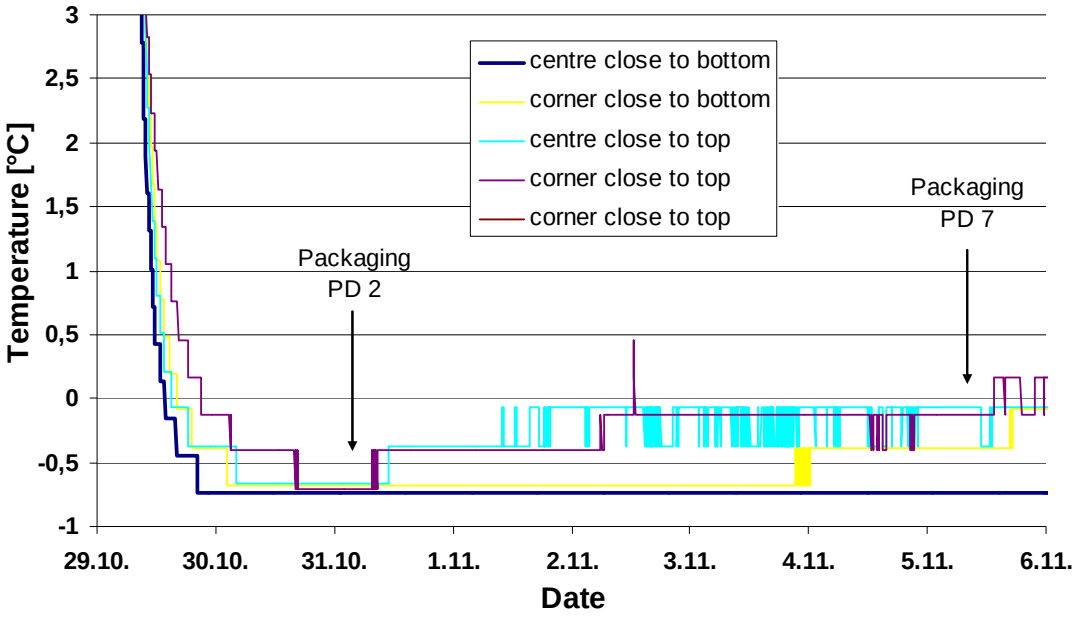

Figure 5: Temperature inside tub (outside the fish) during transport from catch to main processing.

As can be seen in Figure 5, similar cooling rates were observed at different locations in the tub. The temperature inside the tubs had reached a steady state approximately 12-15 hours after the fish and ice were set in the tubs. The average temperature in the tub was approximately half a degree higher close to the top, where the average temperature was approximately -0.2 °C, than at the bottom, where the average temperature was -0.7 °C. The figure also shows that the variation in temperature was higher close to the top in the tub than close to the bottom due to more environmental effect higher in the tub. This temperature difference must be taken in consideration for the fish processed five days later where quality differences in the raw material can be expected based on its position in the tub.

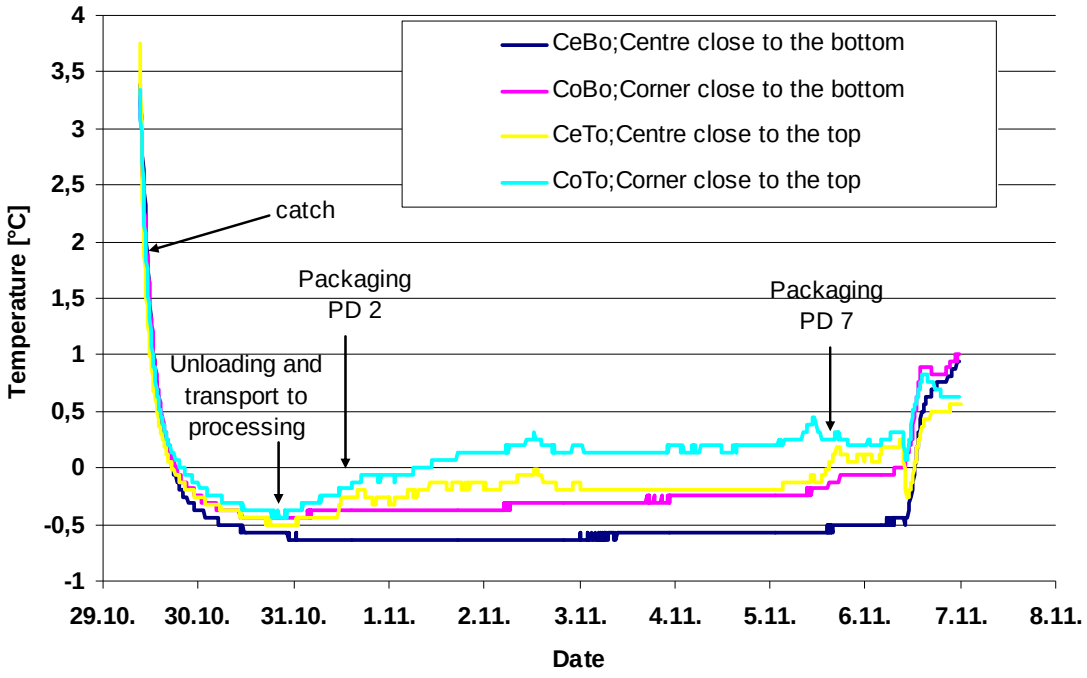

Figure 6. Temperature inside whole cod (product temperature) iced in a fish tub from catch to main processing 2 (PD 2) and 7 (PD 7) days after catch respectively.

As can be seen in Figure 6, very similar cooling rates for whole fish were experienced at different locations in the tub. The time required to chill the product to temperatures below 1 °C was approximately 3 hours and approximately 9 hours were needed to chill the product below 0 °C. According to this study, it takes a bit more than a whole day for the product temperature to reach a completely steady state, using flake ice. Nevertheless, cold management in this part of the chain (from catch to main processing) is found satisfactory. The average fish temperature during storage prior to processing on days 2 and 7 was -0.2 ± 0.1°C and -0.2 ± 0.2°C, respectively.

3.1.2 Temperature through processing

The temperature through processing was measured at three points in the processing line. The first point was before filleting and skinning, the second after skinning and the last one right before packing. The number of measurements at each point ranged from 20 to

40 and the average temperature results are presented along their standard deviation (SD) in **Table 3**.

Table 3: Temperature through processing two days post catch

Measuring point in process line	Before filleting and skinning(10:20 AM)	After skinning (10:50 AM)	Before packing (11:05 AM)
Temperature (°C ± SD)	0.5 ± 0.4	1.4 ± 0.4	2.8 ± 0.6

On average, the product temperature raised by 2.3 °C during the approximately 45 minutes that processing lasted. The average ambient temperature in the processing hall during that period was 9 ± 2 °C.

3.1.3 Temperature through transport and storage

Temperature mapping was also performed during the storage, by monitoring the ambient air temperature in the cooling storage at various positions in the storage room with Stowaway temperature loggers and in the product by placing iButton temperature loggers under the fish in the bottom of a few packs. The results of this temperature mapping can be seen in Figure 7 to Figure 8. The average temperature of the loins from packaging through storage was -0.8 ± 1.2 °C, while the average cooler ambient temperature during storage at Matís was -0.6 ±1.4 °C.

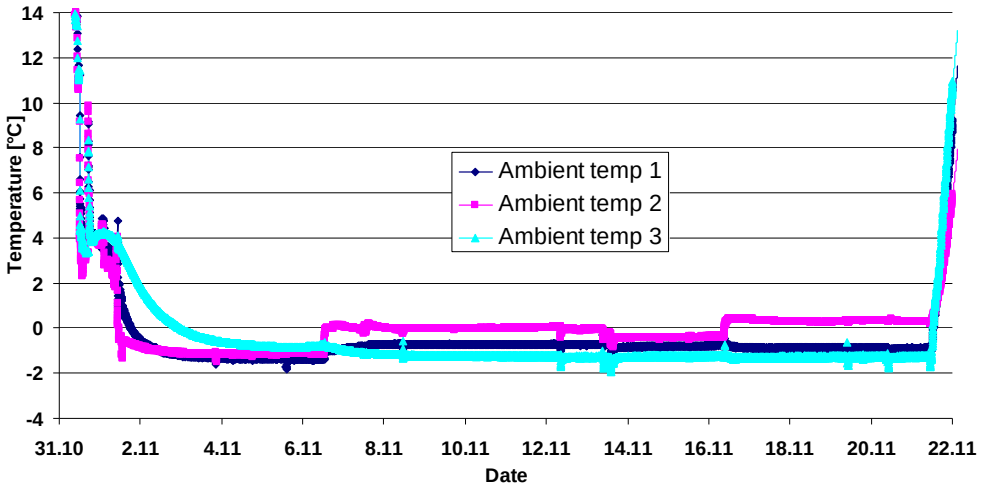

Figure 7: Ambient air temperature through transport and storage.

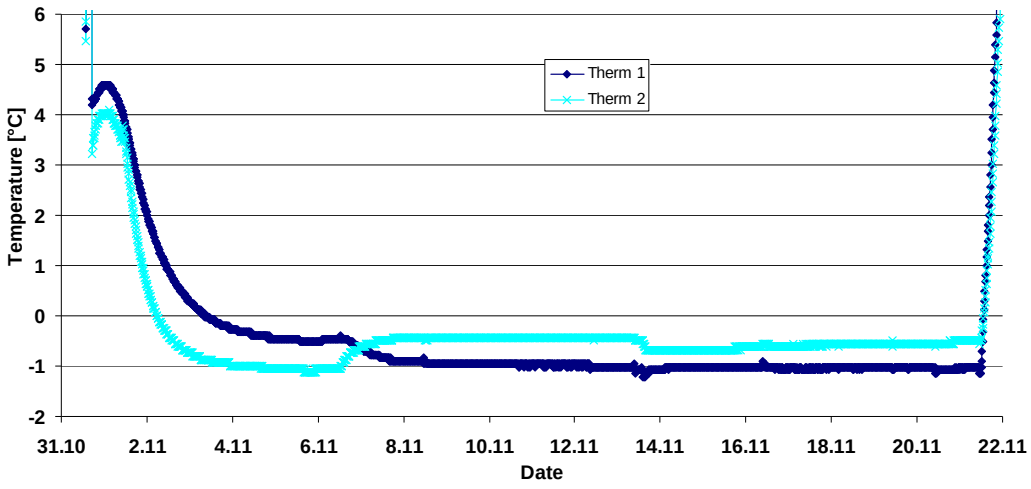

Figure 8: Temperature of cod loins from packaging through transport and storage.

3.2 Gas measurements

The fish loins were packed under modified atmosphere with a target gas composition of 50% CO_2, 5% O_2 and 45% N_2. The initial gas composition at the packaging location for both packing days (2 and 7) can be seen in Table 4.

Table 4: Mean gas concentration measured in ambient air and in MA-packs at packaging location.

	O_2 [%]	+/-	CO_2 [%]	+/-
PD 2 in ambient air	22,2	0,5	0,3	0,1
PD 7 in ambient air	22,2	0,5	0,4	0,1
PD 2 in MAP	6,9	1,9	46,3	2,6
PD 7 in MAP	13,5	0,1	44,8	0,1

Table 4 shows that the average oxygen concentration in the packages was 6.9 ± 1.9 % on packing day 2 but 13.5 ± 0.1 % on packing day 7. The average carbon dioxide concentration was 46.3 ± 2.6 % and 44.8 ± 0.1 % respectively. The gas composition in the ambient air was monitored during the packaging to ensure normal results from the measurements. The gas composition in the ambient air was 22.2 ± 0.5 % oxygen and 0.4 ± 0.1 % carbon dioxide, which are normal composition values for air.

The gas composition in the MA-packed samples was monitored through the sampling period. The results from this analysis can be seen in Figure 9 and Figure 10. This was also done to prevent the effect on results from packages with leakage. The amount of

leaking MA-packages turned out to be within 6% in the study. Such packages were not used for further analysis.

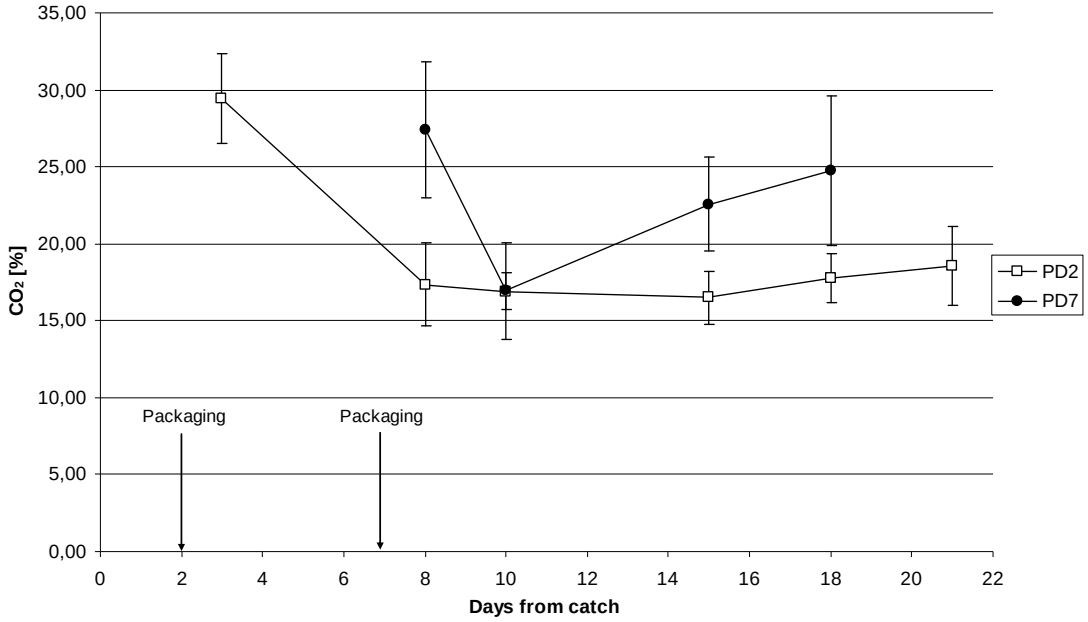

Figure 9: Change in CO_2 concentration in MA-packs during storage for fish packed two days (PD 2) and seven days (PD 7) post catch. Average values are shown and bars indicate standard deviation.

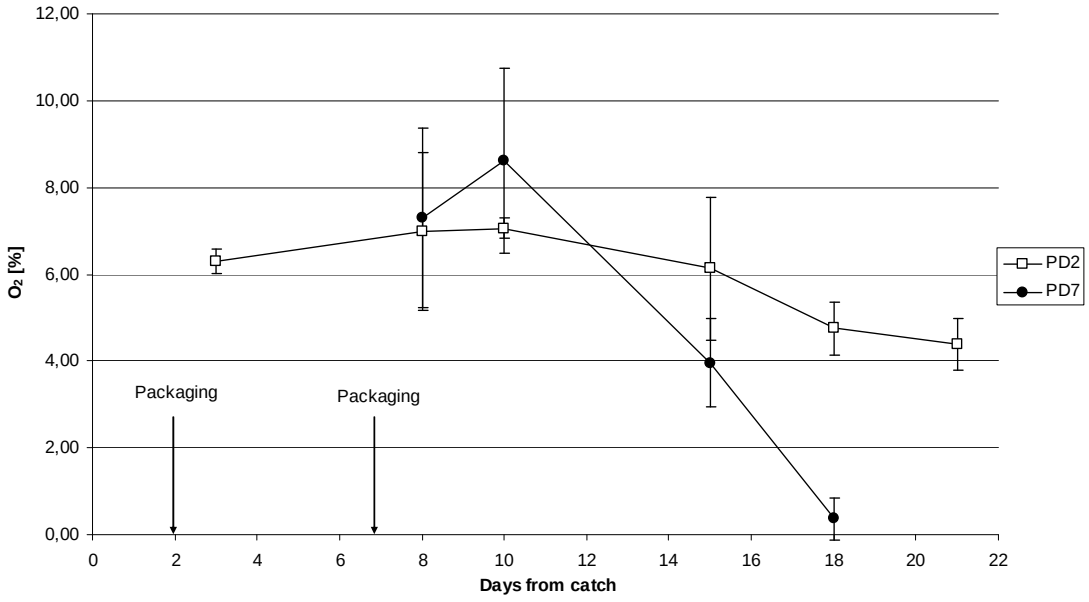

Figure 10: Change in O_2 concentration in MA-packs during storage for fish packed two days (PD 2) and seven days (PD 7) post catch. Average values are shown and bars indicate standard deviation.

Figure 9 shows that the carbon dioxide concentration in the MA-packs decreased rapidly after packaging, but only one day after packaging the carbon dioxide had lowered from 44.8 % and 46.3 % for packaging days 2 and 7, respectively, to 29.4% and 27.4% on days 3 and 8 respectively. A further CO_2 drop (Δ10%) was observed on the second sampling day in the packages' headspace. Part of this rapid decrease in carbon dioxide is certainly due to the absorption of carbon dioxide in the muscle tissue, but this extended drop could be explained by loss through the package. A few days after packaging the carbon dioxide composition reached a fairly steady state. When carbon dioxide absorbs into the water phase of the muscle oxygen is released. Aerobic microorganisms utilize this oxygen for proliferation and hence lead to further CO_2 production. This explains why the CO_2 content increased while the O_2 content decreased during the storage time.

3.3 Drip measurements

The water drip from the samples was monitored through the storage period. The results can be seen in Figure 11.

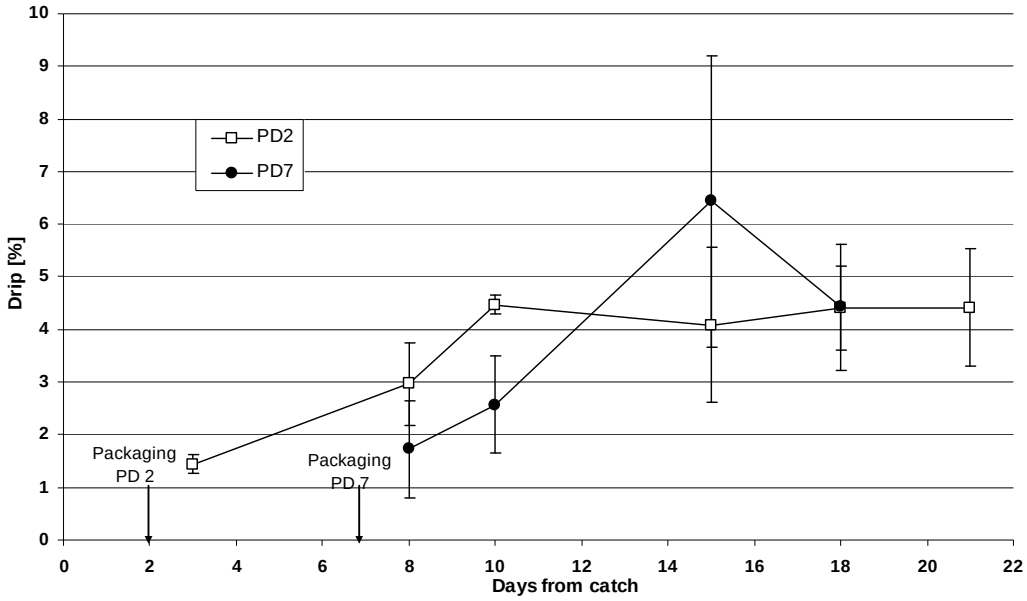

Figure 11: Drip increase in MAP packed fish loins during storage for fish packed two days after catch (PD 2) and 7 days after catch (PD 7).

The figure shows that drip increased in the fish loins packed under MA with storage time, but drip was measured to be 4-5% at the end of the storage time in this study for both

sample groups. A drip of 6.5% was measured in PD 7 on day 15. This higher value can likely be explained by individual difference between the samples, but only 6 cod loins were used for this measurement at each sampling point. It is interesting to see that the drip increased in a similar way for both groups and reached similar maximal drip values at the end of the storage time. The increase in drip with storage time is explained by water loss from the muscle due to degradation of muscle proteins caused by the spoilage mechanisms. However, MAP is known to lead to higher losses than storage in i.e. Styrofoam, due to protein denaturation and decreased water holding capacity of the muscle at lower pH.

3.4 Microbial measurements

Results form microbial counts are shown on Figure 12 to Figure 17. Total psychrotrophic viable counts (TVC) are shown on Figure 12. Minor differences were observed between the two media used and if any, counts on mLH were slightly higher than on IA. The day of packaging had however major effects on TVC. Fillets packed under MA after 2 days of iced storage of whole cod remained below log 4/g from day 3 until day 8 after which an increase was observed and reached maximum numbers on day 15 when TVC was about log 6/g. After that some decline in TVC was observed. Initial counts in fillets MA-packed after 7 days of iced storage of whole cod were however over log 5/g (day 8 from catch). On days 15 to 18 TVC had exceeded log 7/g.

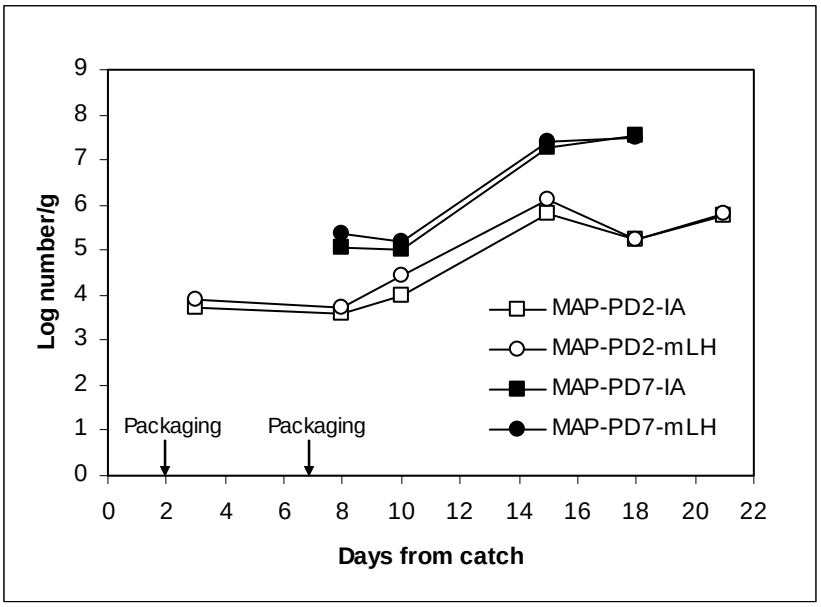

Figure 12: Total psychrotrophic viable counts (TVC) in loins packed under MA 2 and 7 days from catch. Average values of duplicate samples are shown.

Proliferation of H_2S-producing bacteria is shown on Figure 13. The same trend was observed as for TVC but not as drastic. It is interesting to note that initial counts on day 3 were very low (<log 2/g) indicating very low numbers of spoilage bacteria present at that time. Maximum levels reached towards the end of storage were around log 5/g.

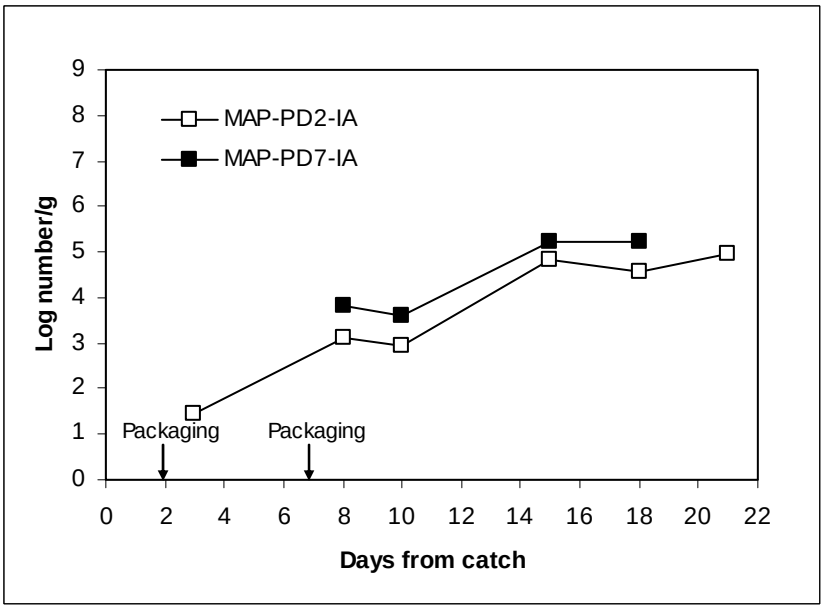

Figure 13: Growth of H_2S-producing bacteria in loins packed under MA 2 and 7 days from catch.

Counts of *Pseudomonas* spp. are shown on Figure 14. Initial counts in both groups were about log 3/g. Minor increase in counts occurred over the storage period, indicating that these bacteria play a minor role in the spoilage of MAP cod loins regardless of packaging day.

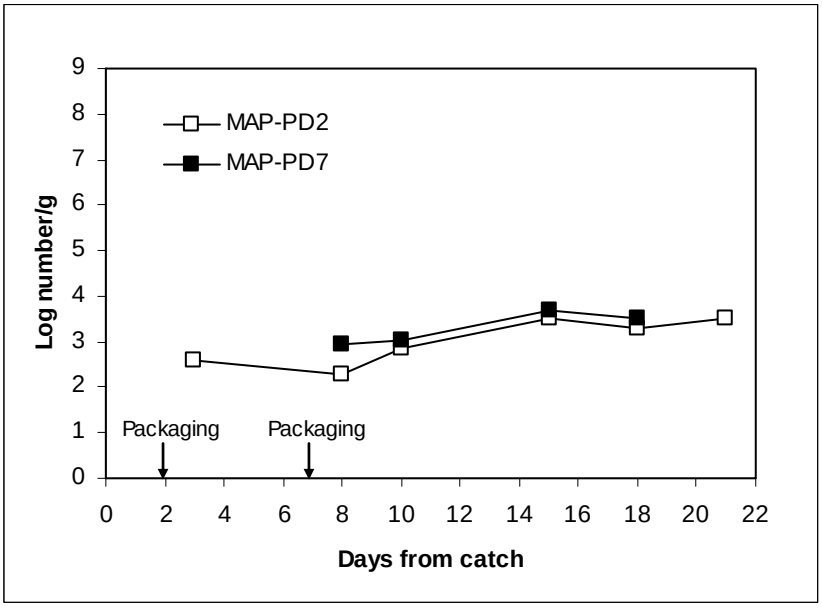

Figure 14: Growth of *Pseudomonas* spp. in loins packed under MA 2 and 7 days from catch.

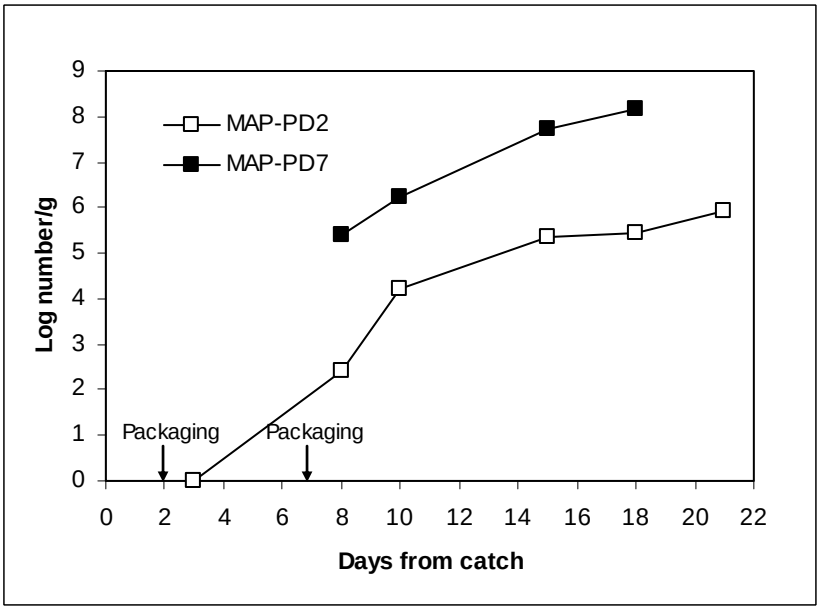

Figure 15: Growth of *Photobacterium phosphoreum* in loins packed under MA 2 and 7 days from catch.

One day after packaging (day 2) *P. phosphoreum* were not detected in the samples (<log 1.3/g) (Figure 15). The number of *P. phosphoreum* the day following the latter packaging day (day 8) was however higher or about log 5.4/g. That same day (day 8) the count of *P. phosphoreum* in loins packed on day 2 was log 2.4/g. After day 8, an increase was observed in both groups, reaching on day 18th of storage log 5.5/g in loins packed on day 2 but log 8.2/g in loins packed on day 7. These results indicate strongly that *P. phosphoreum* is one of the main spoilage organisms in MA-packed cod.

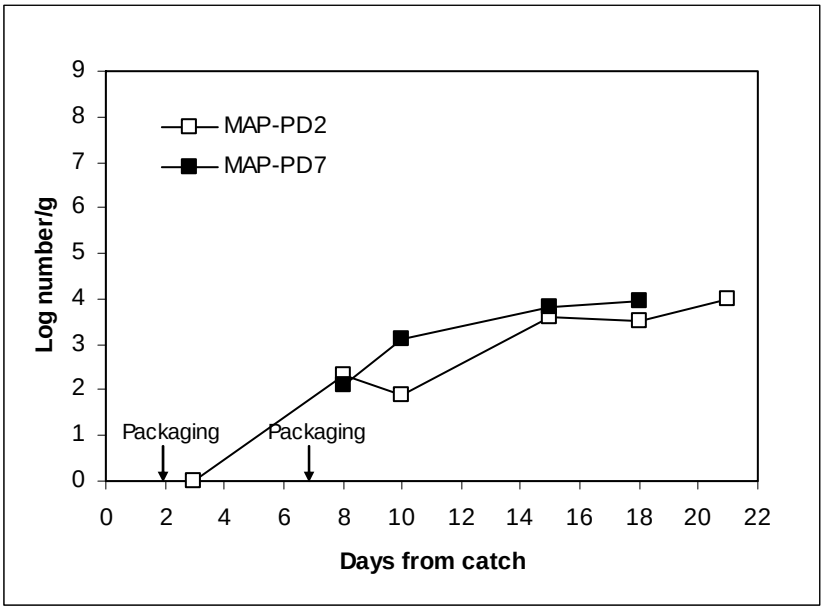

Figure 16: Growth of lactic acid bacteria (LAB) in loins packed under MA 2 and 7 days from catch.

A day after packaging (on day 2) no lactic acid bacteria (LAB) were detected in the samples (<log 1.3/g) (Figure 16). The numbers of LAB a day after the latter packaging day (day 8) were however similar in both groups, just exceeding log 2/g. Thereafter slightly higher numbers of LAB were obtained in the loins packed after 7 days of iced storage. After 18 days of storage the number of LAB had not yet exceeded log 4/g, indicating that they play a minor role in the spoilage of MAP cod.

The effect of delayed processing (7 days post catch) prior to MAP clearly showed that a bacterial selection occurred during storage of iced whole fish, resulting in the most rapid

proliferation of *P. phosphoreum*, followed by H_2S-producing bacteria while little growth was observed for pseudomonads. This difference can be explained by the requirements of some bacterial groups. Pseudomonads are aerobic bacteria, while H_2S-producing bacteria like *Shewanella putrefaciens* tolerate better lower oxygen tension as encountered in the flesh of whole fish and *P. phosphoreum* can grow similarly well with or without oxygen. Analysis of the raw material flesh obtained from whole fish at the latter packaging day confirmed that the counts seen in newly packed loins on day 8 were due to bacterial growth in the flesh of whole cod rather than to contamination of the loins during processing. The results therefore demonstrate that delaying processing of raw material is undesirable if it is intended to be MA-packed, as the main spoilage bacterium *P. phosphoreum*, which was otherwise found at very low levels early post catch, will rapidly proliferate to dominate over the microflora. Other recent studies have shown that superchilling has a negative effect on the growth of *P. phosphoreum* (Olafsdottir et al., 2006).

3.5 Quantitative PCR analysis

Figure 17 compares the *Pseudomonas* counts obtained by cultivation on modified CFC medium (number of pink colonies determined) or estimated by a quantitative PCR (qPCR) method, targeting the carbamoyl phosphate synthase gene (*car*A) with SYBR green based real-time PCR. Apart from the initial sampling point on day 3 (PD 2) and the last sampling day for PD 7, a good correlation was obtained for both the datasets derived from PD 2 and PD 7 as estimated using Pearson correlation coefficient (rP = 0.88 and rP=0.94 respectively).

But generally, qPCR data agreed well to the *Pseudomonas* counts obtained on modified CFC medium. However, the possibility that overestimated counts were occasionally obtained on modified CFC medium cannot be excluded as determination of exact number of pink colonies on crowded plates can prove difficult due to the change of the agar colour to pink.

This is a new method recently being developed at Matís and is still in a validation process. What this method offers in comparison to cultivation methods is speed, being

able to give results 4-5 hours after sample has been received. In the context to the present study, the results show that the quantitative method has the potential of replacing cultivation on CFC agar in the future.

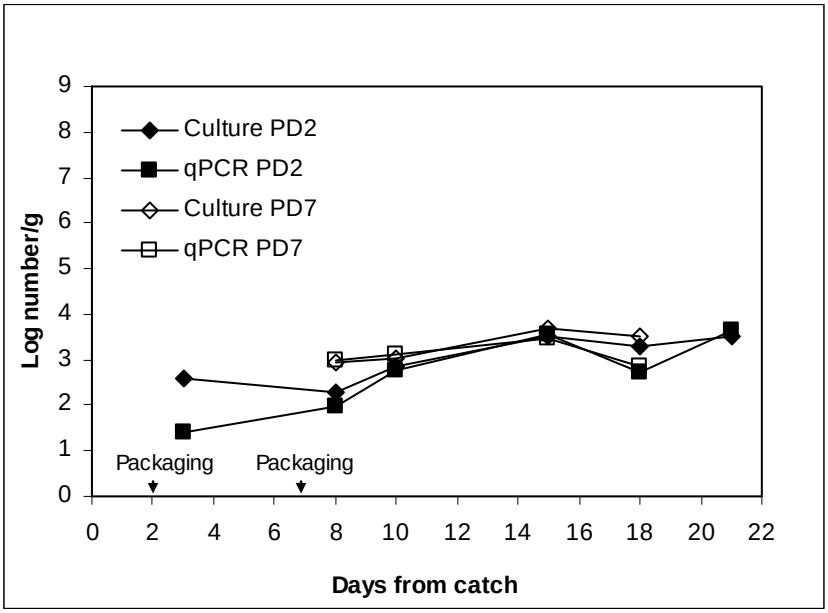

Figure 17: Counts of *Pseudomonas* spp. in loins packed under MA after 2 and 7 days from catch measured by cultural method vs. qPCR method.

3.6 Sensory evaluation

The freshness of the raw material clearly influenced the sensory characteristics of the MA-packed fillets. An example of sensory attributes characteristic at the beginning of storage time is shown in Figure 18. The day after MA packaging, the fillets from fish processed two days after catch (PD 2) received scores for sweet and metallic flavour around 50 (three days from catch), but fillets from fish processed seven days after catch (PD 7) received scores for sweet and metallic flavour around 35 (eight days from catch). On the 15th day after catch, the fillets from group PD 2 received scores around 30, but fillets from group PD 7 received scores around 20. An example of sensory attributes characteristic at the end of storage (indicators of spoilage) is shown in Figure 19. A hint of sour flavour was detected, but TMA flavour was obvious on day 15 after catch for

group PD 7. Sour and TMA flavours were not detectable in group PD 2 on that sampling day. Only hints of those flavours were detected in group PD 2 on day 18 and 21.

When the average QDA score for spoilage related attributes is above 20 (on the scale 0 to 100), most panellists detect them, which indicates that the sample is approaching the end of sensory shelf life. These limits have been used in determination of maximum shelf life of desalted cod (Magnússon et al., 2006), farmed Atlantic salmon (Sveinsdottir et al., 2002) and cod fillets (Bonilla et al., 2007). Based on these criteria, the expected shelf life of the superchilled cod loins from packaging could be roughly calculated as 4-8 days if whole, gutted cod is filleted and MA-packed seven days post catch, but 19 days or even longer if cod is filleted and MA-packed two days post catch.

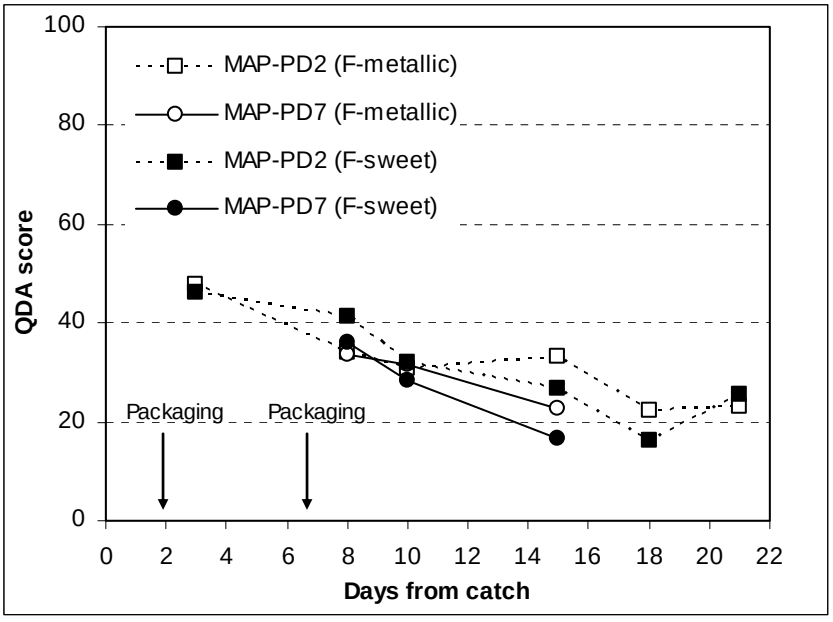

Figure 18: Average sensory scores for metallic and sweet flavour. F = flavour.

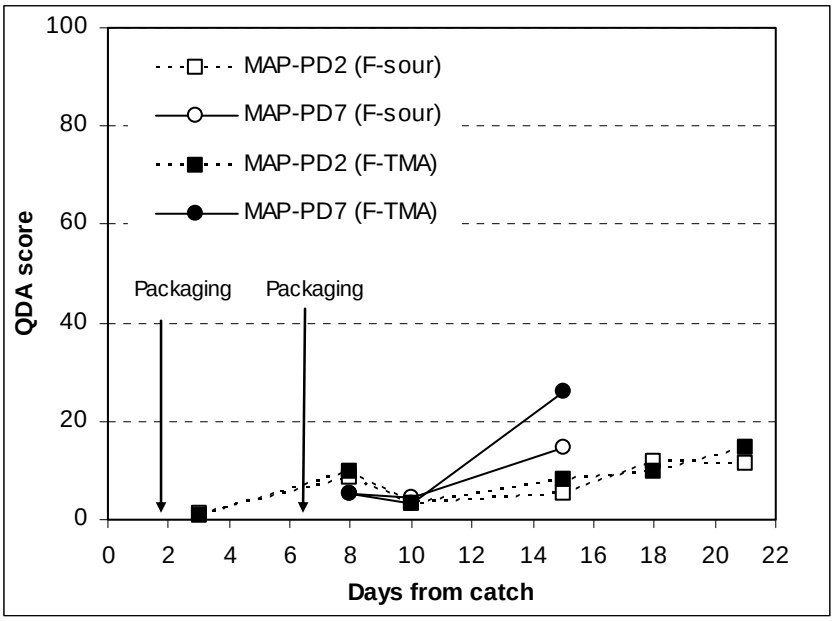

Figure 19: Average sensory scores for sour and TMA flavour. F = flavour.

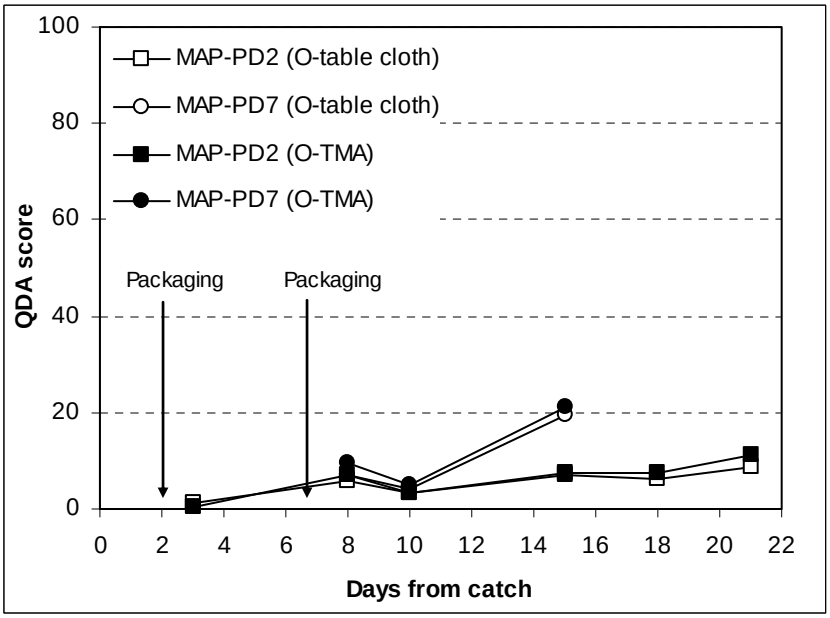

Figure 20: Average sensory scores for table cloth and TMA odour. O = odour.

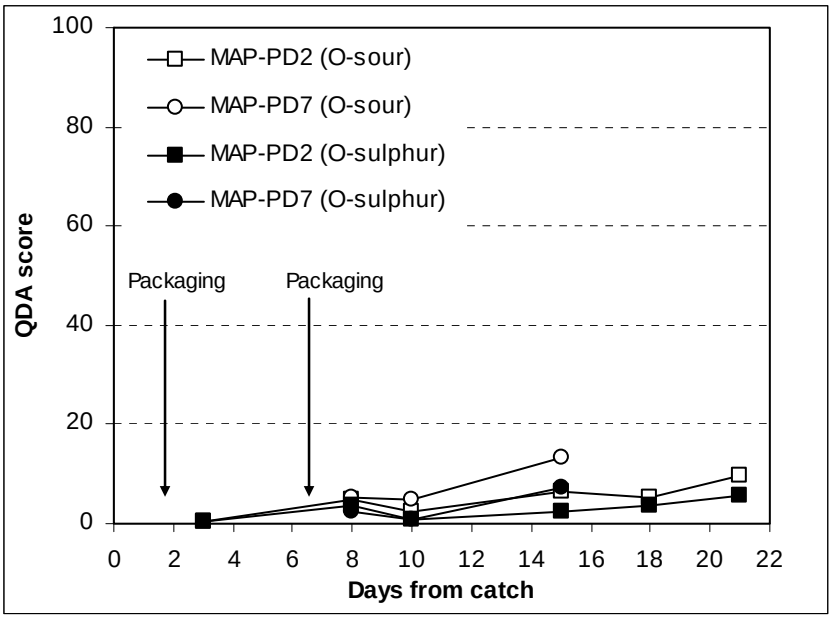

Figure 21: Average sensory scores for sour and sulphur odour. O = odour.

Most sensory attributes were different between groups and changed significantly within each group with storage time (Table 5).

Newly packed fillets of PD 2 had very characteristic sweet and shellfish/algae-like odours and obvious meat and vanilla/warm milk odours. Sweet and shellfish odours were also very characteristic for newly packed fillets of PD 7, and were still evident after 15 days from catch. Meat and vanilla/warm milk odours were obvious in PD 2 until 15 days of storage when only hints of these attributes were detected. Odour of boiled potatoes was evident in both groups throughout the storage time, but frozen storage odour was not detected. Hints of table cloth odour were detected in group PD 7 on day 15. Hints of TMA and sour odours were first detected on day 21 in group PD 2. TMA odour was detected and hint of sour odour was detected on day 15 in group PD7 (see Figure 20-21). The two groups did not differ with regard to appearance, but with storage time, the colour of group PD 2 became slightly darker and discoloured.

Hints of salty flavour were detected in both groups. Newly packed fillets of PD 2 had very characteristic metallic and sweet flavour and these attributes were evident through the storage time. These attributes were slightly less characteristic in the flavour of newly packed fillets of PD 7, and on day 15, metallic flavour was detectable, but only hint of sweet flavour was detected. Meat flavour was evident through the storage time in

both groups. Frozen storage flavour was not detected in either group. A hint of pungent flavour was detected in group PD 2 already on day 8 It was only detectable on day 15 in group PD 7. Hints of off-flavour, sour and TMA flavours were detected on days 18-21 in group PD 2. In group PD 7, these flavour attributes were evident on day 15, except sour flavour whereas only hint of this attribute was detected.

Both sample groups had flaky texture and this attribute did not change with storage time. Generally, the texture did not change much with storage time, though newly packed fillets in group PD 2 had the most juicy texture. The texture of group PD 2 was atypical on day 15, as it was less soft, tender and mushy, but more meaty compared to the groups on most other sampling days. Generally, the samples were rather soft, juicy and tender, rather mushy and meaty but not so clammy or rubbery.

Table 5: Average sensory scores (QDA scale 0-100%) for the two sample groups with storage time. Different letters show significant differences within a line.

Sensory attribute		MAP-PD2						MAP-PD7		
Days from catch:		**3**	**8**	**10**	**15**	**18**	**21**	**8**	**10**	**15**
Odour										
sweet	***	51 ^{a}	41 ^{ac}	39 ^{ac}	41 ^{ac}	35 ^{bc}	45 ^{ac}	39 ^{ac}	38 ^{ac}	29 ^{d}
shellfish, algae	***	47 ^{a}	33 ^{ab}	36 ^{ab}	32 ^{ab}	33 ^{ab}	33 ^{ab}	35 ^{ab}	37 ^{ab}	27 ^{bc}
meat	***	30 ^{a}	23 ^{ac}	27 ^{ab}	28 ^{a}	34 ^{a}	27 ^{ab}	28 ^{a}	27 ^{ab}	17 ^{bd}
vanilla/warm milk	***	35 ^{a}	28 ^{a}	34 ^{a}	28 ^{a}	20 ^{a}	28 ^{a}	28 ^{a}	30 ^{a}	16 ^{b}
boiled potatoes	ms	23	27	28	23	24	30	25	27	23
frozen storage		1	6	1	4	5	7	4	3	5
table cloth	***	1 ^{b}	6 ^{b}	3 ^{b}	7 ^{b}	6 ^{b}	9 ^{b}	7 ^{b}	4 ^{b}	19 ^{a}
TMA	***	0 ^{cd}	7 ^{cd}	3 ^{cd}	8 ^{cd}	7 ^{cd}	11 ^{bc}	9 ^{cd}	5 ^{cd}	21 ^{b}
sour	***	1 ^{b}	5 ^{b}	2 ^{b}	6 ^{b}	5 ^{b}	10 ^{b}	5 ^{b}	5 ^{b}	13 ^{b}
sulphur	***	1 ^{b}	3 ^{b}	1 ^{b}	3 ^{b}	4 ^{b}	5 ^{b}	2 ^{b}	1 ^{b}	7 ^{b}
Appearance										
colour	*	17	28	18 ^{b}	27	34	32 ^{a}	30	24	32
appearance	*	21	27	22	31	39	33	29	27	32
white precipitation		23	27	28	31	26	24	29	23	27
Flavour										
salt	*	5	14 ^{a}	5	11	11	11	14	6	10 ^{b}
metallic	***	48 ^{a}	34 ^{b}	31 ^{b}	33 ^{b}	22 ^{bc}	23 ^{ce}	34 ^{b}	31 ^{b}	23 ^{bc}
sweet	***	46 ^{a}	41 ^{ab}	32 ^{ad}	27 ^{cdf}	16 ^{dg}	26 ^{dg}	36 ^{ac}	28 ^{bcde}	16 ^{fgh}
meat	**	29	26	28	33 ^{a}	22	25	23	25	16 ^{b}
frozen storage	*	1	4	5	11	8	10	5	4	9
pungent	***	2 ^{b}	11 ^{b}	11 ^{b}	13 ^{b}	16 ^{b}	18 ^{b}	10 ^{b}	5 ^{b}	19 ^{b}
sour	***	1 ^{b}	9 ^{b}	3 ^{b}	5 ^{b}	12 ^{b}	11 ^{b}	5 ^{b}	4 ^{b}	15 ^{b}
TMA	***	1 ^{b}	10 ^{b}	3 ^{b}	8 ^{b}	10 ^{b}	15 ^{b}	5 ^{b}	3 ^{b}	26 ^{a}
off-flavour	***	1 ^{b}	11 ^{b}	7 ^{b}	9 ^{b}	13 ^{b}	15 ^{b}	10 ^{b}	5 ^{b}	27 ^{a}
Texture										
flakiness		48	42	40	48	33	43	42	42	48
soft	**	62 ^{a}	62 ^{a}	51	40 ^{b}	53 ^{a}	54	65 ^{a}	56	57 ^{a}
juicy	***	66 ^{a}	58 ^{b}	54 ^{b}	49 ^{b}	53 ^{b}	48 ^{b}	56 ^{b}	54 ^{b}	54 ^{b}
tender	**	50	57	42 ^{b}	38 ^{b}	45	49	51	54	54
mushy	***	28 ^{bc}	47 ^{ab}	38	31 ^{cd}	47 ^{ab}	44 ^{ab}	54 ^{a}	40	45 ^{ab}
meaty mouthfeel	**	40 ^{b}	35 ^{b}	40 ^{b}	53 ^{a}	36 ^{b}	40 ^{b}	36 ^{b}	43 ^{b}	38 ^{b}
clammy		26	30	31	38	23	32	31	29	33
rubbery		18	22	23	23	19	19	21	23	23

ms (marginal significance, p = 0,05-0,10); * (p < 0,05); ** (p < 0,01); * (p < 0,001)**

3.7 Chemical and physical properties of the raw material

The chemical and physical properties of the whole bled and gutted fish were analyzed to assess the quality of the raw material. Samples for analysis were taken from the muscle structure only. Table 6 shows that the water content of the raw material was 81.6 ± 0.4 % at the beginning of the study, with a salt content of 0.2 ± 0.1 % and water holding capacity of 89.5%, indicating good quality of the fish. Total Volatile Base Nitrogen (TVB-N) and trimethylamine (TMA) concentration in the raw material was low (10.4 ± 0.7 mg TVB-N / 100 g sample and 1.7 mg TMA / 100 g sample), which indicates that the fish was fresh. The raw material was stored whole on flake ice between the two packaging days. The chemical properties of the whole fish were assessed again at the second packaging day (PD 7) showing similar water and salt content as in the original measurements, but higher water holding capacity (WHC), as well as higher TVB-N and TMA values. The increase in water holding capacity in the raw material between the two packing days can possibly be explained by water drip from the muscle stored on flake ice. Freely moving water is lost more easily due to drip, leaving tighter bound water in the muscle and thus leading to an increase in overall sample water holding capacity.

Table 6: Chemical properties of the raw material.

Sample	Sampling day	Water [%]	Salt [%]	WHC [%]	TVB-N [mg/100g]	TMA [mg/100g]
Raw material PD 2	3	81,6 ± 0,4	0,2 ± 0,1	89,5	10,4 ± 0,7	1,7
Raw material PD 7	8	81,7 ± 0,4	0,3 ± 0,1	95	14,8 ± 0,7	4,6

3.8 Chemical and physical properties of cod loins – storage experiment

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

Results from measurements of TVB-N and TMA are shown on Figures 22 and 23. These results are very much in line with results from total microbial counts, counts of H_2S-producing bacteria and especially counts of *Photobacterium phosphoreum* (Figure 15). As discussed earlier, the number of *P. phosphoreum* in cod loins a day after packaging on day 7 (day 8) was very high or about log 5.4/g while on that same day *P. phosphoreum* in

cod loins packed on day 2 was only log 2.4/g. *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, 1995 a,b). In fact on day 15 after catch, a considerable increase was measured in TVB-N in the PD 7 fish, mainly explained by TMA production. Concurrently, higher levels of *P. phosphoreum* (log 7-8/g) were found on that sampling day compared to the levels of H_2S-producing bacteria (log 5/g). The low increase in volatile amines observed in PD 2 fish between days 18-21 is in agreement with growing *P. phosphoreum* (from log 5 to 6/g) during this period. These results further support that *P. phosphoreum* is one of the main spoilage organisms in cod packed under MA.

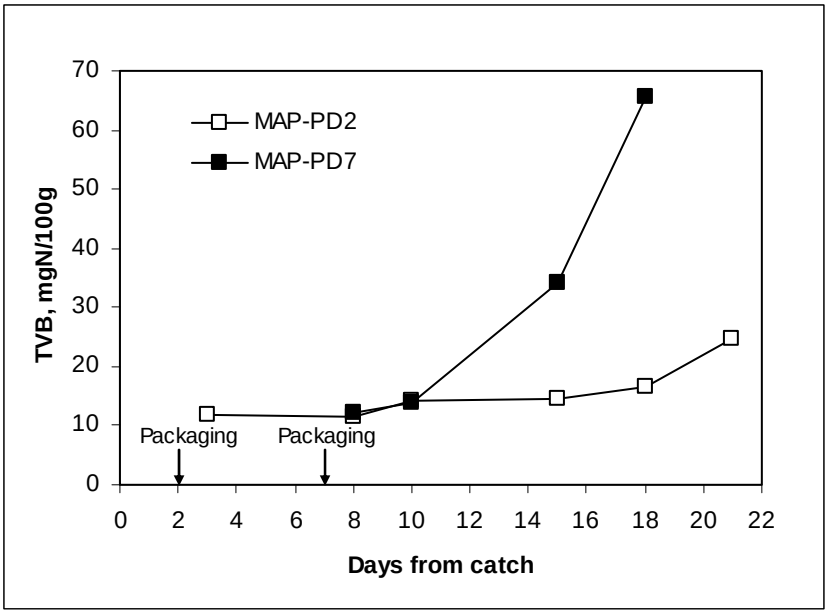

Figure 22: Total Volatile Base Nitrogen (TVB-N) content during storage.

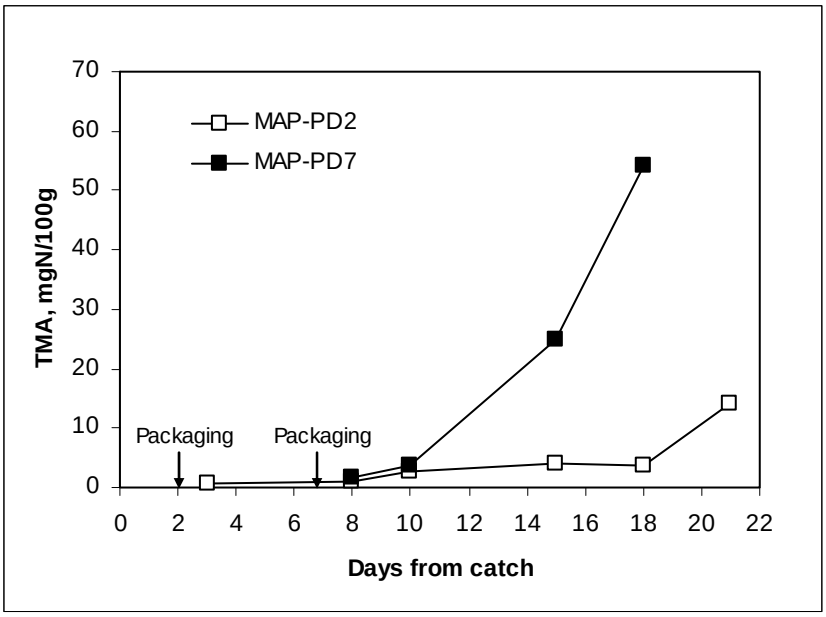

Figure 23: Production of trimethylamine (TMA) during storage.

3.8.2 pH- measurements

Results from pH measurements are shown on Figure 24. Initial pH of the raw material was 6.90 (PD 2) and 6.80 (PD 7). Upon MA-packaging, a decrease in pH was expected due to dissolution of carbon dioxide into the water phase of the muscle. On the sampling day following packaging, an average pH value of 6.70 ± 0.14 (PD 2) and 6.55 ± 0.07 (PD 7) was found. As storage proceeded a marked increase was seen in pH, reaching 7.2 in the loins packed on day 7. Formation of basic spoilage compounds as described above is the main reason for this rise in pH. A more stable pH was observed in the PD 2 fish as a lesser amine production was measured up to day 21.

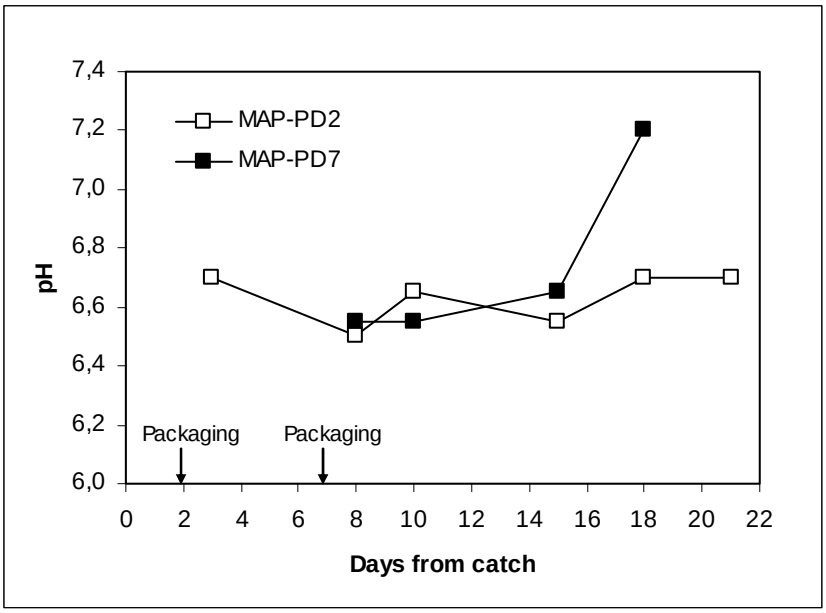

Figure 24: Acidity (pH) in loins packed under MA 2 and 7 days from catch.

3.8.3 Water content, salt content and water holding capacity (WHC)

The physical properties of water was monitored through the study by measuring the water and salt content, as well as the water holding capacity (WHC) at sampling. According to Figure 25 a small lowering trend can be seen in the water content for samples packed under MA 2 days after catch in relation to the drip loss measured in the samples. More fluctuations in the water content were on the other hand observed in the samples packed under MA 7 days after catch, indicating larger differences in water content between samples in the group packed 7 days after catch than in the group packed 2 days after catch. This is also in agreement with the drip results, which were more fluctuating in the samples packed 7 days after catch. These fluctuations in drip and water content between samples within the PD 7 group can possibly be explained by the fact that the fish were taken at different depth within the tubs at sampling, but drip is known to increase due to added load.

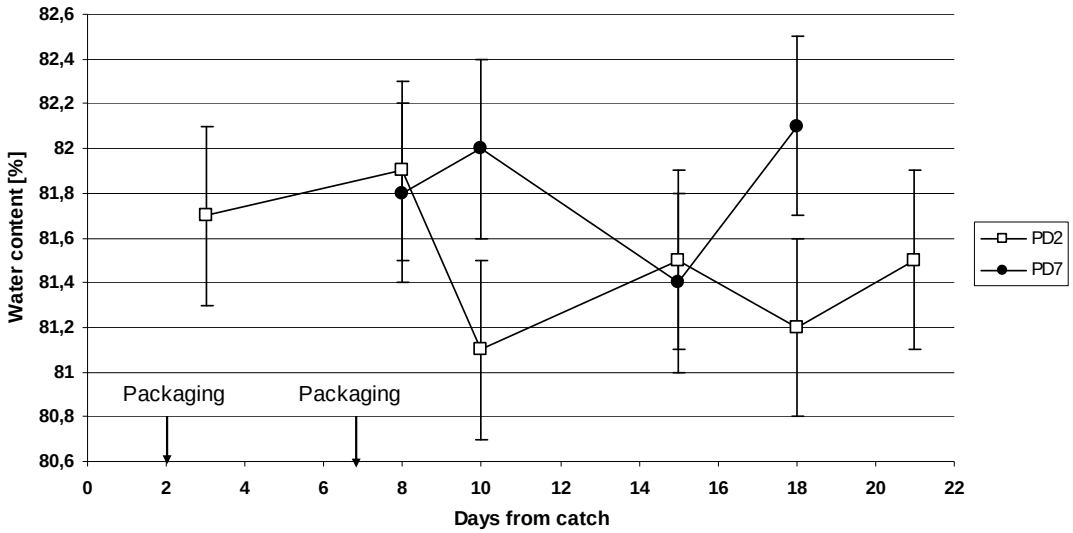

Figure 25: Changes in water content in cod loins during storage for fish packed two days (PD 2) and 7 days (PD 7) after catch.

The salt composition was measured in the samples giving a mean salt value of 0.3 ± 0.1 % in both the samples packed two days (PD 2) and seven days (PD 7) after catch. These are normal salt values for untreated fresh cod muscle and no variation was found in the salt content with storage time.

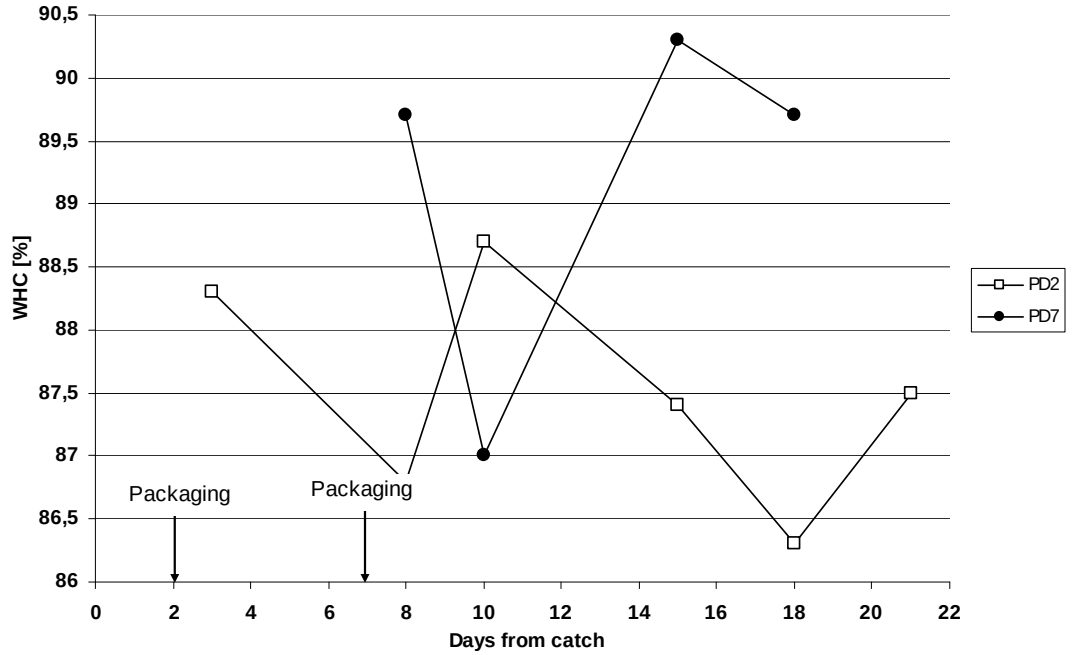

Figure 26: Changes in water holding capacity (WHC) in cod loins during storage for fish packed two days (PD 2) and 7 days (PD 7) after catch.

Figure 26 shows the changes in water holding capacity (WHC) in the cod loins from both groups during storage. Samples packed 7 days after catch show generally higher water holding capacity values except on day 10. This higher water holding capacity in the samples packed 7 days after catch coincided with the higher water content in these samples. Like for the water content, a general decrease of water holding capacity can be seen with storage time indicating a lower ability to retain water in the muscle structure.

3.9 Nuclear Magnetic Resonance

Low field Nuclear Magnetic Resonance (LF-NMR) was used to measure the relaxation times of the samples during storage. The results can be viewed on Figure 27 to Figure 30.

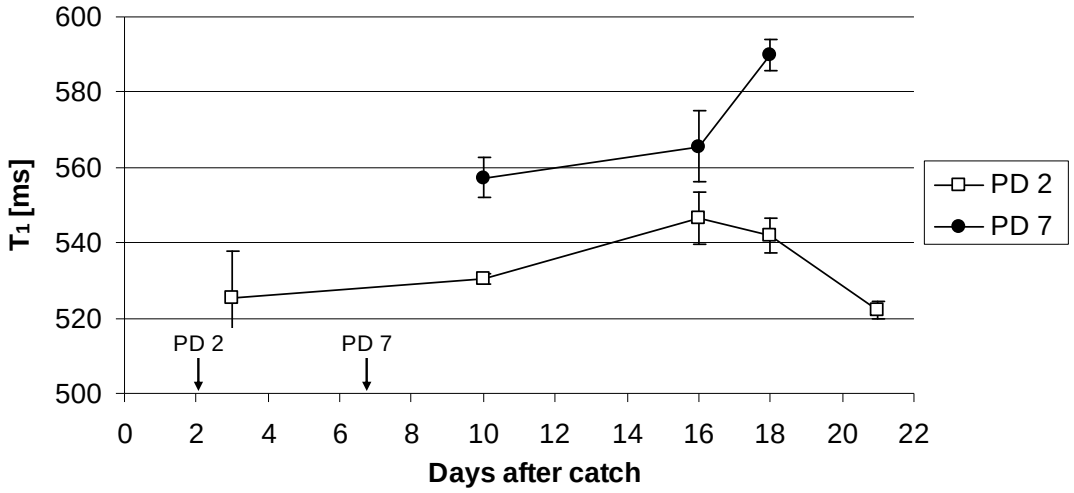

Figure 27: Longitudinal relaxation times, T_1 with storage time.

Figure 27 shows how the longitudinal relaxation time, T_1, changed with storage time in the two groups. The samples packed 7 days after catch showed higher relaxation times than samples packed 2 days after catch, indicating stronger bindings of the water molecules to their surroundings in samples packed at a later stage. This is in agreement with the generally higher water holding capacity and water content in the samples during storage in the samples packed 7 days after catch.

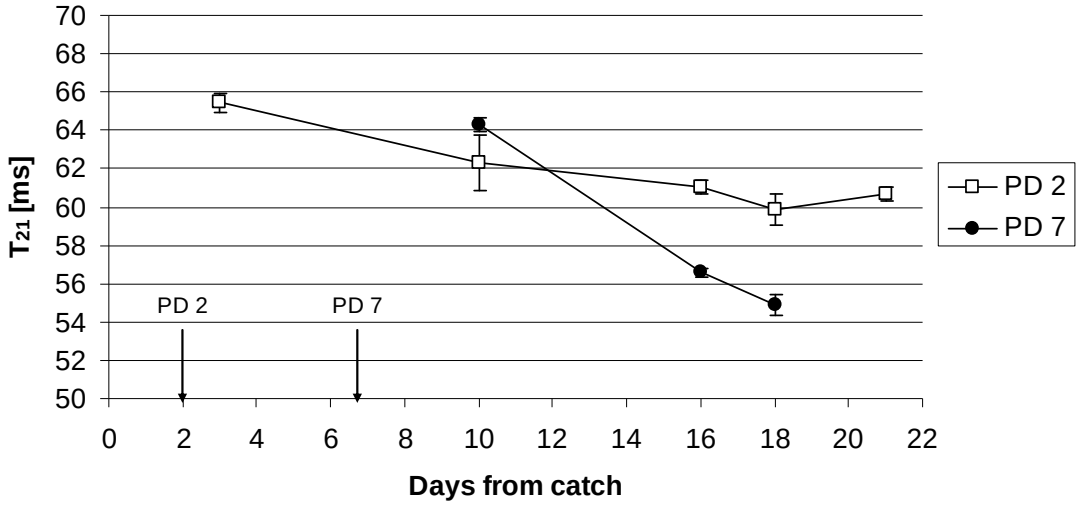

Figure 28: Shorter transverse relaxation time, T_{21} with storage time.

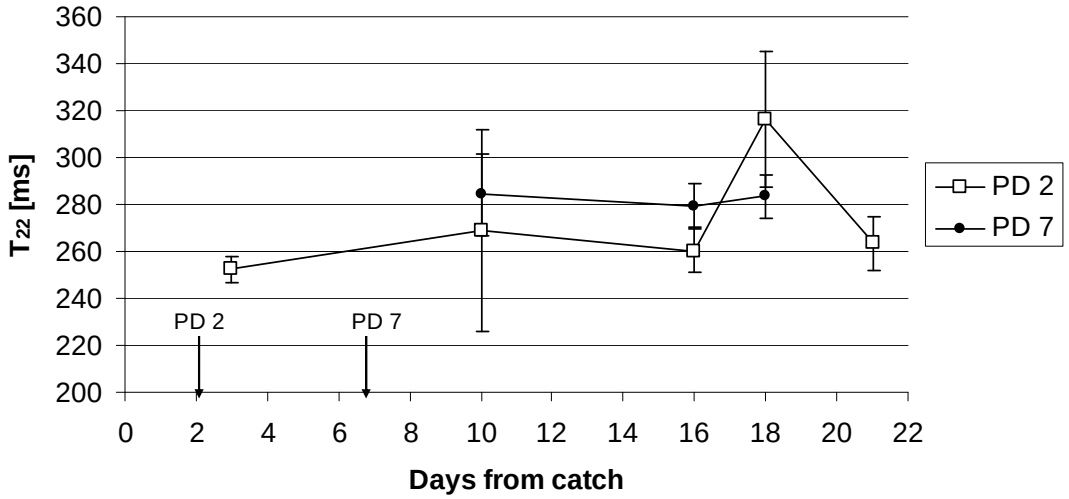

Figure 29: Longer transverse relaxation time, T_{22} with storage time.

Figure 28 and Figure 29 show the transverse relaxation times measured in the samples during storage. The data was fitted with a bi-exponential curve giving two transverse relaxation times, T_{21} corresponding to tightly bound myofibrillar water and T_{22} corresponding to more freely moving water between the myofibrills. Figure 26 clearly shows that a faster decrease in the shorter transverse relaxation time was observed during storage of PD 7 cod loins compared to PD 2 fish, and hence faster increasing restriction of water molecules in the PD 7 samples than in the PD 2 samples within the myofibrillar structure. This is in correlation to the measurements of the longitudinal relaxation time,

T_1. According to Figure 30 the relative amount of freely moving water ranged from 93.5 to 96.5 %, with slightly higher values in samples packed 7 days after catch than in samples packed 2 days after catch. The change in this relative amount of freely moving water did not change significantly during storage in neither group.

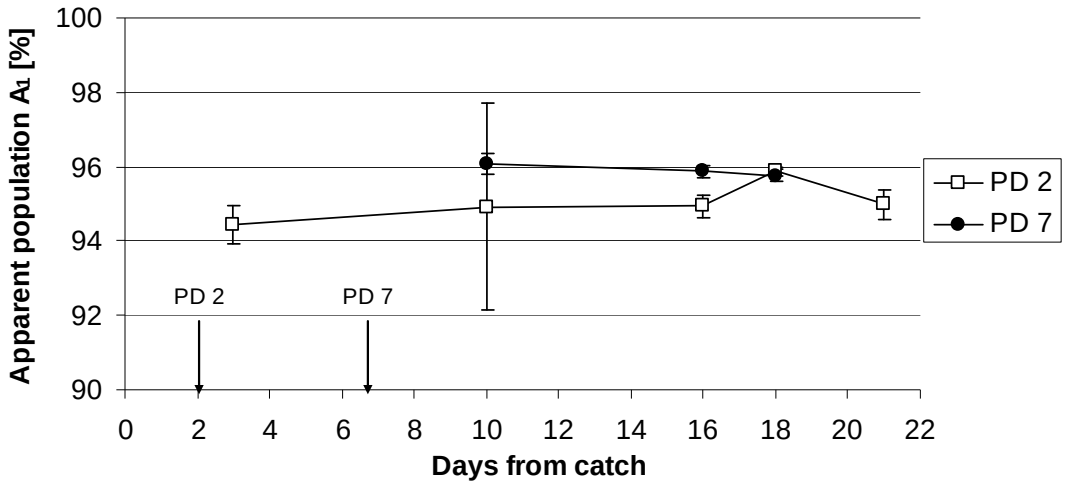

Figure 30: Apparent population of tightly bound water with storage time.

4 CONCLUSIONS

The freshness of the raw material clearly influenced the sensory characteristics. Filleting and MA-packing two days after catch resulted in more prominent freshness sensory characteristics (sweet and metallic flavour) the first days of storage. In addition, sensory indicators of spoilage, such as TMA flavour, became evident much later compared to MA-packed fillets from raw material processed seven days after catch.

The expected shelf life of the MA-packed cod loins could be roughly calculated as 4-8 days when processed from 7 days old whole, gutted cod, but 19 days or more if the cod was filleted and MA-packed two days post catch. This reduced shelf life of MAP products processed at a later stage was well demonstrated by the sensory characteristics evaluated, as well as explained by the temperature profile of the whole fish prior to processing, microbial development and volatile amine production observed. The results therefore demonstrated that delaying processing of raw material is undesirable if it is intended to be MA-packed and sold as more valuable products.

5 ACKNOWLEDGEMENTS

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