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Effect of Transport Packaging and Repackaging into Modified Atmosphere on Shelf Life and Quality of Thawed Atlantic Cod Loins

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Boxes of expanded polystyrene (EPS, with or without ice added), corrugated cardboard (CC, with or without ice added) and polypropylene (PP, gel-ice inside the lid) were used in a case study, for transport of cod loins. Shelf life and quality were analysed after repackaging into modified atmosphere by use of culture dependent microbial methods, analysis of bacterial 16S rRNA sequences, sensory descriptive analysis and analysis of volatile components. The CC boxes showed faster temperature reduction compared to the EPS boxes when no ice added. Still, the microbial shelf life did not differ between transport packaging materials, but adding ice resulted in prolonged microbial shelf life. The sensory shelf lives were similar between all the treatments of MAP, but indications of prolonged sensory shelf life for the CC boxes both with and without ice added, compared to the EPS without ice added, were found. The EPS with added ice compared to the EPS without ice added did also show indication of prolonged sensory shelf life. The PP box seemed to have similar quality preservation as the CC samples with added ice. At the end of shelf life at least two different bacteria genera were detected on the MAP products: *Psychrobacter*, *Shewanella*, *Carnobacterium*, *Pseudomonas* and/or *Acinetobacter*. *Photobacterium* was presented in only minor levels, probably related to inactivation because of freezing. Summarized, this study showed that transport packaging concepts of corrugated cardboard boxes and polypropylene boxes preserved quality equally well as the EPS boxes prior to repackaging and further storage in MAP. Q2

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KEY WORDS: bacteria; modified atmosphere packaging; odour; shelf life; transport packaging; volatile components

INTRODUCTION

Consumers demand raw fish products with high quality that are easy to prepare. Quite common, the packaging sites of single consumer sized products are situated separately from the fish fillet processing site, which results in transport of fillets over longer distances prior to repackaging. Correct and optimal handling at every stage through the production chain is therefore crucial to ensure preservation of quality. As raw fish is a perishable product, bacterial load, packaging method and temperature prior packaging and during transport and storage can affect quality and shelf life. The most common packaging material for raw fish during transport is expanded polystyrene (EPS) boxes, in units 10–20 kg, with added 3–5 kg ice. Alternative packaging solutions for bulk packaging of fresh or raw fish are demanded by the fish industry, mainly because of the space demanded when handling EPS boxes. Different current solutions to preserve the quality of fresh fish are available, like high density polyethylene

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(HDPE) boxes, corrugated polypropylene (PP) boxes, conical and reusable plastic boxes (also HDPE, or PP) and fibre-based materials, such as corrugated cardboard and solid cardboard. Important properties for bulk packaging materials are insulation, shock strength, water resistance and how to handle it. The presence of wet ice inside transport boxes, in addition to the chilling capacity in the transport vehicles, is a common way of ensuring low core temperature of the fish during transport. Improved solutions by replacing ice with edible products can also be possible to use, which result in increased transport efficiency and reduction of the CO_2 load.[1]

Furthermore, it is a pronounced interest in selling the fish pre-packaged into consumer sized packages, either by use of air inside the package, vacuum packaging, skin pack or modified atmosphere packaging (MAP). The MAP method by its content of carbon dioxide (CO_2) inhibits bacterial growth and thus facilitates prolonged shelf life.[2–6] MAP of farmed Atlantic cod has also shown that high levels of oxygen (O_2) together with CO_2, give optimal quality preservation,[7,8] which can also be applied to wild caught cod. CO_2 absorbs into the fish muscle after packaging, and a certain gas volume to product volume ratio (g/p ratio) is important to ensure sufficient partial pressure and effect of the CO_2. Additionally, the fish product has to be in good hygienic state, which is a presumption to achieve an optimal effect of MAP.[9,10]

Fresh fish is contaminated by a diverse mixture of bacteria during harvesting and processing. Little is known about the total microbial flora of raw fish[11,12] and how it affects sensory quality and shelf life. Volatile components can be formed by bacteria and by autolytic reactions during storage, and are influenced by the initial quality of the fish, storage temperature and packaging conditions. Bacterial metabolism and development of volatile components can affect the sensory quality of fish products, e.g. both *Photobacterium phosphoreum* and *Shewanella putrefaciens* can reduce trimethylamine oxide (TMAO) to trimethylamine (TMA), causing fishy odour.[13] Furthermore, fluctuating temperatures or temperature increase results in increased growth of spoilage bacteria and shorten the time of shelf life,[6,14,15] and temperature reduction from 5 °C till 0 °C can double time of shelf life.[16]

To our knowledge, evaluation of different transport packaging materials, with and without ice added, and subsequent repackaging into small units of MA-packaged cod loins have previously not been published. The objective of this case study was to investigate the effect of different transport packaging concepts relevant for the fish industry, and how quality and shelf life was preserved during further storage (repackaging) in MAP.

MATERIALS AND METHODS

Fish materials

Atlantic cod (*Gadus morhua*) were caught at the North Cape fishing ground by use of long line. After catching, the fish were gutted on board the fishing vessel and directly frozen pre-rigour in blocks (25 kg) in plate freezers, and stored for about 10 months at −23 °C. Thawing was performed in 2 °C freshwater (17 h) by use of a thawing tank (Stette DF530-C, Peter Stette AS, Skodje, Norway); subsequently, the neck was cut, and fillets cut into loins and packaged as 10 kg units of loins per box (packaging; see below).

Packaging methods, materials and storage

Packaging for transport. Three different packaging materials (boxes) were used in this study based on solutions the fishing industry requested: Corrugated cardboard (CC) box (Smurfit Kappa Norpapp, Hønefoss, Norway), expanded polystyrene (EPS) box (Vartdal Plastindustri, Vartdal, Norway), both of 10-kg sizings, and reusable box made of polypropylene (PP, Coolblue Box Company, Grimsby, England) of 25-kg sizing. The cardboard box had an exterior dimension of 60 × 40 × 17 cm, a wall thickness of 7 mm and a liquid absorbent pad in the bottom of the box. The EPS box had an exterior dimension of 60 × 40 × 13 cm, a wall thickness of 45 mm and drainage holes (no absorbent pad). The CC and EPS boxes were either packaged using standard amount of ice (4 kg) added on top, or with no ice. The PP box had an exterior dimension (conical) of 80 × 30/40 × 19 cm, and the lid contained gel-ice inside (no wet ices was added). The core temperature of the loins for all treatments was at time of packaging (prior adding ice) 4.4 ± 0.5 °C. The expanded polystyrene (EPS) boxes (n = 4), the

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corrugated cardboard (CC) boxes (n = 4) and the polypropylene (PP) boxes (n = 4) were placed beside each other on one separate Euro pallet during transport (three layers of boxes). The temperature inside the truck during transport (20 h) to Ås (Norway), with arrival the day after, was 1.9 ± 1.2 °C. A further storage period was then performed to give similar storage conditions as a parallel transport to a processing plant at the European continent. The temperature inside the refrigerating room during the three days of intermediate storage (prior repackaging) was 0.2 ± 0.4 °C.

Repackaging into consumer sized packages. Repackaging into modified atmosphere was performed four days after thawing and packaging for transport. The loins were randomly taken from the respectively transport boxes. Two – two and a half loins were put in each tray (400 g), which constituted a gas Q3 volume to product volume ratio of about 2/1. The pre-mixed packaging gas contained 40% CO_2 and 60% O_2 (Yara AS, Oslo, Norway). A tray packaging machine was used for the MA packaging (Polimoon 511VG, Promens, Kristiansand, Norway).

The plastic trays were made of APET/PE (Wihuri Oy, Wipak, Nastola, Finland), thermoformed by JiHå Plast AB (Karlskoga, Sweden) with a top web consisting of PETP/PE/EVOH/PE (Biaxer 65 XX HFP AF, Wihuri Oy, Wipak). The trays had 50-mm drawing depth. The oxygen transmission rate (OTR) was measured to be 0.05 cm^3 O_2/(package·day) (1143-ml trays) at 1.5 °C and 100% humidity, using the AOIR-method.[17] Each tray contained a liquid absorbing pad of 12.5 × 9 cm (Dri-Loc® Absorbent Pad, Sealed Air Corporation, Epernon Cedex, France), with the fish samples placed on the non-perforated side. The absorbency of the pad is 50 ml.

As a control, samples from the 'EPS with ice' were also packed with air, in similar trays as the MAP samples, but with no gas mix added, and needle holes were applied on the top web to ensure air access, in order to imitate packaging at some retail stores today.

Sampling was performed 3, 7, 10 and 15 days after repackaging. There were four replicates per packaging treatment at each sampling time. The temperature during storage of the repackaged samples (15 days) was monitored to be 1.7 ± 0.7 °C.

See Table 1 for an overview of the packaging treatments. T1

Analyses

Temperature. The temperature loggers used were Ecolog TN4-L (Elpro-Buchs AG, Buchs, Switzerland) to measure core temperature of the loins prior packaging into transport boxes, and for continuous logging of the temperature inside the truck and the refrigerating room for temporary storage and after repackaging into MAP. EBI-125A/85A loggers (Ebro Electronic, Ingolstadt, Germany) were used to measure the core temperature of the loins during transport and further refrigerating storage (a logger placed in a loin in centre of the boxes). The accuracy of the loggers is 0.1 °C and 0.3 °C, respectively. Measurements were done every 10 min.

Headspace gas analysis. The CO_2 and O_2 were analysed at each sampling time by a CheckMate 9900 O2/CO_2 analyser (PBI Dansensor, Ringsted, Denmark).

Culture-dependent analyses of bacteria (plate count). Samples of 3 × 3 cm and 1-cm depth were taken from one loin per package/treatment, diluted by approximately 90-ml peptone water until 1/10 dilution was attained, and run in Stomacher for 60 s. Appropriate 10-fold dilutions were made and

Table 1. Experimental design: packaging treatment—for transport (à 10 kg) and repackaging into consumer sized packages (à 400 g).

Transport packaging (4-day storage)		Repackaging (15-day storage)	
Transport packaging material	*Ice/level of ice*	*Packaging method*	*Sample name*
Expanded polystyrene (EPS)	With wet ice added	MAP	'EPS with ice'
Expanded polystyrene (EPS)	Without ice	MAP	'EPS without ice'
Corrugated cardboard (CC)	With wet ice added	MAP	'CC with ice'
Corrugated cardboard (CC)	Without ice	MAP	'CC without ice'
Polypropylene (PP)	Gel-ice inside lid	MAP	'PP'
Expanded polystyrene (EPS)	With wet ice added	Air (control)	'EPS with ice/air'

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spread on agar plates: Iron agar (Oxoid, Basingstoke, Hampshire, U.K.) for total bacterial count and for H_2S-producing bacteria (counting black colonies), and CFC (Cephaloridine Fucidin Cetrimide) agar for Pseudomonas bacteria. All plates were incubated at 15 °C for 6 days.

Culture independent analyses of bacteria. Bacterial DNA extraction was performed directly from the fish matrix. Analyses were performed 10 days after repackaging into MAP (14 days after thawing). The Stomacher solutions (50 ml) were first frozen and then thawed in a refrigerator for DNA isolation. DNA extraction, cloning and sequencing of 16S rRNA gene were performed as described in Hansen, Mørkøre *et al.* [*8]. Taxonomic assignments were done using searches in RDP II (Ribosomal Database Project, SeqMatch, http://rdp.cme.msu.edu/classifier/classifier.jsp) and BLAST, Megablast (www.ncbi.nlm.nih.gov/blast).

Analyses of volatile compounds. Dynamic headspace/GC-MS analyses of volatile compounds were performed on samples from the packages store for 10 days after repackaging into MAP (14 days after thawing). Samples were cut from the same loin as performed for the bacterial analyses. Volatiles were analysed as earlier described by Hansen, Mørkøre *et al.* [*8] on 5-g homogenate and expressed as µg/g sample.

Sensory analysis. The sensory panel consisted of 12 trained assessors[18] with an average of 15 years of experience in sensory analysis. The assessments were conducted according to the general guideline for establishing a sensory profile,[19] and the evaluations were carried out in a sensory laboratory designed according to the guidelines in ISO 8589[20] with separate booths and with electronic registration of data (Compusense Five, Version 4.6, Guelph, Ontario, Canada). The assessors were trained and calibrated on fresh cod samples with the purpose of mutual understanding of the attribute definition and an agreement of intensity score given to the samples. The samples were taken from the second loin in the packages, served as raw vacuum packaged samples of about 3 cm^3, and the intensity of each odour attribute was perceived by sniffing into the newly opened plastic bag. The panel evaluated the samples at each sampling time during the 15 days of storage (after repackaging into MAP). The samples were evaluated for the intensity of the sensory attributes: acidic, sea water, metallic, cloying, sulphur, ammonia, sour, unfresh and rancid odour. Appearance was analysed by means of yellowness and whiteness.

The panellists recorded their results on a 15-cm non-structured continuous scale with the left side of the scale corresponding to the lowest intensity, and the right side corresponding to the highest intensity. The computer transformed the responses into numbers between 1 = low intensity and 9 = high intensity.

Liquid loss. Results are given as the weight increase of the liquid absorber as % of initial muscle weight.

Muscle pH. The pH was analysed using a Knick pH meter (Knick GmbH & Co, Berlin, Germany) and a muscle electrode S/N 5290739 (Mettler Toledo, Urdorf, Switzerland). The analyses were performed with triplicate measurements of each sample.

Statistics

The effect of the experimental factor 'transport packaging', with six levels, were analysed: EPS with ice, EPS without ice, CC with ice, CC without ice and PP with gel-ice included in the lid, all after being repackaged into MAP. Additionally, there was one treatment (EPS with ice) packaged in trays containing air. These six levels were analysed separately for each day during refrigerating storage after repackaging into MAP, using analysis of variance (ANOVA), with Tukey's multiple comparisons test where applicable ($p < 0.05$). PLS2 (Partial Least Squares with 2 blocks) was used for the multivariate analyses. Statistical Software used was: SAS 9.2 (SAS Institute, Inc., Cary NC, USA) for the ANOVA/Tukey's test, SYSTAT 12 (SYSTAT Software, Inc., San Jose CA, USA) for the bacteria counts plots and Unscrambler X (CAMO AS, Oslo, Norway) for the PLS2 analyses.

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RESULTS AND DISCUSSION

Storage conditions

During transport and intermediate storage, the 'EPS without ice' and the 'CC without ice' reached 0.5 °C after 38 and 29 h, respectively. On the other hand, CC boxes and EPS boxes (both added wet ice) reached 0 °C similarly, within 4 h. The PP boxes (with gel-ice inside the lid) had quite similar temperature profiles compared to 'CC without ice' and reached 0.5 °C after 28 h.

The gas mixture changed during storage from 40% CO_2 and 60% O_2 (32% CO_2 and 65% O_2 immediately after packaging) to 13% CO_2 and 83% O_2 (measured after three days of storage). The fish muscle absorbs CO_2 until equilibrium is attained in MA packages. Consequently the partial pressure of CO_2 will reduce.[21] Because of this reduction some under-pressure was detected. The gas mixture in headspace during storage was similar for all the MA packages, which was as expected.

Microbiological shelf life

Total bacterial count and H_2S-producing bacteria of the cod loins, immediately after thawing and processing at the plant, was 4.2 ± 0.3 log cfu/g and 1.5 ± 0.2 log cfu/g, respectively. The only difference between bacterial count after transport and intermediate storage was between the 'EPS with ice' and the 'CC without ice' (Day 0, Table 2). Despite faster temperature reduction for the 'CC without ice' **T2** compared to the 'EPS without ice', no differences were found in bacterial count. After repackaging into MAP, both 'EPS without ice' and 'CC without ice' exceeded microbiological shelf life after 7 days of storage, according to the total bacterial count limit of 6 log cfu/g.[22] The 'EPS with ice' and the 'CC with ice' had similar bacterial counts after transport, intermediate storage and repackaging, and they did also achieve microbiological shelf life of 10 days. The 'PP' did also achieve a microbial shelf life of approximately 10 days, despite similar temperature reduction capacity to the 'CC without ice'.

The 'EPS with ice/air' samples had, not surprisingly, the highest bacterial growth during storage compared to the MAP samples. Still, wrapping of fresh fish fillets for consumer packaging is quite common at deli markets. The microbiological shelf life was only about three days after repackaging into consumer packages (based on a shelf life limit of 6 log cfu/g), which was seven days after thawing/filleting. This corresponds to the sensory shelf life. Koutsoumanis and Nychas[23] suggest a pseudomonads level of 10^7 cfu/g as limit of shelf life. This would have resulted in shelf life of about 15 days in the present study for the MAP samples (according to the CFC counts). Related to the sensory analyses (see below), this was probably not the case in the present study. However, such pseudomonads level seemed to be relevant for the air stored samples 'EPS with ice/air', which favoured a higher part of *Pseudomonas* sp. showed by the 16S rDNA analyses (Table 3). Still the count numbers **T3** of *Pseudomonas* (CFC agar) showed too long shelf life according to the intensity scores of the sensory attributes. Overestimation of Pseudomonas levels is also previously reported.[24]

Studies performed on MAP salmon[5,25] show positive correlations between the total bacterial count and the negatively associated sour odour of raw fillets, and they found a shift in sensory score at bacterial count of log 6 cfu/g. In the present study, similar change was found at about log 7 cfu/g of total bacterial count. Still, different compositions of the microbial community can affect at which bacterial level changes in sensory quality can be detected. Indications of negative correlation were seen between the intensity of acidic odour and total bacterial count. However, loss of positively associated odour might be less dependent to bacterial growth and metabolism as the negatively associated odours, because the initial quality loss in fish is primarily caused by autolytic change.[26]

Microbiota

It is likely to assume that the microbial communities dominating after 10 days of storage were similar to what was found after, e.g. 7 days of storage, as demonstrated for vacuum and MAP raw salmon products after 7 and 10 days of storage.[27] This means that the different microbial communities of the cod samples, showed in Table 3, probably limited quality and shelf life. Shelf life of 'EPS with ice' seemed to be limited by one dominating bacteria (*Psychrobacter*), and the other samples reached

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Table 2. Development of total bacterial count (log cfu/g on iron agar), *Pseudomonas* (log cfu/g on CFC agar) and H_2S-producing bacteria (log cfu/g on iron agar, black colonies) (mean ± SE, n = 4) of thawed cod loins in different transport packaging solutions and repackaging into MAP (control; air) and further storage (2 °C). Day 0 = repackaging into MAP (four days after thawing). For each row, entries with the same letter attached to them are not significantly different at the 0.05 level according to Tukey's Multiple Comparisons Test.

		EPS with ice/MAP	*EPS without ice/MAP*	*CC with ice/MAP*	*CC without ice/MAP*	*PP*[a]*/MAP*	*Control* (*EPS with ice/air*)
Total count	Day 0	3.62 ± 0.19b	3.94 ± 0.49ab	3.94 ± 0.23ab	4.46 ± 0.09a	4.26 ± 0.38ab	3.62 ± 0.19b
	Day 3	4.57 ± 0.24b	5.37 ± 0.51ab	4.45 ± 0.64b	4.79 ± 0.37b	5.07 ± 0.45b	6.08 ± 0.33a
	Day 7	5.41 ± 0.18d	6.82 ± 0.55b	5.19 ± 0.53d	6.31 ± 0.43bc	5.58 ± 0.17cd	8.00 ± 0.00a
	Day 10	6.22 ± 0.28c	7.32 ± 0.37b	6.48 ± 0.43bc	7.39 ± 0.60b	6.76 ± 0.71bc	9.00 ± 0.00a
	Day 15	7.00 ± 0.62c	8.20 ± 0.22b	7.51 ± 0.72bc	8.37 ± 0.40ab	7.98 ± 0.68bc	9.51 ± 0.09a
Pseudomonas	Day 0	2.84 ± 0.23bc	3.49 ± 0.34ab	3.08 ± 0.19bc	3.71 ± 0.03a	3.43 ± 0.27ab	2.84 ± 0.23bc
	Day 3	3.67 ± 0.30b	4.29 ± 0.43ab	3.40 ± 1.00b	4.19 ± 0.41ab	4.31 ± 0.46ab	4.97 ± 0.20a
	Day 7	4.92 ± 0.26c	6.02 ± 0.47b	4.79 ± 0.49c	5.75 ± 0.19b	5.47 ± 0.36bc	7.40 ± 0.24a
	Day 10	5.62 ± 0.21c	6.61 ± 0.31b	5.81 ± 0.17c	6.63 ± 0.14b	6.19 ± 0.45bc	8.00 ± 0.00a
	Day 15	6.44 ± 0.46b	6.82 ± 0.24b	7.01 ± 0.61b	7.41 ± 0.50b	6.91 ± 0.45b	8.85 ± 0.30a
H_2S-bacteria	Day 0	3.12 ± 0.57	3.36 ± 0.16	3.43 ± 0.23	3.04 ± 0.30	3.65 ± 0.25	3.12 ± 0.57
	Day 3	4.14 ± 0.27b	4.74 ± 0.73b	4.18 ± 0.68b	3.99 ± 0.52ab	4.58 ± 0.34ab	5.59 ± 0.05a
	Day 7	5.01 ± 0.18b	5.68 ± 0.50ab	4.74 ± 0.64b	5.56 ± 0.81ab	5.04 ± 0.19b	6.29 ± 0.30a
	Day 10	5.75 ± 0.30b	6.68 ± 0.40ab	6.01 ± 0.55b	6.73 ± 0.96ab	6.15 ± 0.89b	8.04 ± 0.17a
	Day 15	6.01 ± 1.19b	7.81 ± 0.27ab	6.96 ± 1.33b	7.72 ± 0.32ab	7.51 ± 1.01ab	8.93 ± 0.16a

[a]Contains ice inside the lid.

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Table 3. Sequence analyses of bacterial DNA from thawed cod loins in different transport packaging solutions and repackaging into MAP (control; air) and further storage (2 °C), isolated 10 days after repackaging (=14 days after thawing). The results are given as percent of the total amount of sequences from each packaging method. The partial 16S rRNA gene sequences showed 97%–100% identity to the different bacteria groups with search in BLAST (www.ncbi.nlj.nih.gov/blast).

	EPS with ice/MAP	*EPS without ice/MAP*	*CC with ice/MAP*	*CC without ice/MAP*	*PP*[a]*/MAP*	*Control (EPS with ice/air)*
Psychrobacter	86	31	37	38	15	19
Shewanella	3	3	33	30	12	24
Acinetobacter	0	13	0	4	15	30
Carnobacterium	0	25	3	13	39	0
Photobacterium	0	16	3	4	0	0
Pseudomonas	0	6	7	0	19	22
Psychromonas	0	3	10	9	0	0
Pseudoalteromonas	3	0	7	2	0	0
Phascolarctobacterium	0	3	0	0	0	0
Acidilobus	0	0	0	0	0	3
Uncl. Rhodobact.	0	0	0	0	0	3
Lactobacillus	3	0	0	0	0	0
Unclassified Gamma-proteobacterium	3	0	0	0	0	0
Log CFU g^{-1}, total viable count	6.2	7.2	6.7	7.9	6.9	9.0
Sequences (n)	29	32	30	47	26	37

[a]Contains ice inside the lid.

end of shelf life because of at least two different genus of bacteria, and especially showed by the 'EPS with ice/air' samples, in which the *Acinetobacter* and *Pseudomonas* correlated to the negative associated odour attributes (Figures 1 a, b). *Psychrobacter*, *Shewanella*, *Carnobacterium*, *Pseudomonas*, **F1** *Acinetobacter*, *Photobacterium* and *Psychromonas* more or less formed a mixed culture at end of shelf life for the other samples. The 'EPS without ice', which had the highest temperature during transport and temporary storage, had higher levels of *Carnobacterium*, *Photobacterium* and *Acinetobacter* compared to 'EPS with ice' and 'CC with ice'. *Carnobacterium* is reported to have a low spoiling potential[28], and is reported in earlier studies of MAP fish, both with N_2/CO_2 [29,30] and O_2/CO_2.[8] *Shewanella* bacteria develop both trimethylamine (TMA) and H_2S, and can be sensitive to CO_2.[31] The detection of *Shewanella* after storage in modified atmosphere containing CO_2 could be explained by the presence of oxygen, still, an indication of toxicity of high levels of oxygen is reported,[8] and such toxicity is also shown for *Shewanella*[32] and for some bacteria in general.[33] Therefore, the present results show that *Shewanella* is able to grow both with CO_2 and high-O_2 present. The CO_2 level could have been too low (13% CO_2) to inhibit growth of *Shewanella*, as *Shewanella* was presented in all the treatments.

Earlier findings show that MAP samples have been dominated by *P. phosphoreum* (79–100%) on MAP products[5,8] and that *P. phosphoreum* can be expected to be found at high levels both in temperature abused cod products[34] and in cod loins at low temperature storage (both air and MA storage).[35] In the presented study, the 'EPS without ice' had the highest occurrence of *Photobacterium*, yet only 16%, which might be related to the frozen storage, as *Photobacterium* is reported to be eliminated after freezing,[36] and *Photobacterium* might not be a common bacterium in the processing environment.

The presented samples of 'EPS with ice' probably reached end of shelf life caused by *Psychrobacter*. However, *Psychrobacter* might be less harmfully related to quality degradation, as *Psychrobacter* were not correlated to the negative associated attributes (Figure 1 a, b). Additionally, the presented study shows that *Psychrobacter* was able to grow and surpass other bacteria in modified atmosphere condition containing CO_2 and O_2. To our knowledge *Psychrobacter* is previously not described on raw fish fillet products (MAP and air), as a spoilage bacteria, like on salt-cured and dried salt-cured cod products.[37] Gennari, Tomaselli *et al.* [*38] report *Psychrobacter immobilis* as a minor spoiler detected on sardine skin and gills. Related to this, a microbiota dominated by *Psychrobacter* might enable higher quality during storage, which could have been the case in the presented study for the 'EPS with ice' (86% of *Psychrobacter* after 10 days of storage, Table 3).

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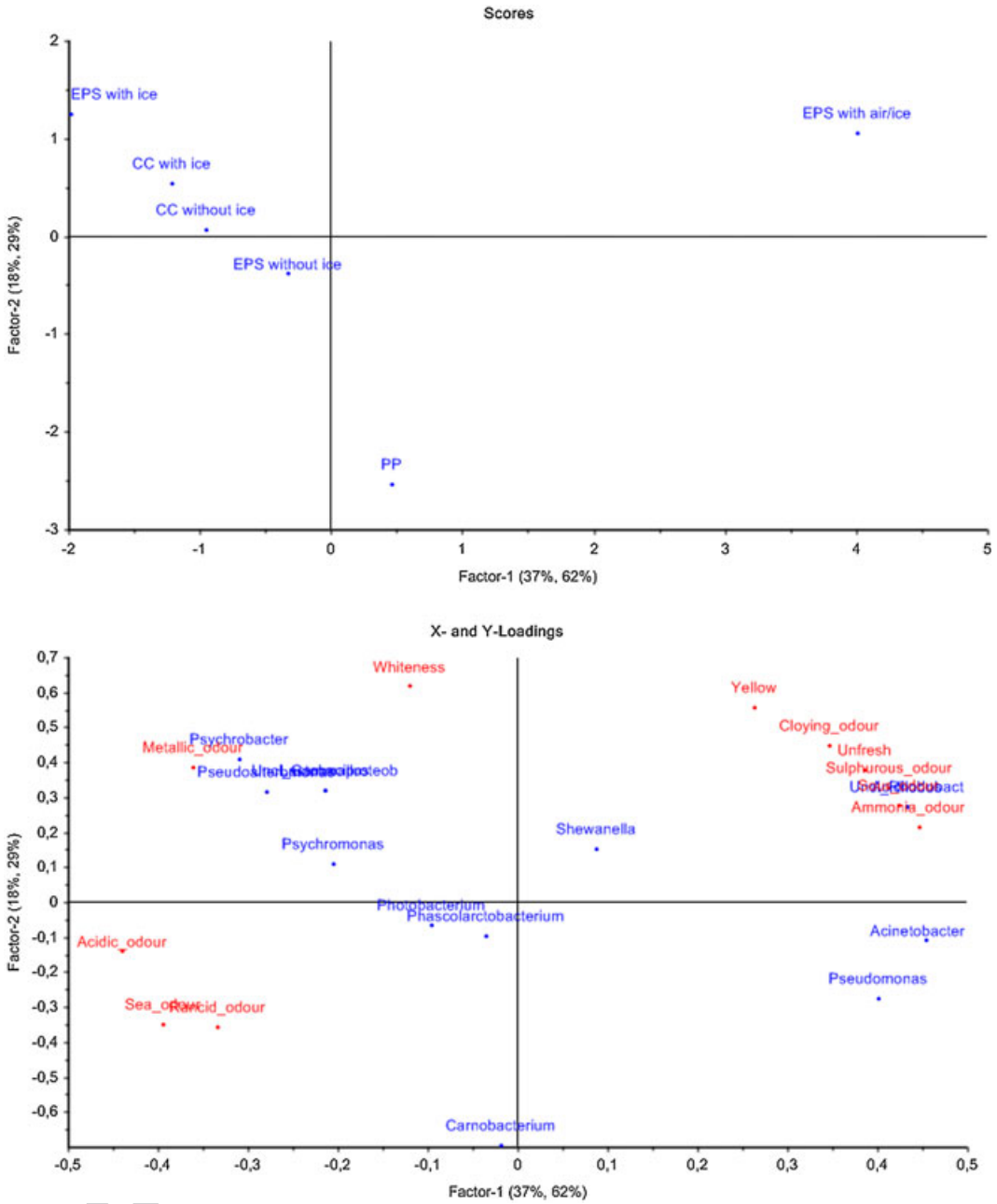

Figure 1. a, b. Score plot (a) and correlation loading plot (b), wiht X = microbial community and Y = odour. Data used are from 10 days after repackaging into MAP (2 °C storage). Q8

Volatile components

TMA is well known to be produced during bacterial metabolism, e.g. by *Photobacterium* and *Shewanella* from the TMAO (trimethyl amine oxide) to TMA (trimethyl amine), that negatively affects quality.[13,31] Still, similar levels of TMA were detected both for the MAP samples and the 'EPS with ice/air' samples (Table 4) even high-oxygen level is reported to reduce the formation of TMA.[7,8,38] T4 'EPS with ice', dominated by *Psychrobacter*, had similar levels of TMA as the 'EPS without ice', which had the highest level of *Photobacterium*. According to Özugul and Özugul[39] both *Psychrobacter* and *Photobacterium* are able to produce TMA. Similarly, *Acinetobacter*, which

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Table 4. Volatile components (μg/g) (mean ± SE, n = 4) of thawed cod loins in different transport packaging solutions and repackaging into MAP (control; air) and further storage (2 °C), isolated 10 days after repackaging (=14 days after thawing). For each row, entries with the same letter attached to them are not significantly different at the 0.05 level according to Tukey's Multiple Comparisons Test.

	EPS with ice/MAP	*EPS without ice/MAP*	*CC with ice/MAP*	*CC without ice/MAP*	*PP[a]/MAP*	*Control (EPS with ice/air)*
Trimethyl amine	0.329 ± 0.468	0.300 ± 0.204	0.180 ± 0.174	0.195 ± 0.140	0.061 ± 0.061	0.414 ± 0.447
Dimethyl disulphide	0.022 ± 0.034	0.006 ± 0.002	0.007 ± 0.003	0.027 ± 0.046	0.075 ± 0.142	1.320 ± 2.447
Dimethyl trisulphide	0.030 ± 0.035	0.010 ± 0.007	0.016 ± 0.008	0.007 ± 0.003	0.064 ± 0.115	0.283 ± 0.456
2-Pentanone	0.005 ± 0.001	0.010 ± 0.003	0.006 ± 0.006	0.006 ± 0.003	0.041 ± 0.072	0.123 ± 0.229
3-methyl-1-butanol	0.020 ± 0.014bc	0.080 ± 0.042a	0.023 ± 0.010bc	0.039 ± 0.026bc	0.011 ± 0.009c	0.074 ± 0.028ab
2-Butanone	0.009 ± 0.005abc	0.009 ± 0.005ab	0.000 ± 0.000c	0.003 ± 0.005bc	n.d.	0.017 ± 0.005a
1-Penten-3-ol	0.049 ± 0.026	0.023 ± 0.011	0.034 ± 0.010	0.021 ± 0.006	0.071 ± 0.094	0.007 ± 0.006

[a]Contains ice inside the lid.
n.d.: not detected.

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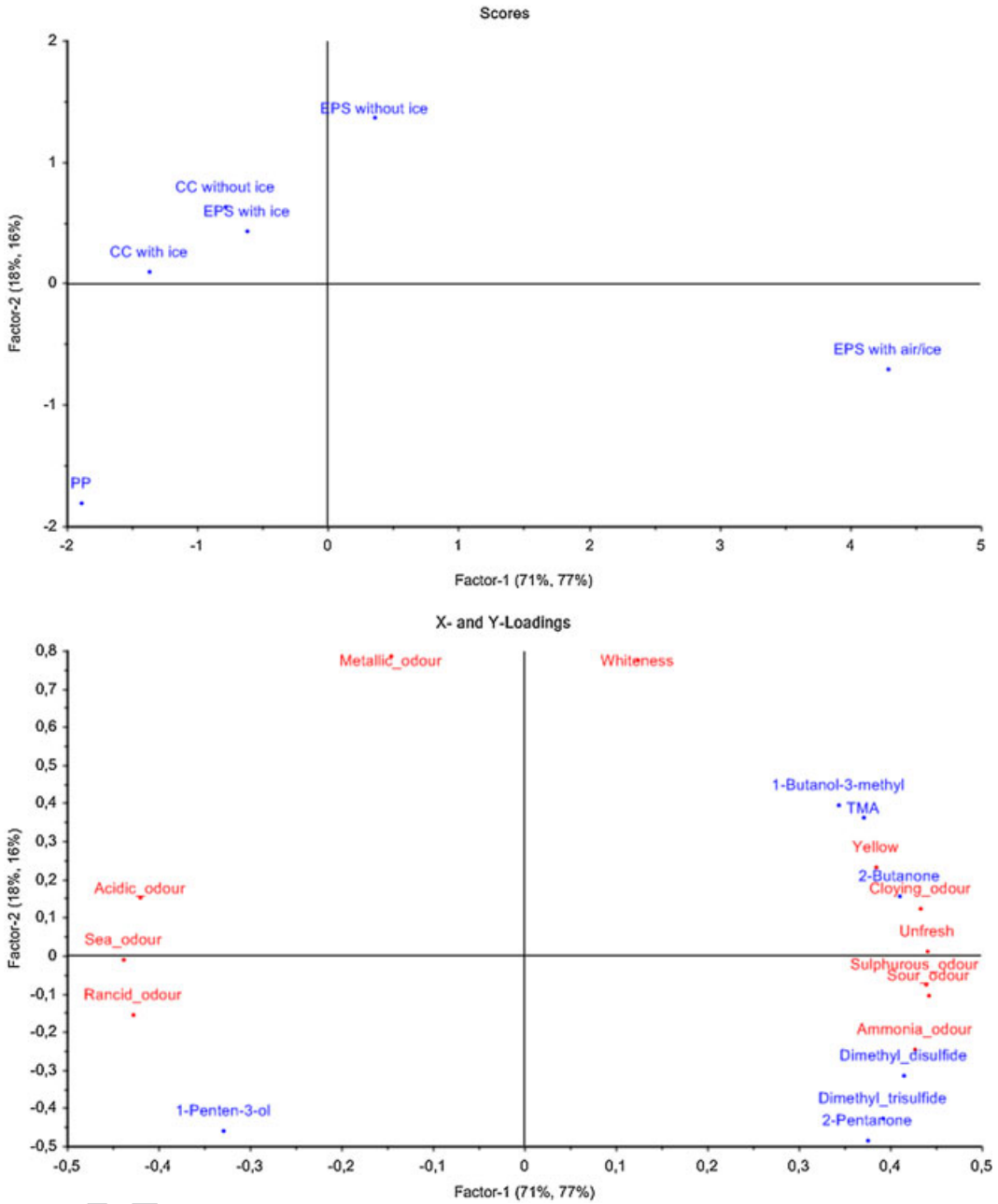

Figure 2. a, b. Score plot (a) and correlation loading plot (b), wiht X = volatile compounds and Y = odour. Data used are from 10 days after repackaging into MAP (2 °C storage).

especially dominated the 'EPS with ice/air' samples, may also produce TMA, according to Barrett and Kwan [*41]

Sulphide producing bacteria (SPB) such as *Shewanella baltica*, *Pseudomonas mephitica* and *Citrobacter freundii* are reported to convert sulphur-containing amino acids from degraded fish muscle proteins into sulphides.[40–42] The components dimethyl disulphide and dimethyl trisulphide, probably developed during bacterial growth and metabolism, were high for the 'EPS with ice/air' samples (Table 4), but did not significantly differ between treatments because of the high standard deviations. The 3-methyl-1-butanol and 2-butanone were significantly highest for the 'EPS with ice/air' samples, probably caused by higher bacterial activity compared to the MAP samples.

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Table 5. Sensory scores (four of the attributes) (1 = low intensity, 9 = high intensity) (mean ± SE, n = 4) of thawed cod loins in different transport packaging solutions and repackaging into MAP (control; air) and further storage (2 °C). For each row, entries with the same letter attached to them are not significantly different at the 0.05 level according to Tukey's Multiple Comparisons Test.

		EPS with ice/MAP	*EPS without ice/MAP*	*CC with ice/MAP*	*CC without ice/MAP*	*PP*[a]*/MAP*	*Control (EPS with ice/air)*
Acidic	Day 3	2.6 ± 0.7	2.6 ± 0.7	2.2 ± 0.5	2.8 ± 0.3	2.1 ± 0.2	2.0 ± 0.5
	Day 7	2.7 ± 0.8a	1.9 ± 0.1ab	2.8 ± 0.3a	2.6 ± 0.3a	2.6 ± 0.4a	1.0 ± 0.0b
	Day 10	2.4 ± 0.7a	1.8 ± 0.4ab	2.3 ± 0.2a	2.3 ± 0.8a	2.2 ± 0.6a	1.0 ± 0.0b
	Day 15	1.5 ± 0.4	1.3 ± 0.4	1.7 ± 1.0	1.6 ± 0.2	1.3 ± 0.2	1.0 ± 0.0
Seawater	Day 3	2.2 ± 0.5	2.2 ± 0.6	2.2 ± 0.5	2.6 ± 0.3	2.0 ± 0.2	1.8 ± 0.2
	Day 7	2.3 ± 0.6a	1.7 ± 0.2ab	2.2 ± 0.4a	2.1 ± 0.3a	2.3 ± 0.3a	1.1 ± 0.1b
	Day 10	2.0 ± 0.6a	1.7 ± 0.5ab	1.9 ± 0.2ab	1.8 ± 0.7ab	2.0 ± 0.5ab	1.0 ± 0.0b
	Day 15	1.5 ± 0.5	1.2 ± 0.2	1.6 ± 0.8	1.5 ± 0.2	1.1 ± 0.1	1.0 ± 0.0
Cloying	Day 3	3.3 ± 0.6	3.3 ± 0.4	3.7 ± 1.1	3.2 ± 0.3	3.5 ± 0.3	3.6 ± 0.5
	Day 7	2.8 ± 0.8b	3.7 ± 0.1b	2.9 ± 0.2b	3.0 ± 0.4b	3.1 ± 0.6b	6.4 ± 0.4a
	Day 10	3.5 ± 1.2b	4.3 ± 0.5b	3.7 ± 0.1b	3.5 ± 0.9b	3.8 ± 0.8b	6.8 ± 0.3a
	Day 15	4.9 ± 0.3	5.7 ± 1.3	5.1 ± 1.7	5.4 ± 0.6	6.2 ± 0.8	7.5 ± 0.2
Sour	Day 3	2.5 ± 0.8	2.7 ± 0.3	3.5 ± 0.8	2.6 ± 0.4	2.9 ± 0.6	3.1 ± 0.4
	Day 7	2.1 ± 0.4b	3.5 ± 0.2b	2.1 ± 0.6b	2.2 ± 0.4b	2.1 ± 0.5b	6.2 ± 0.2a
	Day 10	2.4 ± 0.9b	3.8 ± 1.2b	2.3 ± 0.6b	2.4 ± 1.0b	2.3 ± 0.1b	7.8 ± 0.2a
	Day 15	2.8 ± 0.3	5.9 ± 2.0	3.8 ± 1.5	4.5 ± 0.6	5.6 ± 2.0	8.4 ± 0.4

[a]Contains ice inside the lid.

Figure 2 a, b shows a clustering of volatile components and sensory attributes, showed by correlations between dimethyl disulphide, dimethyl trisulphide, TMA, 3-methyl 1-butanol, 2-butanone, 2-pentanone, and the sulphurous odour, ammonia odour, sour odour and the off-odour, clearly pronounced for the 'EPS with ice/air' samples. This corresponds to what Olafsdottir, Jonsdottir *et al.* [*45] have described as microbial spoilage odours. It also indicates that 'EPS without ice' differed to the 'CC without ice', 'EPS with ice' and the 'CC with ice'. **F2**

Sensory quality

There were no differences between the MAP samples during storage for the sensory attributes (Table 5), despite higher bacterial count numbers for the samples transported without ice prior repackaging into MAP. No differences might also be caused by relatively high individual variations within treatments. Still, there were indications of lower intensities of negatively associated attributes (like the cloying and sour odour), and higher intensities of the positively associated once (acidic and seawater odour) for the 'EPS with ice' compared to the 'EPS without ice'. This corresponds to a loss of freshness affected by temperature conditions during transport and the first period of storage, reported by Lauzon and others.[38] **T5**

The MAP samples, which contained the antibacterial CO_2 gas, preserved quality better than storage in air, as shown in previous studies.[6,43] Because of quite stable sensory scores during storage, the sensory shelf life for the 'EPS with ice' seemed to be about 15 days (Table 5), but according to high levels of standard error, not significantly different to the other treatments of MAP.

'EPS with ice/air' samples had highest intensity of the negatively associated attributes detected from day 7 (cloying and sour) and day 10 (sulphur, ammonia and unfresh odour). The intensity scores were at these time points, doubled compared to day 3, and end of sensory shelf life for 'EPS with ice/air' was probably reached after three days of storage (7 days after thawing), which corresponds to the microbiological shelf life.

The yellowness changed during storage, but only for the 'EPS with ice/air', which had the highest intensity of yellowness after 10 days of storage (14 days after thawing), which correspond to earlier findings.[44]

pH

The pH levels found for the different samples corresponded to earlier reported studies on Atlantic cod caught by long line.[45] The pH for the different treatments varied between 6.6 and 6.0 after three days

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Table 6. Liquid loss (mean ± SE, n = 4) of thawed cod loins in different transport packaging solutions and repackaging into MAP (control; air) and further storage (2 °C). For each row, entries with the same letter attached to them are not significantly different at the 0.05 level according to Tukey's Multiple Comparisons Test.

	EPS with ice/MAP	*EPS without ice/MAP*	*CC with ice/MAP*	*CC without ice/MAP*	*PP*[a]*/MAP*	*Control (EPS with ice/air)*
Day 3	5.1 ± 0.5ab	5.0 ± 0.3ab	6.0 ± 0.3a	4.6 ± 0.5bc	5.4 ± 0.4c	3.7 ± 1.0c
Day 7	5.7 ± 0.4bc	5.5 ± 0.4bc	6.4 ± 0.1a	5.3 ± 0.3 cd	6.1 ± 0.2ab	4.8 ± 0.1d
Day 10	5.7 ± 0.2ab	6.0 ± 0.5a	6.5 ± 0.4a	5.8 ± 0.3a	6.5 ± 0.3a	4.7 ± 0.9b
Day 15	6.0 ± 0.2bc	5.9 ± 0.1bc	7.2 ± 0.6a	6.0 ± 0.1b	6.3 ± 0.1b	5.2 ± 0.6c

[a]Contains ice inside the lid.

of storage. The MAP samples had relative stable pH during storage. The 'EPS with ice/air' samples had higher pH values compared to MAP, probably related to bacterial activity and the denaturation of proteins (detected at Day 10 and Day 15). Some results show reduced pH when CO_2 presented as HCO_3^- is dissolved into the fish muscle.[21] However, this seems not to be consistent, as also no effect on pH is reported.[8,46]

Liquid loss

Some of the MAP samples showed higher levels of liquid loss compared to the samples stored in air (Table 6). This might be because of vacuum effect, as a result of CO_2 absorption into the product. Such **T6** vacuum effect is also reported for MAP chicken,[47] and increased liquid loss is also reported for wolffish packaged in modified atmosphere compared to storage in air.[2] Some of the liquid loss might also have taken place during the transport and temporary storage prior repackaging into MAP. A slight increase in liquid loss during storage was found, which corresponds to prior studies.[48,49] Liquid loss can affect sensory quality (juiciness and hardness). However, this was not investigated in the presented study.

CONCLUSIONS

The presented study shows that both corrugated cardboard and polypropylene boxes preserve quality of the fish product as good as expanded polystyrene. The sensory attributes normally disliked by consumers even indicated longer shelf life for the 'CC without ice' compared to 'EPS without ice', probably caused by differences in time of temperature reduction from 4 °C to 0 °C during transport and temporary storage. *Psychrobacter* seemed to be a dominating bacterium detected after ten days in modified atmosphere conditions (CO_2/O_2), especially for the 'EPS with ice', which seemed to positively affect time of shelf life. Only one treatment of MAP had presence of *Photobacterium* of significance. *Pseudomonas* and *Acinetobacter* were probably the quality limiting bacteria of the samples stored in air, resulting in only three days of shelf life. Because of the microbiota detected, it is especially for the 'EPS with ice' reasonable to assume that time of shelf life was almost 15 days because of relatively stable sensory scores during storage. The samples transported without ice reached end of shelf life at about 7 days after repackaging into MAP, regardless of type of packaging materials.

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