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Effect of cooling and packaging methods on the quality deterioration of redfish fillets

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Report summary

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Titill / Title	**Effect of cooling and packaging methods on the quality deterioration of redfish fillets**		
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Ágrip á íslensku:	Markmið tilraunarinnar var að meta áhrif krapaískælingar eftir flökun og/eða pökkun í lofttæmdar umbúðir á gæðarýrnun ferskra karfaflaka. Flökin voru geymd við -1 °C í 6 daga til að herma eftir vel útfærðum sjóflutningi í frauðplastkössum og svo við 2 °C líkt og gerist eftir afhendingu erlendis og geymslu í smásölu. Fylgst var með vöru- og umhverfishitastigi frá pökkun og framkvæmt skynmat, örveru- og efnamælingar. Fiskurinn var veiddur að vorlagi og unninn 6 dögum eftir veiði. Niðurstöður sýna að gæði hráefnisins voru ekki sem best við pökkun þar sem þránunarferli (PV og TBARS) var komið vel af stað. Þetta skýrir væntanlega hvers vegna engin af þessum kæliaðferðum leiddi til geymsluþolsaukningar. Einnig kom í ljós að enginn ávinningur fékkst við að kæla flökin óvarin í krapaís þar sem örveruvöxtur og myndun TVB-N og TMA í flökunum gerðist hraðar við frekari geymslu. Hins vegar virðist vera ákjósanlegra að kæla lofttæmd pökkuð flök í krapaís því þessi aðferð leiddi til hægari vaxtar skemmdarörvera, lægra magns TMA og hægara þránunarferlis. *Photobacterium phosphoreum* er mikilvæg í skemmdarferli ferskra karfaflaka, óháð pökkunaraðferð.		
Lykilorð á íslensku:	*Karfaflök – Vakúm pökkun - Undirkæling – Krapaís - Gæðarýrnun - Geymsluþol – Skemmdarörverur – Þránun*		

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Summary in English:	The aim of this study was to evaluate the effect of slurry ice cooling in process (post-filleting) and packaging method (+/- oxygen) on the quality deterioration of skinned redfish fillets during storage in expanded polystyrene boxes simulating well-performed sea freight transportation (6 days at -1 °C) followed by storage at the retailer (2 °C). Also, to assess the use of vacuum-packaging to protect the fillets from direct contact with the cooling medium (slurry ice) and to achieve superchilling following extended treatment. Temperature monitoring as well as sensory, chemical and microbial analyses were performed. The fish was caught in the spring and processed 6 days post catch. The results show that quality of the fillets was not optimal at packaging, due to the detection of primary and secondary oxidation products. This may have been the reason why shelf life extension was not achieved by any of the methods evaluated. Further, there was no advantage of cooling the fillets unpacked since this method stimulated microbial growth and formation of basic amines. On the other hand, slurry ice cooling of vacuum-packaged fillets led to a slower microbial development, the lowest TMA level and delayed autoxidation. Finally, the importance of *Photobacterium phosphoreum* in the spoilage process of redfish fillets, independently of the packaging method, was demonstrated.
English keywords:	*Redfish fillets – Vacuum packaging - Superchilling – Slurry ice - Quality deterioration - Shelf life – Microbial spoilage – Oxidation*

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1. Aim of storage trial

The aim of the storage trial was to evaluate the effect of liquid cooling in process (post-filleting) and packaging method (+/- oxygen) on the quality deterioration of skinned redfish fillets during storage in expanded polystyrene (EPS) boxes simulating well temperature-controlled sea freight transportation (6 days at -1 °C) followed by storage at the retailer (2 °C). Also, to assess the use of vacuum-packaging to protect the fillets from direct contact with the cooling medium (slurry ice) and to achieve superchilling following extended treatment.

2. Experimental design

2.1 Fish processing, packaging and post-packaging treatments

Redfish (*Sebastes marinus*) was caught in May 2010 by the fishing vessel Sturlaugur H. Böðvarsson. The fish was bled, rinsed, iced in tubs and stored chilled until processed 6 days later at HB Grandi (Reykjavík, Iceland). At the processing plant, the following steps were undergone: beheading, filleting, skinning, and thereafter about half of the batch was cooled in slurry ice (around -1 °C, 1-1.5% NaCl) before packaging in EPS boxes (inner size: 35.6x21.6x6.5 cm). The different treatments evaluated are listed in Table 1. Samples from treatments AE and BE (4 boxes of 3 kg fillets, each) were received at Matis at 10 am and placed in the storage chamber (-1 °C). Fillets to be vacuum-packed (VP) were received in 5 kg boxes, A-fillets (4.4-5.4 °C) and B-fillets (1.5-2.5 °C). Fillets (1.5 kg) were inserted in vacuum bags (coextruded PA/PE, 250x400x0.12 mm; Plastprent, Reykjavík), vacuumed and sealed, after which liquid cooling was applied for 40 min (AV) or 25 min (BV) before placing two bags in an EPS box. Experimental time was set to 11 am (time 0) for graphical presentation.

Table 1. Definition of treatments evaluated

Treatment code	In process fillet treatment	Packaging	Post-packaging treatment	Storage temperature during 12-day period
AE	no cooling, A	3-kg EPS	none	-1 °C (6d); 2 °C
AV	no cooling, A	1.5-kg VP, 2 bags per EPS box	40-min liquid cooling	-1 °C (6d); 2 °C
BE	liquid cooling, B	3-kg EPS	none	-1 °C (6d); 2 °C
BV	liquid cooling, B	1.5-kg VP, 2 bags per EPS box	25-min liquid cooling	-1 °C (6d); 2 °C

VP, vacuum-packaged

To monitor the product temperature from packaging, temperature loggers (iButton DS1922L, Maxim Integrated Products Inc, USA) were aseptically inserted at three positions (bottom corner inside package, bottom centre underneath the fish bulk, and centre top fillet) in one EPS box for AE and BE treatments. For AV and BV treatments, two loggers (bag corner and centre) were inserted during packaging. This means that 2 loggers were monitoring the bottom and upper corner positions in the box, while two other loggers measured the fish temperature at the bottom and top centres. The ambient temperature was similarly recorded with two loggers positioned on two sides of the selected packages. The logger accuracy was of ±0.5 °C and its resolution of 0.0625 °C. Temperature was recorded every 5 min. In addition, temperature was monitored in the cooling medium used post-packaging at Matís. Two loggers were sinked to lower and upper positions in the slurry ice, mapping one half of the tank for A-fillets and the other one for B-fillets. A fifth logger was placed in the centre of the tub at a lower position.

2.2 Storage at Matís

This study included four test groups (Table 2), two of them aerobically stored in EPS boxes (AE and BE) and two others vacuum-packaged with two bags stored in an EPS box (AV and BV). The boxes were stacked per test group in the storage chamber, with each stack placed on an empty EPS box to avoid direct contact of the filled boxes with the floor. The chamber temperature was set to -1 °C for 6 days, followed by storage at 2 °C for the rest of the trial.

3. Analysis of sensory, microbiological and chemical parameters

3.1 Materials and Methods

3.1.1 **Sampling**

Samples were obtained in duplicate for each group from the upper and lower sections of each box, selecting four fillets from each section for microbiological and chemical analyses (total of 8 fillets per treatment). The rest of loins (about 12) was used for sensory analysis. Regular sampling was performed as described in Table 2.

Table 2. Sample groups, treatments, storage conditions and sampling days

Sample name	Treatment	Storage	Sampling days*
A-raw material	No cooling		0
B-raw material	Liquid cooling in process		0
AE	No cooling	EPS boxes	6, 9, 12
AV	Liquid cooling after VP	VP, 2 bags in EPS boxes	6, 9, 12
BE	Liquid cooling in process	EPS boxes	6, 9, 12
BV	Liquid cooling before and after VP	VP, 2 bags in EPS boxes	6, 9, 12

* Days post-packaging; EPS, expanded polystyrene; VP, vacuum-packaging.

3.1.2 **Sensory evaluation**

Generic Descriptive Analysis (DA), introduced by Stone and Sidel (2004), was used to assess cooked samples of four sample groups of fresh redfish fillets (Table 2). Evaluation using the Torry scheme (Shewan *et al*., 1953) was performed simultaneously. Eight panellists, all trained according to international standards (ISO 1993) including detection and recognition of tastes and odours, trained in the use of scales and in the development and use of descriptors, participated in the sensory evaluation. The members of the panel were familiar and experienced in using the DA method and Torry freshness score sheet. Two training sessions took place prior to the storage trial. The panel was trained in recognition of sensory characteristics of the samples, 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 but for this experiment, only attributes describing spoilage and texture were selected. Fourteen sensory attributes were evaluated as described in Table 3.

Samples weighing ca 40 g were taken from the loin part of the fillets and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 5 min 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 treatment in a random order. A computerised system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording.

Table 3. Sensory attributes for cooked redfish and their description

Sensory attribute	Short name	Description of attribute
Odour		
rancid	o-rancid	Rancid odour
table cloth	o-cloth	Reminds of a table cloth (unclean, damp cloth to clean kitchen table, left for 36 h)
TMA	o-TMA	TMA odour, reminds of dried salted fish, amine
sour	o-sour	Sour odour, sour milk, spoilage sour, acetic acid
sulphur	o-sulphur	Sulphur, matchstick, boiled kale
Flavour		
rancid	f-rancid	Rancid flavour
pungent	f-pungent	Pungent flavour, bitter
sour	f-sour	Sour taste, spoilage sour
TMA	f-TMA	TMA flavour, reminds of dried salted fish, amine
off-flavour	f-off	Strength of off-flavour (spoilage flavour/off-flavour)
Texture		
soft	t-soft	Left end: firm. Right end: soft. Evaluate how firm or soft the fish is during the first bite
juicy	t-juicy	Left end: dry. Right end: juicy. Evaluated after chewing several times: dry - draws juice from the mouth
meaty mouthfeel	t-meaty	Meaty texture, meaty mouthfeel, crude muscle fibers
tender	t-tender	Left end: tough. Right end: tender. Evaluated after chewing several times

3.1.3 **Microbiological analysis**

Fish samples were aseptically minced, assessing two pooled fillets per sample. Two replicate samples were evaluated for each group. Minced flesh (20 g) was mixed with 180 g of chilled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required. Total viable psychrotrophic counts (TVC) were performed 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. Counts of H_2S-producing bacteria (black colonies) were evaluated on IA. Plates were spread-plated and incubated at 17 °C for 5 days. Enumeration of presumptive pseudomonads was performed using modified Cephaloridine Fucidin Cetrimide (mCFC) agar as described by Stanbridge and Board (1994). *Pseudomonas* Agar Base (Oxoid, UK) with CFC selective Agar Supplement (Oxoid) was used and the plates were incubated at 22 °C for 3 days. Mean bacterial numbers are presented as $\log_{10}$ numbers of colony-forming units (cfu) g^{-1} fish.

Estimation of *Photobacterium phosphoreum* (*Pp*) counts was achieved by a quantitative Polymerase Chain Reaction (qPCR) method developed at Matís (E. Reynisson, unpublished). Briefly, 10 ml of the 10-fold diluted fish sample in MRD buffer was frozen at -20 °C for later DNA extraction. For the DNA extraction, the diluted samples were centrifuged at 11000 x g for 7 min to form a pellet. The supernatant was discarded and DNA was recovered from the pellet using the promeganesil KF, Genomic system (MD1460) DNA isolation kit (Promega Corporation, Madison, USA) in combination with King Fisher magnetic beads automatic DNA isolation instrument (Thermo Lab systems, Waltham, USA) according to the manufacturers' recommendations. All PCR reactions were done using the MX 3005p instrument. The PCR was done using Brilliant qPCR master mix (Stratagene, La Jolla, CA, USA). Primers were synthesised and purified with HPLC (MWG, Ebersberg, Germany). The DNA standard used for quantification of *P. phosphoreum* was previously calibrated against the PPDM-Malthus conductance method (Dalgaard *et al*., 1996; Lauzon, 2003) using fish samples from storage trials.

3.1.4 **Chemical analysis: Total Volatile Base Nitrogen (TVB-N), trimethylamine (TMA), pH and salt content**

All chemical analyses were performed in duplicate. The method of Malle and Tao (1987) was used for total volatile bases (TVB-N) and trimethylamine (TMA) measurements in the previously prepared mince. TVB-N was measured by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen) and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic acid (TCA) solution. The distilled TVB-N was collected in boric acid solution and titrated with sulphuric acid solution. TMA was measured in TCA extract by adding 20 ml of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine. The pH was measured in 5 g of minced loins mixed with 5 ml of deionised water using the Radiometer PHM 80. The pH meter was calibrated using the buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25 °C). Salt content was evaluated from two minced fillets for A- and B-raw material using the Volhard titration method (AOAC 976.18, 2000).

3.1.5 **Analysis of total lipids, lipid hydrolysis and oxidation**

Lipid analysis was performed using two loins for each sample, analysing duplicate samples for each treatment. Total lipids (TL) were extracted from 25 g samples with methanol/chloroform/0.88% KCL (1/1/0.5, v/v/v) according to the Bligh and Dyer (1959) method. The lipid content was determined gravimetrically and the results were expressed as

grams lipids per 100 g wet muscle. Free fatty acids (FFA) were determined on the TL extract according to Lowry and Tinsley (1976), with modifications from Bernardez *et al.* (2005). FFA concentration was calculated as micromolar oleic acid based on a standard curve spanning a 2-22 µmol range. Results are expressed as grams FFA per 100 g of lipids.

Assessment of primary lipid oxidation by the determination of the lipid hydroperoxide value (PV) with a modified version of the ferric thiocyanate method (Santha and Decker Eric, 1994) was performed. The results are expressed as mmol lipid hydroperoxides per kg of sample. A modified method of Lemon (1975) was used for measuring thiobarbituric acid reactive substances (TBARS, representing secondary lipid oxidation compounds). A sample (5.0 g) was homogenised with 5.0 ml of TCA extraction solution (7.5% TCA, 0.1% propyl gallate and 0.1% ethylene diamine tetraacetic acid (EDTA) mixture prepared in ultra pure water) using a homogeniser (Ultra-Turrax T-10 basic, IKA, Germany) at maximum speed for 10 s. The homogenised samples were then completed with 5.0 ml TCA extraction solution and centrifuged at 9400 *x g* for 15 min (Model Z323K, Hermle laboratories, Germany). The supernatant (0.5 ml) was collected and mixed with the same volume (0.5 ml) of thiobarbituric acid (0.02 M) and heated in a water bath at 95 °C for 40 min. The samples were cooled down on ice and immediately loaded into 96-wells microplates (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for reading at 530 nm (POLARstar OPTIMA, BMG Labtech, Offenburg, Germany). A standard curve was prepared using tetraethoxypropane. The results are expressed as µmol of malonaldehyde diethylacetal (MDA) per kg of sample.

Tertiary oxidation compounds were investigated by measuring the formation of interaction compounds between primary and secondary lipid oxidation products and nucleophilic molecules (protein-like) present in the fish muscle. The formation of interaction compounds was assessed by the fluorescence ratio. Fluorescence measurements (Perkin Elmer LS 50B) were made at 393/463 and 327/415 nm excitation/emission maxima, according to other researchers (Aubourg *et al*., 1997; Aubourg *et al*., 1998; Aubourg, 1999a,b; Aubourg, 2001). The excitation and emission slit was set at 2.5 nm. The relative fluorescence (RF) was calculated as $RF=F/F_{st}$, where F is the sample fluorescence intensity at each excitation/emission maximum and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/ml in 0.05M H_2SO_4) at the corresponding wavelength. The fluorescence shift (δF) was calculated as the ratio between the two RF values, i.e. $\delta F = RF_{393/463nm} / RF_{327/415nm}$, and was analysed on the organic phase (δF_{or}) resulting from the lipid extraction.

3.1.6 **Data analysis**

The program NCSS 2000 (NCSS, Utah, USA) was used to perform statistical analysis. The general linear model method was used to compare attribute scores from DA method between sample groups and to correct for panellists' effect. The program calculates multiple comparisons using Duncan's multiple comparison test. Analysis of variance (one-way ANOVA) was carried out on the microbial and chemical data. Comparison of data with respect to treatments was performed using the Duncan's multiple comparison test. The significance level was set at 5%.

3.2 Results and Discussion

3.2.1 **Temperature monitoring**

Temperature was monitored at Matís during liquid cooling of VP fish in the cooling tub and during storage. Figure 1 shows how the temperature of the cooling medium (slurry ice) changed in the lower part of the tub upon addition of the 6 AV bags (A-lower tub), increasing from -0.6 to 0.2 °C in the lower part of the tub in 40 min. A similar trend was observed upon addition of the 6 BV bags (B-lower tub), going from -0.8 to 0.2 °C during the first 15 min after which AV bags were removed, resulting in a modified temperature profile due to ice movement. Loggers positioned in the upper part of the tub showed the temperature stability of slurry ice in this area. Mixing of slurry ice would have contributed to a better temperature distribution in the tub.

The ambient and product temperatures of the four groups were monitored during storage. Table 4 summarises the mean product and ambient temperatures recorded during the 12-day period as well as other relevant details, whereas Figure 2 illustrates the temperature profiles taking place. Temperature of fillets not cooled in process (AE) was about 3 °C compared to 0.5 °C for those liquid-cooled in process (BE). Initial temperature of vacuum-packed fillets was slightly higher (T_{AV} = 4.5 °C; T_{BV} = 1.8 °C) probably due to delay in handling at room temperature during preparation and packaging. Slurry ice cooling was done post-packaging and rapidly decreased fish temperature down to 0 °C, slightly faster though for BV than AV due to initial temperature differences (Table 4). Fish stored in bulk in EPS boxes (AE and BE) took longer to reach 0 °C. Superchilled storage condition (-1 °C for 6 days) further decreased fish temperature, reaching the lowest temperature of -0.1 °C for AE, but -0.9 to -0.8 °C for the other treatments.

On day 6, the chamber temperature was increased to 2 °C to simulate chilled storage at a European retailer. Fish temperature started to rise and had reached 0 °C after 6.2 to 6.8 days post packaging/stockage. AE and BE had reached 1 °C earlier than the vacuum-packed treatments (about 0.6 d). Overall, it was found that the average product temperature was higher for AE than the other treatments. Cooling of AV fish in slurry ice post-packaging resulted in a lower temperature profile than BE fish, but the temperature profile of BV fish was similar to that of AV fish. In other words, there was not apparent advantage of cooling the fish unpacked if it can be done post-packaging to reduce the product temperature as desired. The effect of vacuum-packaging on the quality deterioration of the fish during extended storage is reported in the following sections.

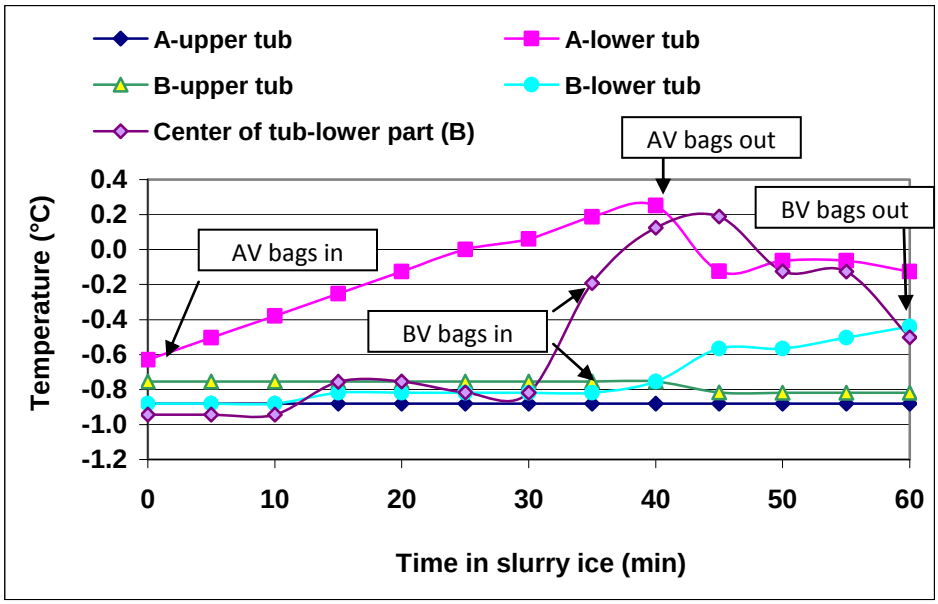

Figure 1. Temperature of slurry ice (SI) in the 460-L tub during cooling of AV and BV treatments. A-upper tub refers to the area where AV bags were cooled and the logger position in SI. The tub had been stored at room temperature for almost 2 h when the cooling treatment ended.

Table 4. Details relating to the temperature profile of the different redfish treatments

Treatment	$T_{initial}$ (°C)	T_{env} (°C)	T_{fish} (°C)	Time to 0 °C (day)	T_{min} (°C)	Time to 1 °C (days)	Time to T_{env} (days)
AE	2.9-3.1	0.4 ± 1.5	0.7 ± 0.9	0.63-0.95	-0.1	8.8	9.9
AV	4.5	0.5 ± 1.5	0.2 ± 1.0	0.01-0.04	-0.9	9.4^{U}-9.6^{L}	10.1
BE	0.5	0.6 ± 1.5	0.3 ± 1.1	0.14	-0.8	8.7	10.1
BV	1.8	0.6 ± 1.5	0.2 ± 1.0	0.007	-0.9	9.3	10.8

$T_{initial}$, temperature of the fillets as recorded early storage; T_{env}, mean environmental temperature; T_{fish}, mean product temperature; T_{min}, lowest product temperature recorded; U, upper position in box; L, lower position in box.

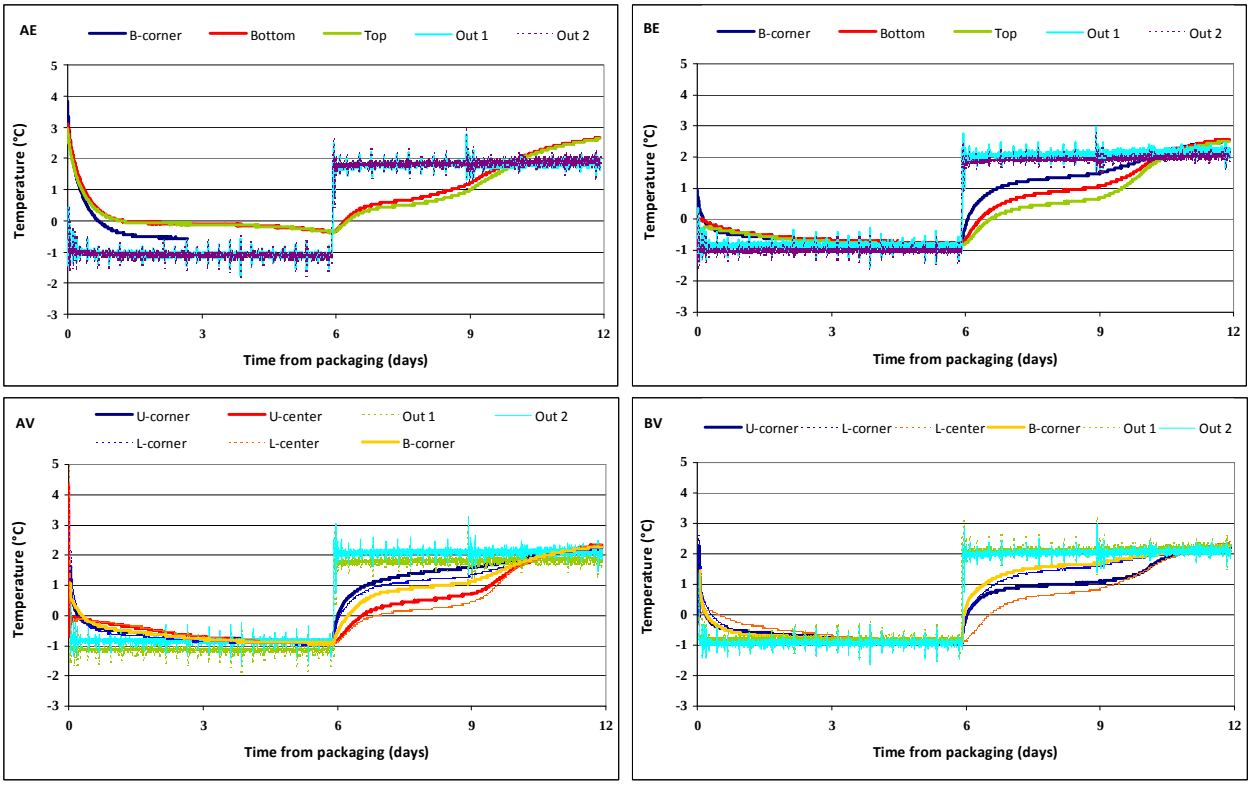

Figure 2. Temperature profile monitored for each treatment (AE, AV, BE and BV) during the 12-day storage period. Product (bottom corner, bottom and top centres) and environmental (out 1 and 2) temperature shown.

3.2.2 **Sensory evaluation**

Sensory evaluation of cooked redfish was performed by two methods; Torry freshness assessment and DA method describing several attributes relating to odour, flavour and texture. The mean Torry scores are shown in Figure 3. On each sampling day there was no significant difference between the treatments (p>0.05). The mean Torry score of 5.5 has been used to indicate end of shelf life and Figure 3 shows that the shelf life could be estimated to about 10 days. After 12 days of storage, all treatments had passed this borderline. Freshness loss is generally characterised by a Torry score of 7 (out of 10, see Appendix I), which is observed to occur at about day 5 and 6 for B- and A-fillets, respectively.

The mean DA scores for the spoilage odours during storage time are shown in Figure 4. The mean values for all attributes and p-values for differences between treatments are shown in Appendix II. Each day is treated as a separate dataset. No significant differences were found on day 0. More meaty mouthfeel was detected in treatments AE and AV than BE on day 6 but the difference was small. Treatment AV received higher scores for table cloth and TMA odours on day 12 than the other treatments. AV also received a significantly higher score for sulphur odour than AE. The DA results for spoilage attributes were consistent with results

from the Torry evaluation, indicating that the shelf life of the fillets was around 10 days for all treatments. Table 5 summarises the freshness and shelf life data intrapolated from the Torry curve for each group.

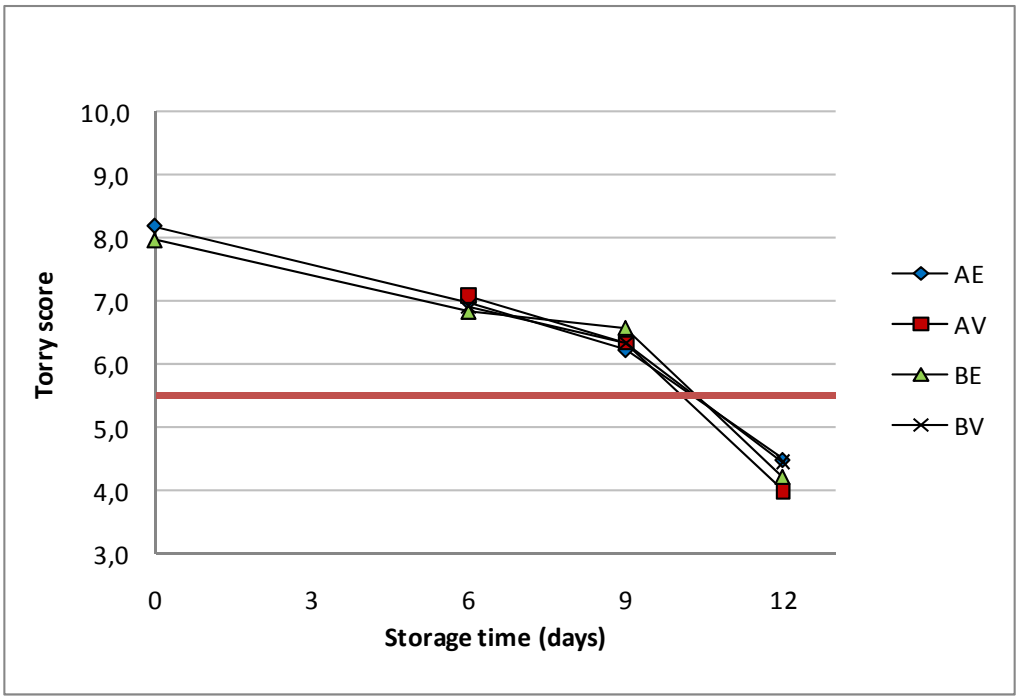

Figure 3. Mean Torry freshness scores for cooked redfish fillets during storage

Table 5. Estimation of the freshness period and shelf life (in days) of redfish fillets based on Torry scores

Treatment	Product temperature (± SD °C)	Freshness period (days)	Shelf life (days)
AE	0.7 ± 0.9	6	10-10.5
AV	0.2 ± 1.0	6	10-10.5
BE	0.3 ± 1.1	5	10-10.5
BV	0.2 ± 1.0	5	10-10.5

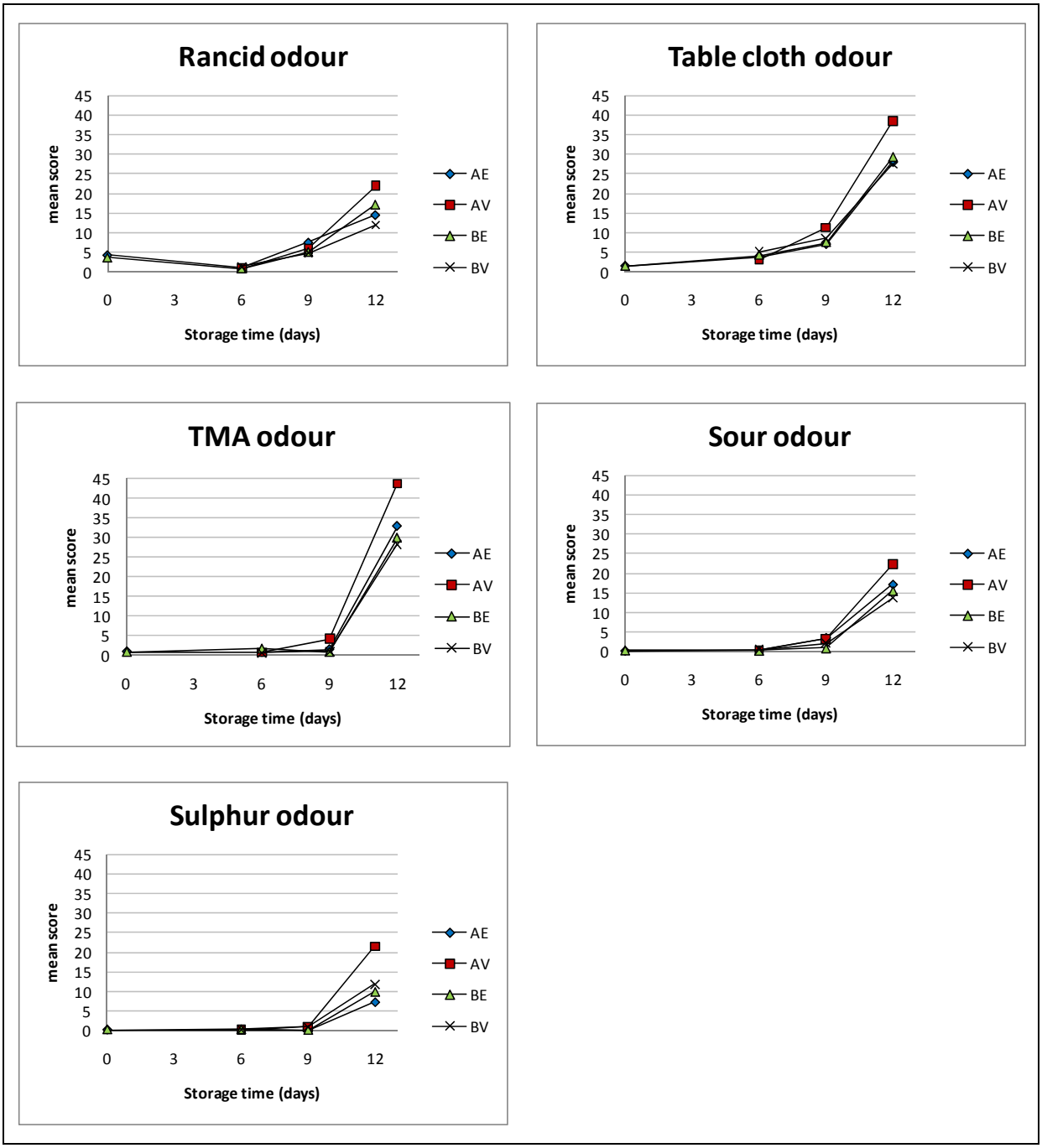

Figure 4. Changes in cooked redfish samples with storage time in mean DA scores for rancid, table cloth, TMA, sour and sulphur odours

3.2.3 **Microbiological analysis**

Three bacterial groups, *Photobacterium phosphoreum*, *Shewanella putrefaciens* (an H_2S-producing bacterium) and pseudomonads, are considered as important spoilage bacteria (named hereafter as specific spoilage organisms, SSO) in fresh, coldwater marine fish stored aerobically (Olafsdottir *et al*., 2006a,b; Reynisson *et al*., 2010), while the growth rate of pseudomonads is much reduced under low oxygen tension. Few studies have reported on

the proliferation of these bacteria in redfish fillets. Figure 5 presents the microbial growth in the different redfish treatments as storage time progressed.

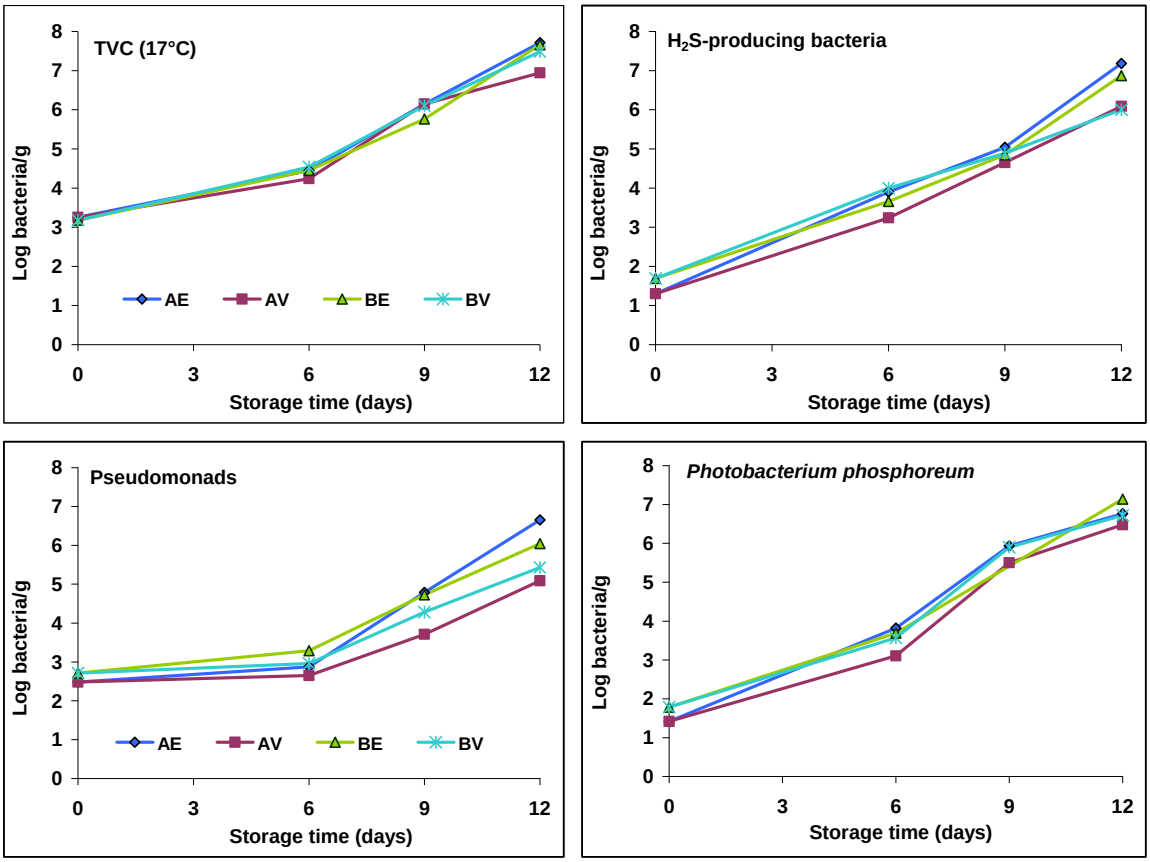

Figure 5. Total viable psychrotrophic counts (TVC-IA) and counts of H_2S-producing bacteria, pseudomonads and *P. phosphoreum* (Pp) in differently treated redfish groups (mean shown, n=2).

Microbiological quality (TVC, 1550 to 1800 cfu g^{-1}) of the fillets was similar for uncooled (A) and cooled (B) raw material, which is satisfactory for a 6-day old fish. However, slightly lower levels of spoilage bacteria were found on uncooled fillets, but this difference was insignificant (*p>0.05*). Liquid cooling of unprotected fillets is known to readily contaminate the fillets if the cooling medium is not properly cooled and renewed during process. Generally, spoilage bacteria are found on fish skin and upon processing, contamination of the fillets distributes them all over the processing line. Pseudomonads were dominating among the SSO investigated, accounting for 17% (A-fish) and 34% (B-fish) of the overall cultivable microbiota (TVC) on the processing/packaging day.

Statistical analysis of microbial data indicated that there was generally no significant difference among the treatments evaluated during the first 9 days of storage (see Appendix III, Table 9). Despite the lower initial levels of *P. phosphoreum* (*Pp*) and H_2S-producing bacteria at packaging, they proliferated at a faster rate than pseudomonads at superchilled

storage. From day 6, following the temperature rise by 3 °C, rapid microbial growth occurred for *Pp*. This coincides well with previous knowledge on the influence of temperature on *Pp* proliferation, being generally the most sensitive to superchilling conditions and the most responsive to increasing temperature among the SSO evaluated (Olafsdottir *et al*., 2006b). On day 9, pseudomonads and H_2S-producing bacteria had reached similar levels in air-stored fish (AE and BE), while *Pp* load was tenfold higher. On the other hand, pseudomonads grew more slowly in vacuum-packed fish (AV and BV). This could be expected since pseudomonads require oxygen for growth. H_2S-producing bacteria (especially *Shewanella putrefaciens*) and *Pp* tolerate well low oxygen tension and are able to produce TMA from TMAO at a faster rate under such condition. The packaging method had the least influence on *Pp* growth since similar growth curves were observed for all treatments, with a slightly lower development for AV treatment. Overall, the results show that a slower microbial development took place in AV fish compared to the other treatments. This is probably due both to the lower contamination with spoilage bacteria initially and the lowest temperature profile up to day 10. The dominance of *Pp* at incipient spoilage (d9), independently of the treatment applied, is also emphasised.

3.2.4 **Chemical analysis: TVB-N, TMA, pH and salt content**

Changes in TVB-N and TMA content as well as pH in minced fish were evaluated throughout storage as shown in Figure 6 and Figure 7, respectively.

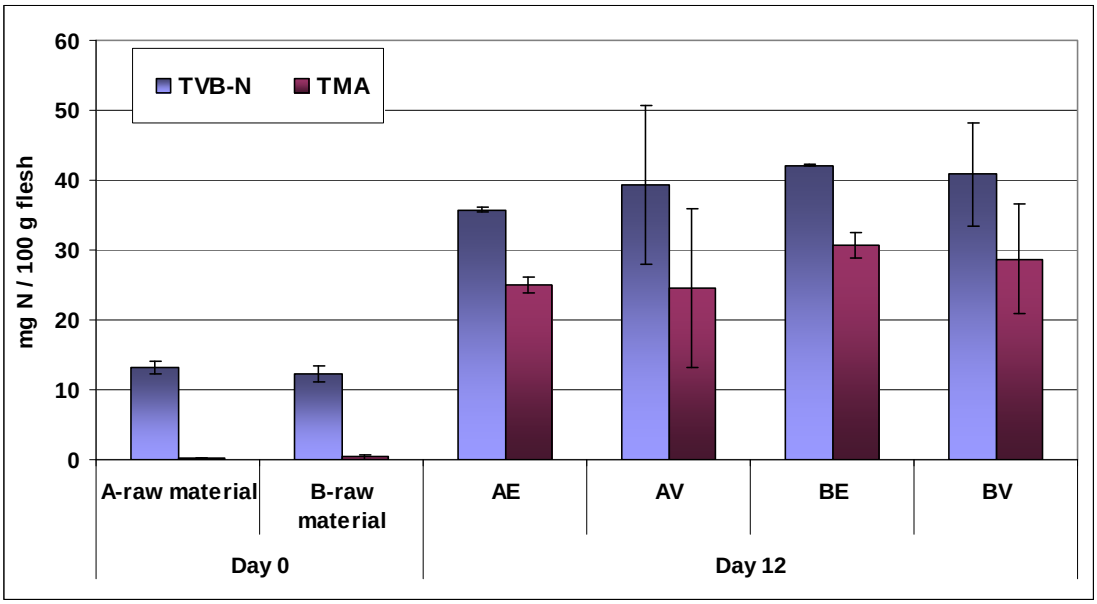

Figure 6. Total Volatile Base Nitrogen (TVB-N) and trimethylamine (TMA) content in differently treated redfish groups (mean ± SD, n=2)

Salt content of fillets was 0.2 ± 0.0% in A-fish but 0.3 ± 0.0% in B-fish. Similar TVB-N and TMA levels were detected in the differently treated fillets on the last sampling day, but generally slightly lower levels were observed in A-fish (*p>0.05***)**. This agrees with the observed counts of *Pp* which is an important TMA producer (Dalgaard, 1995). Interestingly, vacuum-packaging did not significantly increase TVB-N and TMA production in fillets, perhaps due to the low product temperature in these groups. The pH was initially measured as 6.5, but on day 12 it had raised to just below a value of 7 and being slightly lower in vacuum-packed fish. According to EU regulations (EC No 2074/2005), the consumption limit for TVB-N in fillets of S*ebastes* spp. is 25 mg N/ 100 g. This agrees with our findings since this level was exceeded 12 days post-packaging while sensory evaluation deemed the fish to be unfit for consumption on day 10. Average chemical data and statistical analysis are provided in Appendix III (Table 9).

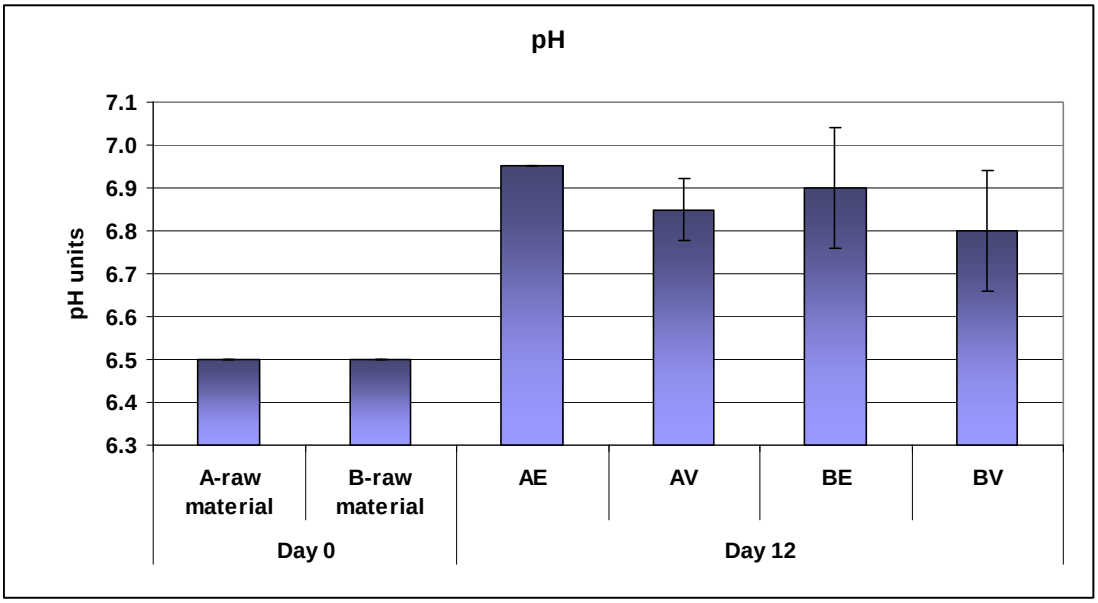

Figure 7. Measurements of pH in differently treated redfish groups (mean ± SD, n=2)

3.2.5 **Lipid analyses**

Figures 8 to 11 present the data obtained by lipid analyses. Total lipids in redfish fillets ranged between 3.15 to 4.43% in A-fish and 3.43 to 4.36% in B-fish. FFA values, a measure of hydrolytic rancidity, reached during storage period were low and ranged between 0.8 and 4.6 g FFA/100 g lipids (Figure 8). The general trend observed is that vacuum packaging contributed to a faster FFA formation, with AV progressing fastest. FFA are known to have detrimental effects on ATPase activity, protein solubility and relative viscosity (Careche and

Tejada, 1994), to cause texture deterioration by interacting with proteins (Mackie, 1993), to be interrelated with lipid oxidation development (Han and Liston, 1987) and to cause taste deterioration (Refsgaard *et al*., 2000). Therefore, their accumulation in foods has been related to some extent to their lack of acceptability. FFA content has been successfully used to assess fish deterioration during frozen storage (de Koning and Mol, 1991) and chilled storage (Barassi *et al*., 1987).

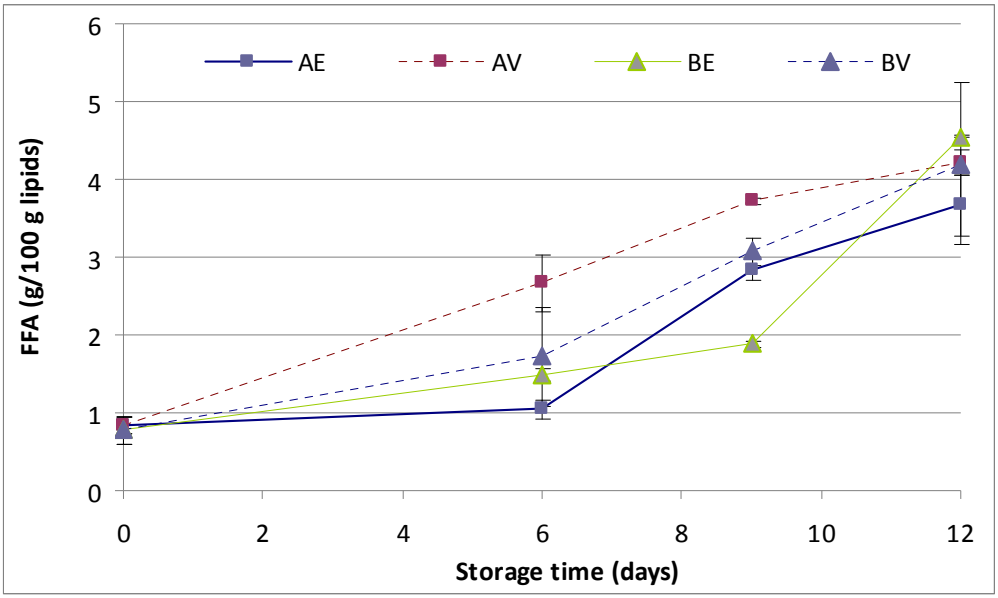

Figure 8. Evolution of FFA content in redfish fillets during storage as influenced by the cooling and packaging methods applied

Assessment of the lipid hydroperoxide value (PV) revealed that the raw material had already undergone some primary oxidation prior to processing as the fish was processed 6 days post catch, but little change in PV apparently took place during storage (Figure 9). Hydroperoxides are odour- and flavourless. An increasing PV may therefore indicate the potential for the formation of secondary oxidation products (aldehydes, ketones, short chain fatty acid and others) with unpleasant odours and flavours. Formation of secondary lipid oxidation compounds, hydroperoxides given by TBARS values (µmol MDA kg^{-1}) in redfish fillets, is presented in Figure 10. TBARS values were high at packaging, being significantly lower in A- than B-raw material. A value higher than 10 µmol MDA/kg fish sample may cause noticeable rancid flavours (Ke et al., 1976). As storage progressed, significant differences in TBARS evolution were noticed among treatments, with lowest values measured in VP samples. The peaking TBARS levels measured in air-stored fish, on days 6 (AE) and 9 (BE), coincided with the highest PV levels detected.

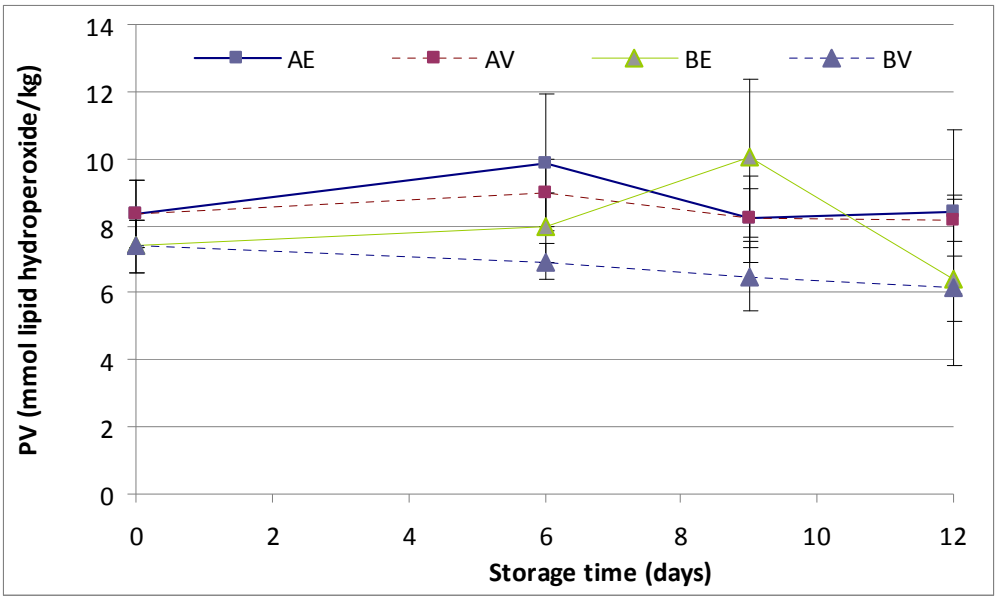

Figure 9. Peroxide values (mmol lipid hydroperoxide/kg fish) in redfish fillets during storage as influenced by the cooling and packaging methods applied

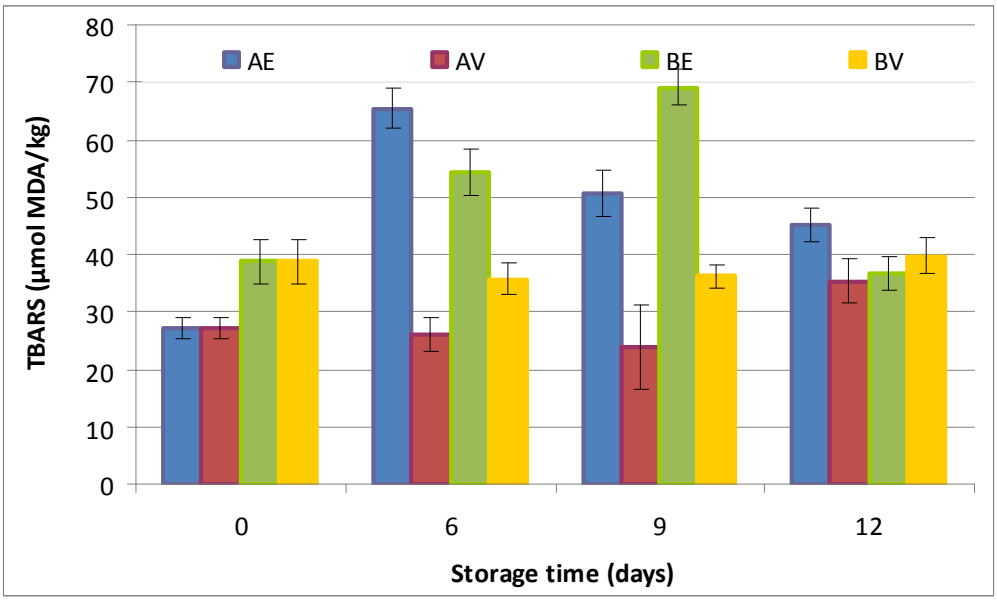

Figure 10. TBARS values (µmol MDA/kg fish) in redfish fillets during storage as influenced by the cooling and packaging methods applied

Tertiary lipid oxidation events were investigated by measuring the formation of interaction compounds between primary and secondary lipid oxidation products and nucleophilic molecules (protein-like) present in the fish muscle. The formation of interaction compounds was assessed by the fluorescence ratio. Studies have shown that fluorescence detection (δF value) is a valid method to assess lipid oxidation (Aubourg *et al.*, 1995; Aubourg *et al.*, 2007; Rodrígez *et al.*, 2009). According to the mean values of the organic phase, a low ratio of tertiary oxidation compounds was measured in the newly processed redfish fillets while a

slight increasing trend was detected in air-stored samples after 9 days of storage concomitantly to the rise in environmental temperature by 3 °C (Figure 11). In fact, a significant increase was only seen in AE-fish on day 12. The electrophilic character of most lipid oxidation compounds leads them to interact with food constituents possessing nucleophilic functions. Such interactions are highly favoured by a temperature increase of oxidised lipids, particularly in protein-rich foodstuffs such as marine source, which have high portion of essential and reactive amino acids such as lysine and methionine. Average lipid data and statistical analysis are provided in Appendix III (Table 10).

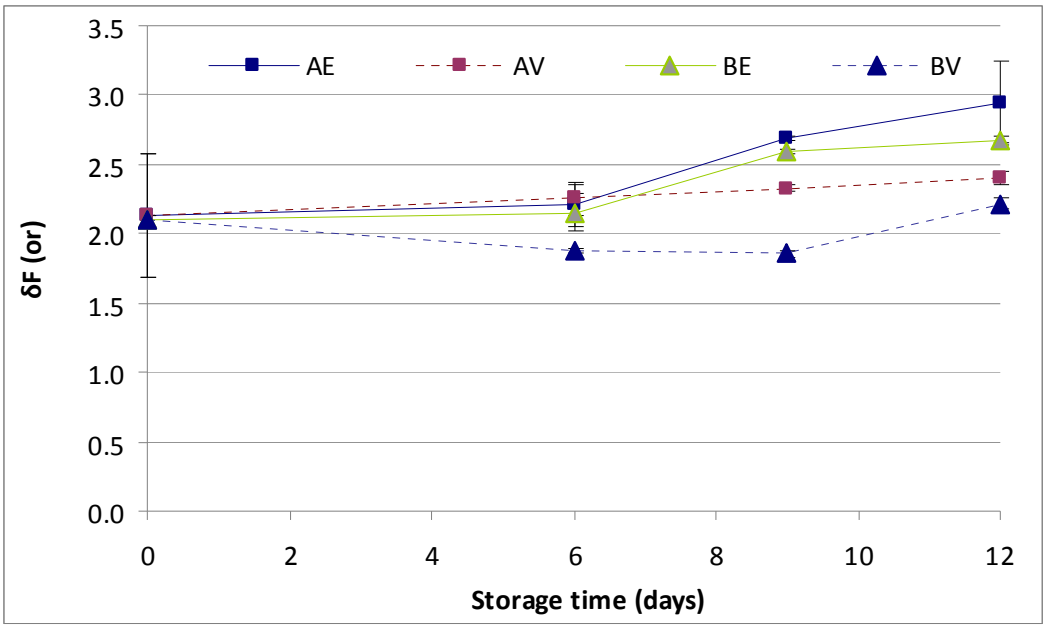

Figure 11. Fluorescence shift ratio of the organic phase resulting from Bligh and Dyer lipid extraction of redfish fillets during storage as influenced by the cooling and packaging methods applied

3.3 Overview of the study and conclusions

The study aimed to assess liquid cooling methods to quickly lower the temperature of redfish fillets before their final packaging and export by sea freight to European markets. To reduce microbial contamination of the fillets and salt uptake upon liquid cooling as well as to delay lipid oxidation, vacuum packaging of the fillets was evaluated. Quality evaluation of the 6-d old raw material (sensory and lipid analyses) indicated that deterioration of the fish was already on its way on the processing day, as demonstrated by the Torry score (8 out of 10) and the detection of primary and secondary lipid oxidation products at packaging. This may explain the little advantage observed for the treatments applied compared to untreated

fillets with respect to quality maintenance. Some of the characteristics of the redfish fillets at packaging as well as values or estimates of microbial and chemical spoilage indicators at (or close to) sensory rejection for the differently treated products are listed in Table 6. This summary will facilitate the overall comparison of the results obtained.

Table 6. Characteristics of redfish fillets at packaging and spoilage-related data following storage

Treatments	**AE NC-EPS**	**AV VP-LC**	**BE LC-EPS**	**BV LC-VP-LC**
Lipid range (%)	3.2 ± 0.2 to 4.4 ± 0.5		3.4 ± 0.5 to 4.4 ± 1.1	
Salt content (%)	0.2 ± 0.0		0.3 ± 0.0	
pH at packaging (units)	6.5 ± 0.0		6.5 ± 0.0	
$T_{initial}$ (°C) of fillets	2.9-3.1	4.5	0.5	1.8
$T_{fish\text{-}average}$ (°C) during storage	0.7 ± 0.9	0.2 ± 1.0	0.3 ± 1.1	0.2 ± 1.0
T_{min} (°C) during storage	-0.1	-0.9	-0.8	-0.9
Freshness period (days)	ca 6	ca 6	ca 5	ca 5
Shelf life (Torry) (days)	ca 10	ca 10	ca 10	ca 10
TVC (**log CFU/g**) at sensory rejection	6.9	6.5	6.8	6.8
Pseudomonads counts (**log CFU/g**)	5.8	4.4	5.4	5.3
H_2S-producing bacteria counts	6.1	5.4	5.8	5.9
Photobacterium phosphoreum counts	6.4	6.0	6.5	6.3
TVB-N (mg N/100g) on d12*	35.8	39.3	42.1	40.8
TMA (mg N/100g) on d12*	25.1	24.5	30.6	28.7
P ratio on d12*	0.70	0.62	0.73	0.70
Lipid hydrolysis (FFA, low values)	slower	fastest	slowest	faster
Primary oxidation products (PV)	little change max d6	little change	little change max d9	little change
Secondary oxidation products (TBARS)	max d6	no increase	max d9	no increase
Tertiary oxidation - Interaction compounds	increase	steady	increase	steady

NC, no cooling; **EPS**, storage in expanded polystyrene boxes; **VP**, vacuum-packed; **LC**, cooled in slurry ice before and/or after vacuum packaging; * at overt spoilage.

In general, the liquid cooling performed at the processing plant allowed for a temperature decrease of about 2.5 °C in BE-fillets. Superchilling of the fillets was only achieved following the extended period of liquid cooling of the vacuum-packed fish performed at Matís. It took about 10 times longer to reach a similar superchilled state for BE-fillets in the cooling chamber while it was never achieved in AE-fillets. Despite the differences in mean product temperature (up to 0.5 °C), similar trends were observed in quality deterioration. However, slight deviations were noticed among treatments which may indicate the possible advantage of the VP method for liquid cooling. This should be verified using fresher raw material.

Liquid cooling performed only after vacuum packaging (AV) contributed to a slower microbial development, the lowest TMA level and delayed autoxidation, i.e. the formation of secondary and tertiary oxidation products in redfish fillets. However, hydrolytic rancidity

(FFA level) was enhanced by vacuum packaging though low values resulted. Liquid cooling of unprotected fillets apparently stimulated microbial growth, especially that of *Pp*, as well as TBV-N and TMA formation. This was observed despite the low mean product temperature for both BE and BV treatments. Finally, the importance of *Pp* in the spoilage process of redfish fillets, independently of the packaging method, was demonstrated.

4. Acknowledgements

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6. APPENDIX I: Scheme for Torry freshness evaluation of cooked redfish

Table 7. **Scoring scale for freshness evaluation of cooked redfish fillets (modified Torry scale)**

Score	Odour	Flavour
10	Initially weak odour of boiled cod liver, fresh oil, starchy	Boiled cod liver, watery, metallic.
9	Shellfish, seaweed, boiled meat, oil, cod liver	Oily, boiled cod liver, sweet, meaty characteristic.
8	Loss of odour, neutral odour	Sweet/ characteristic flavours but reduced in intensity.
7	Woodshavings, woodsap, vanillin	Neutral
6	Condensed milk, boiled potato	Insipid
5	Milk jug odours, boiled clothes- like	Slight sourness, trace of "off"-flavours, rancid
4	Lactic acid, sour milk TMA	Slight bitterness, sour, "off"-flavours, TMA, rancid
3	Lower fatty acids (eg acetic or butyric acid) composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide, rancid

7. APPENDIX II: Statistical analysis of sensory data

Table 8. Mean scores for sensory attributes and p-values for difference between groups. Different letters within a column per day show significant difference between groups (p<0.05). D = days from filleting/packaging.

	o-rancid	o-cloth	o-TMA	o-sour	o-sulphur	f-rancid	f-pungent	f-sour	f-TMA	f-off	t-soft	t-juicy	t-meaty	t-tender
D0														
AE	4	2	1	0	0	4	5	1	1	1	51	51	36	51
BE	4	1	1	1	0	3	2	1	1	1	53	53	34	53
p-value	0,743	0,844	0,828	0,301	0,661	0,745	0,312	0,456	0,710	0,686	0,746	0,758	0,631	0,779
D6														
AE	1	4	1	0	0	4	7	1	1	3	52	51	48 **a**	48
AV	1	3	1	0	0	2	12	2	2	4	51	47	49 **a**	46
BE	1	4	2	0	0	3	11	0	1	3	54	51	41 **b**	49
BV	1	5	1	1	0	1	12	1	2	3	52	51	46	48
p-value	0,844	0,703	0,629	0,410	0,880	0,543	0,222	0,206	0,747	0,875	0,857	0,577	0,027	0,677
D9														
AE	8	7	1 **b**	4	0	8	14 **a**	4	3	5	57	52	35	51
AV	6	11	4 **a**	3	1	5	12	2	3	7	54	52	33	57
BE	5	8	1 **b**	1	0	6	8 **b**	4	2	2	57	47	34	49
BV	5	9	1 **b**	2	1	6	8	5	3	4	55	48	35	52
p-value	0,432	0,536	0,015	0,568	0,273	0,688	0,034	0,726	0,860	0,522	0,602	0,477	0,784	0,187
D12 *														
AE	15	28 **b**	33 **b**	17	7 **b**									
AV	22	38 **a**	44 **a**	22	22 **a**									
BE	17	29 **b**	30 **b**	16	10									
BV	12	27 **b**	28 **b**	14	12									
p-value	0,158	0,026	0,031	0,290	0,045									

8. APPENDIX III: Statistical analysis of microbial and chemical data

Table 9. Mean data **for** microbial and chemical analyses **and p-values for difference between** treatments**. Different letters within a column indicate significant difference among samples (*p*<0.05); d = days from filleting/packaging.**

Groups	TVC-IA (log cfu g^{-1})	H_2S-prod. (log cfu g^{-1})	PCR-Pp (log cfu g^{-1})	*Pseud.* (log cfu g^{-1})	pH (units)	TVB-N (mg N $100g^{-1}$)	TMA (mg N $100g^{-1}$)
p value	*0.000*	*0.000*	*0.000*	*0.000*	*0.006*	*0.004*	*0.004*
A-fillets d0	3.3 ± 0.1 a	1.2 ± 0.2 a	1.4 ± 0.8 a	2.5 ± 0.1 a	6.50 ± 0.00 a	13.1 ± 1.0 a	0.3 ± 0.0 a
B-fillets d0	3.2 ± 0.1 a	1.7 ± 0.1 a	1.8 ± 0.4 a	2.7 ± 0.1 ab	6.50 ± 0.00 a	12.3 ± 1.1 a	0.4 ± 0.3 a
AE-d6	4.4 ± 0.2 b	3.9 ± 0.1 b	3.8 ± 0.5 bc	2.9 ± 0.4 ab			
AV-d6	4.2 ± 0.1 b	3.2 ± 0.1 b	3.1 ± 0.0 b	2.7 ± 0.1 ab			
BE-d6	4.5 ± 0.1 b	3.7 ± 0.1 b	3.7 ± 0.7 bc	3.3 ± 0.2 bc			
BV-d6	4.5 ± 0.7 b	4.0 ± 0.4 b	3.6 ± 1.2 bc	3.0 ± 0.2 ab			
AE-d9	6.1 ± 0.1 c	5.0 ± 0.4 c	5.9 ± 0.3 de	4.8 ± 0.1 de			
AV-d9	6.1 ± 0.2 c	4.7 ± 0.5 c	5.5 ± 0.2 de	3.7 ± 0.5 c			
BE-d9	5.8 ± 0.3 c	4.8 ± 0.0 c	4.9 ± 0.9 cd	4.7 ± 0.0 de			
BV-d9	6.1 ± 0.1 c	4.9 ± 0.6 c	5.9 ± 0.2 de	4.3 ± 0.0 d			
AE-d12	7.7 ± 0.1 e	7.2 ± 0.2 e	6.8 ± 0.0 de	6.7 ± 0.3 h	6.95 ± 0.00 b	35.8 ± 0.3 b	25.1 ± 1.1 b
AV-d12	6.9 ± 0.2 d	6.1 ± 0.1 d	6.5 ± 0.5 de	5.1 ± 0.2 ef	6.85 ± 0.07 b	39.3 ± 11.3 b	24.5 ± 11.4 b
BE-d12	7.7 ± 0.0 e	6.9 ± 0.0 e	7.1 ± 0.1 e	6.0 ± 0.0 g	6.90 ± 0.14 b	42.1 ± 1.8 b	30.6 ± 1.2 b
BV-d12	7.5 ± 0.0 e	6.0 ± 0.0 d	6.7 ± 0.0 de	5.4 ± 0.1 f	6.80 ± 0.14 b	40.8 ± 7.4 b	28.7 ± 7.9 b

Table 10. Mean data **for analysis of** lipid deterioration **and p-values for difference between** treatment**s. Different letters within a column indicate significant difference among samples (*p*<0.05); d = days from filleting/packaging.**

Groups	FFA (g/100 g TL)	PV (mmol/kg fish)	TBARS (MDA/kg fish)	Tertiary prod. $\delta F_{organic}$
p value	*0.000*	*0.000*	*0.000*	*0.002*
A-fillets d0	0.84 ± 0.10 a	8.36 ± 1.00 ab	27.05 ± 1.86 a	2.13 ± 0.45 ab
B-fillets d0	0.78 ± 0.18 a	7.40 ± 0.78 ab	38.76 ± 3.87 b	2.09 ± 0.04 ab
AE-d6	1.05 ± 0.12 a	9.88 ± 2.03 b	65.40 ± 3.42 d	2.20 ± 0.15 ab
AV-d6	2.67 ± 0.36 bd	8.95 ± 1.00 ab	26.02 ± 2.86 a	2.25 ± 0.12 ab
BE-d6	1.49 ± 0.08 a	7.95 ± 1.03 ab	54.35 ± 3.93 d	2.15 ± 0.14 ab
BV-d6	1.72 ± 0.63 ab	6.92 ± 0.55 a	35.61 ± 2.75 b	1.88 ± 0.02 a
AE-d9	2.85 ± 0.14 cd	8.21 ± 0.89 ab	50.66 ± 3.90 d	2.69 ± 0.02 bc
AV-d9	3.72 ± 0.03 de	8.19 ± 1.30 ab	23.89 ± 7.28 a	2.33 ± 0.02 ab
BE-d9	1.88 ± 0.04 ac	10.04 ± 2.35 b	69.15 ± 3.13 d	2.60 ± 0.02 bc
BV-d9	3.07 ± 0.18 d	6.47 ± 1.03 a	36.17 ± 2.12 b	1.86 ± 0.02 a
AE-d12	3.66 ± 0.39 de	8.42 ± 2.45 ab	45.06 ± 3.03 c	2.95 ± 0.31 c
AV-d12	4.22 ± 0.17 e	8.14 ± 0.63 ab	35.41 ± 3.93 b	2.41 ± 0.05 ab
BE-d12	4.55 ± 0.02 e	6.38 ± 2.55 a	36.56 ± 2.97 b	2.68 ± 0.03 bc
BV-d12	4.20 ± 1.04 e	6.13 ± 0.95 a	39.80 ± 3.10 b	2.22 ± 0.04 ab