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Changes in visual and textural quality in the redfish species (*Sebastes marinus*) during different storage regimes

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Report summary

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Titill / Title	**Changes in visual and textural quality in the redfish species (*Sebastes marinus*) during different storage regimes** / Tilraunir gegn blettamyndun í ferskum karfaflökum		
Höfundar / Authors	Heather Philp, Ragnheiður Sveinþórsdóttir, Anna Hjaltadóttir		
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Ágrip á íslensku:	Fisktegundin *Sebastes marinus* eða karfi eins og hún er kölluð í daglegu tali var viðfangsefni þessa verkefnis þar sem markmiðið var að finna orsök og leysa það vandamál sem blettamyndun er á ferskum karfaflökum. Þessir blettir sem myndast á ferskum karfaflökum eru gulleytir og myndast innan fimm daga frá vinnslu flakanna, það skapar vandamál vegna flutningstíma þeirra og skerðir gæði þeirra vegna sjónrænna áhrifa. Rannsóknirnar sem voru framkvæmdar í verkefninu ná yfir þessa fimm daga sem tekur blettina að myndast. Í tilraunum þar sem reynt var að koma í veg fyrir blettamyndunina var ferskum karfaflökum pakkað annars vegar í frauðplastkassa þar sem motta á botninum leysti út koltvíoxíð á meðan hinn hermdi flutningur átti sér stað og hins vegar var flökunum pakkað einu og einu í lofttæmdar umbúðir þar sem var einnig motta undir þeim sem hleypti út koltvíoxíði. Niðurstöðurnar voru þær að með þessum umbúnaði flakanna var komið í veg fyrir oxun lípíða í holdinu en bæði sjónræn áhrif og áferð flakanna versnuðu. Önnur tilraun var þá gerð þar sem karfi var blóðgaður um leið og hann var tekinn um borð í veiðiskipið og hann borinn saman við karfa sem kom óblóðgaður að landi (eins og venjan er) yfir fimm daga tímabil. Niðurstöðurnar urðu þær að blettirnir voru minna áberandi í fiskinum sem hafði verið blóðgaður um borð í veiðiskipinu. Lokaniðurstöður urðu þær að líklegur orsakavaldur þessara gulleytu bletta sem myndast á ferskum karfaflökum sé tengd niðurbroti á litarefnum sem innihalda járn s.s. blóðrauða og mýóglóbini.		
Lykilorð á íslensku:	*Karfi, karfaflök, blettamyndun, útflutningur*		

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Summary in English:	The species *Sebastes marinus*, commonly known as redfish, is the subject of a series of experiments aimed at determining the cause and mitigation of the appearance of yellowish stains on the surface of processed fillets. These detract from the visual quality and occur within five days of processing, thus precluding their transport to customer by sea and reducing their potential value. An investigation of progression described the appearance of the staining over a five day period. An attempt to prevent the staining was carried out by packing the fillets in two forms of modified atmosphere, one where the fillets were maintained in standard boxes with the addition of carbon dioxide releasing pads, and one where the fillets were individually sealed in vacuum bags with carbon dioxide releasing pads. It was found that the packaging prevented oxidation of lipids in the muscle but the visual and textural quality was greatly reduced. A further investigation monitored the appearance of stains in fish that had previously been bled at sea. It was found that the yellowish stains were less apparent in the bled fish compared to those that had not been bled. In addition, the textural quality was again reduced suggesting this may be a most suitable method for improving the quality such that the fresh fillets may be transported by sea. It is proposed that the likely cause is related to the breakdown of iron-containing pigments such as haemoglobin and myoglobin.
English keywords:	*Redfish, redfish fillets, yellowish staining, export*

Contents

Introduction

The species *Sebastes marinus*, commonly known as redfish or ocean perch is a very valuable fishery product in Iceland; from demersals, it is second only to cod in terms of value (www.statice.is). When transported whole to market, it has a very long shelf life in the region of 9 -11 days (Mausse, 2000) but when processed into fillets, the product has a shelf life of only 4 days before unsightly yellowish stains begin to appear, reducing the visual quality dramatically and causing problems for producers. Consequently, the current options for sales are: to dispatch whole to buyers in another country for them to process themselves, to process into fillets and freeze immediately, or to process into fillets and dispatch by air in order to get the product to the customer within the shelf life period. The first two options represent a considerable loss of potential value to the producers while the third is a prohibitively expensive means of getting the product to the customer.

It has been theorised that the discolouration of the fillets is caused by the oxidation of fats in the muscle; redfish are considered a 'semi-fatty' fish (FAO, 1989) so this would certainly support the hypothesis. Alternatively, it has been found that species of fish that follow a diet rich in carotenoids frequently display yellowing on the surface of the fillet after processing (Choubert et al, 2011). The most important food for this species in North Atlantic waters is krill, a small crustacean which is known to have high concentrations of the carotenoid astaxanthin (Ali-Nehari et al, 2011). For this reason, it is worth to explore the possibility of a dietary influence on the fillet appearance.

The project was undertaken in two phases. The first used temperature controlled chambers to simulate the refrigerated transport conditions that fillets dispatched by sea would be exposed to in order to identify the stage at which the stains appeared. The next step was to carry out a series of experiments with different types of modified atmosphere packaging and at-sea processing in order to determine the effect on visual quality during the transport period and to assess the potential for mitigation of the staining process.

Methods

Sample collection

The first stage, exploring the development of stains over time, was carried out using fresh fish from the most recent haul collected on the morning of the vessel landing. The fillets were prepared by hand by trained staff at the fishing company, Vinnslustöðin hf. Following standard procedure, 6 sets of 10 fillets were packed into a 5kg expanded polystyrene boxes (EPS) lined with a plastic sheet and iced heavily. They were transported to the temperature controlled chamber which had been set to 4°C. Immediately, and over the following five days, a box containing 10 fillets was taken to the laboratory and photographed using a Canon 40D camera with macro lens and flash. The photography was undertaken using a stand to hold the camera at a distance of 35 cm from the fillet and the whole equipment was placed to a box lined with white polypropylene sheet.

The next stage of the experiment tested the effect of the addition of carbon dioxide releasing pads developed by the German Institute of Food and Technology and supplied by the company McAirlads. Five fillets were placed in individual vacuum bags with an absorbent pad, five with an absorbent + carbon dioxide releasing, five in an EPS box lined at the base with a large absorbent pad and covered with a plastic liner before being iced and sealed outside with a vacuum bag, and five in an EPS box lined at the base with a large absorbent and carbon dioxide releasing pad, covered with a plastic liner and iced before being sealed outside with a vacuum bag. All boxes were placed to the temperature controlled chamber for a period of five days before being removed, individually labelled and placed to the nitrogen storage container until analysis could be undertaken at the laboratory.

Laboratory analyses

Carotenoid analysis was performed using sections dissected from the loins of the five fillets in each group with duplicates being undertaken from each. Total lipids were extracted according to the Folch (Folch et al, 1956) method. Briefly, 5 g tissue was homogenized using three 10s bursts at full speed with 100ml of solvent solution using chloroform-methanol (2:1 v/v). The homogenate was filtered with #4 Whatman paper and washed several times before adding to a solution of 0.9% NaCl. The mixture was agitated for 10 minutes in an orbital

shaker, following which the contents were placed to a separating funnel and allowed to stand for 10 minutes. The lower phase was drained; the samples were then placed to a Buschi rotovap and chloroform was evaporated under vacuum.

The carotenoid content assessed using the procedure of Takeungwongtrakul et al, 2012. Between 30-50 mg of lipid was mixed with 10ml of petroleum ether and the mixture allowed to stand for 30 minutes. Absorbance was measured spectrophotometrically at 468nm and the concentration (C) of the carotenoid in the sample calculated using the following equation:

C (μg/g lipids) = Abs_{468} x Volume of extract x Dilution factor/0.2 x Sample weight (g)

Where 0.2 is the Abs_{468} of 1 μg/ml standard astaxanthin

Oxidation analysis was undertaken again in duplicate for each sample using samples dissected from the loin area of the fillet. Primary lipid oxidation was assessed by the determination of the lipid hydroperoxide value (PV) using a modified version of the ferric thiocyanate method (Shantha and Decker, 1994). The results are expressed as mmol lipid hydroperoxides per kg of sample. A modified method of Lemon (1975) was used for measuring thiobarbaturic acid reactive substances (TBARs, representing secondary oxidation compounds). Briefly, 5g of sample was homogenised with 5.0ml of TCA extraction solution (7.5% TCA, 0.1% propyl gallate and 0.1% ethylene diamine tetraacetic acid (EDTA) mixture prepared in distilled water using an Ultra-Turrax T-25 basic (IKA, Germany) at maximum speed for 10 s. The homogenised samples were completed with 5.0ml TCA extraction solution and centrifuged at 9400 *x g* for 15 mins. 5.0ml of supernatant was collected and mixed with an equal volume of Thiobarbaturic acid (0.02M) and heated in a water bath at 95°C for 40 minutes. The samples were cooled on ice and read immediately at 530nm using a Lambda EZ210 spectrophotometer and measured against a standard curve prepared using tetraethoxypropane. The results are expressed as μmol of malonaldehyde diethylacetal (MDA) per kg of sample.

Results

Changes in visual and textural quality during simulated sea-transport

Figure 1. Appearance of processed fillets on Day 1 post-processing.

Immediately after processing the fillets, the appearance of the muscle is glossy and pinkish-white, and any residual traces of blood is red. The fillets feel firm to the touch and there is no indication of gaping between the flakes.

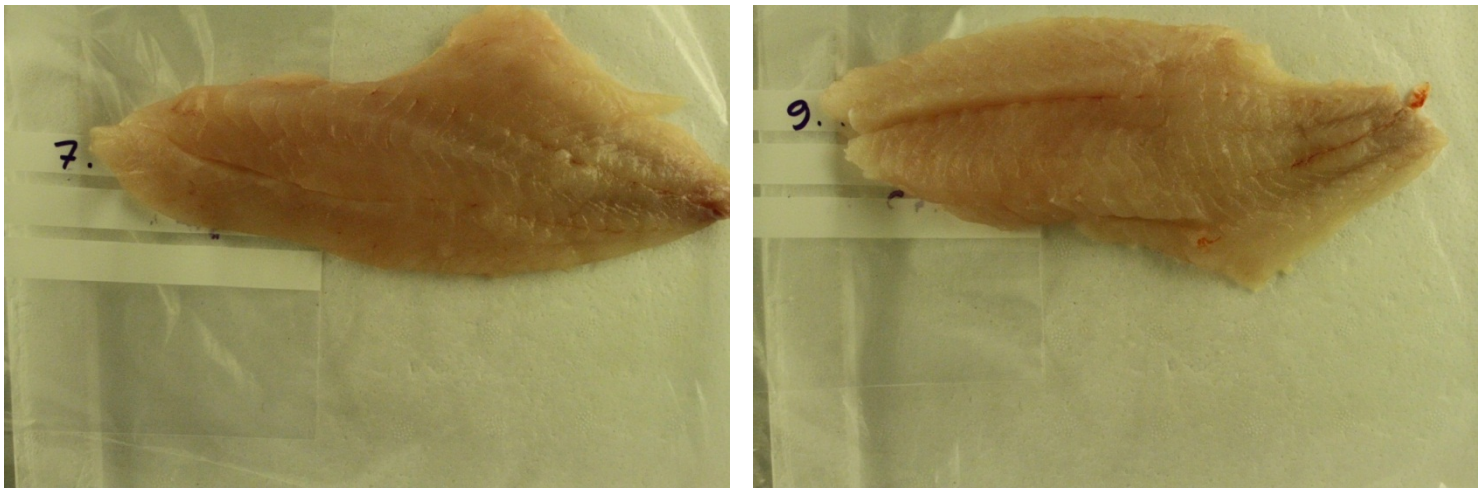


Figure 2. Appearance of fillets on Day 2 post-processing

After 24 hours of simulated transport, the fillets still feel firm to the touch but the residual traces of blood have darkened to a red-brown and the pinkish-white colour previously observed has become more dull.

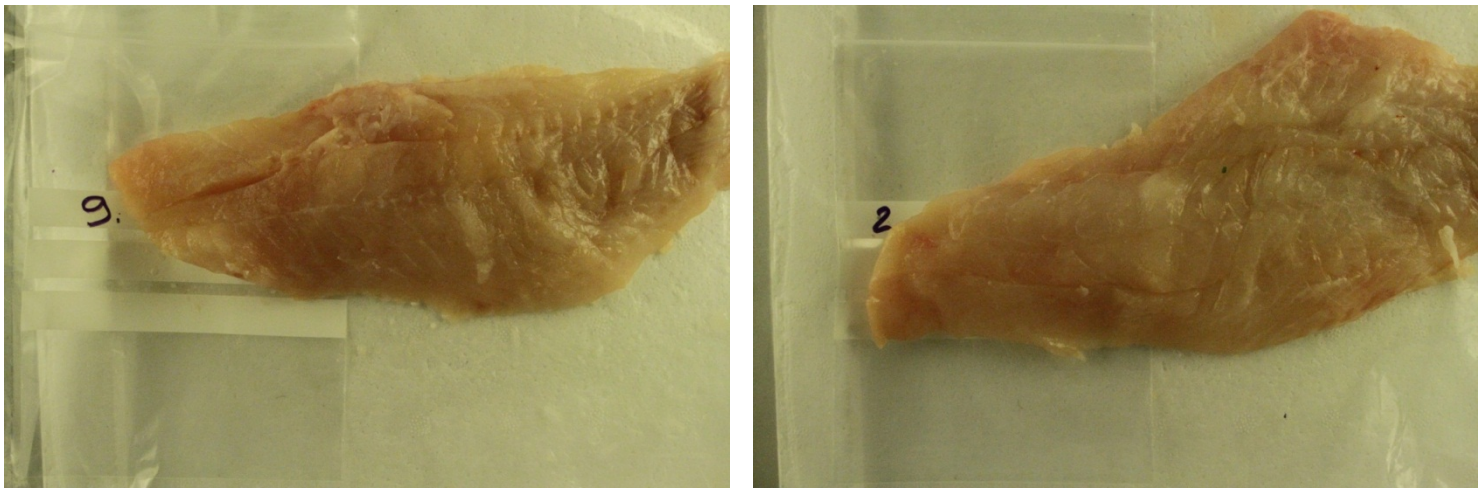


Figure 3. Appearance of fillets on Day 4 post-processing.

By the fourth day, the pinkish colour is observed only in patches and the surface is dull with hints of yellow. The texture has become much less firm and handling the fillets even with care causes indentations to appear.

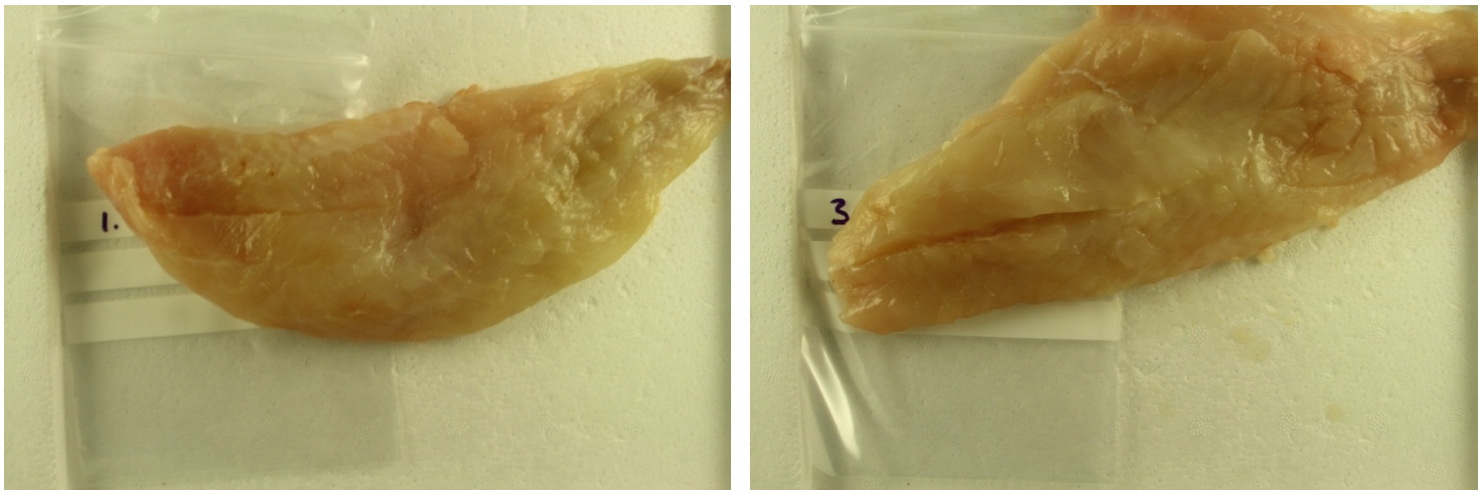


Figure 4. Appearance of the fillets on Day 5 post-processing

On the fifth day post-processing, the surface of the fillets shows clear yellowish staining. The muscle is still dull pinkish in places but this has faded to a pale colour. The texture is soft and handling leaves indentations where the muscle compresses.

Addition of carbon-dioxide releasing pads to standard packing boxes vs standard packing method

Control EPS boxes (fish pads only)

Figure 5. Fillets were placed skin side up over an absorbant pad to soak the melt-water from ice during transportation

Figure 6. Fillets stored in standard packaging for five days. The fillets displayed some yellowish stains on the surface and loss of firmness consistent with earlier observations.

Figure 7. Fillets stored in standard packaging for 5 days. The pinkish colour was dull and faded, and there was indications of yellowing on the surface.

Figure 8. Fillets stored in standard packaging for 5 days. The pinkish colour was dull and faded, and there was indications of yellowing on the surface.

Carbon dioxide releasing pads added to standard packaging for five days simulated transport

Figure 9. Fillets stored in standard packaging with the addition of carbon-dioxide releasing pads. Upon opening the box, it was immediately apparent that these fillets were different in colour to those packed with absorbant pads only. The skin side (pictured above) showed areas of opaque white and there were pronounced patches of yellow staining.

Figure 10. Fillets stored in standard packaging with the addition of carbon-dioxide releasing pads. As noted above, the white areas were opaque and the texture when handled was soft and jelly-like.

Figure 11. Fillets stored in standard packaging with the addition of carbon-dioxide releasing pads. As noted above, there was pronounced yellowing on the surface of the fillets.

Fillets packed in individual vacuum bags with the addition of absorbant pads only.

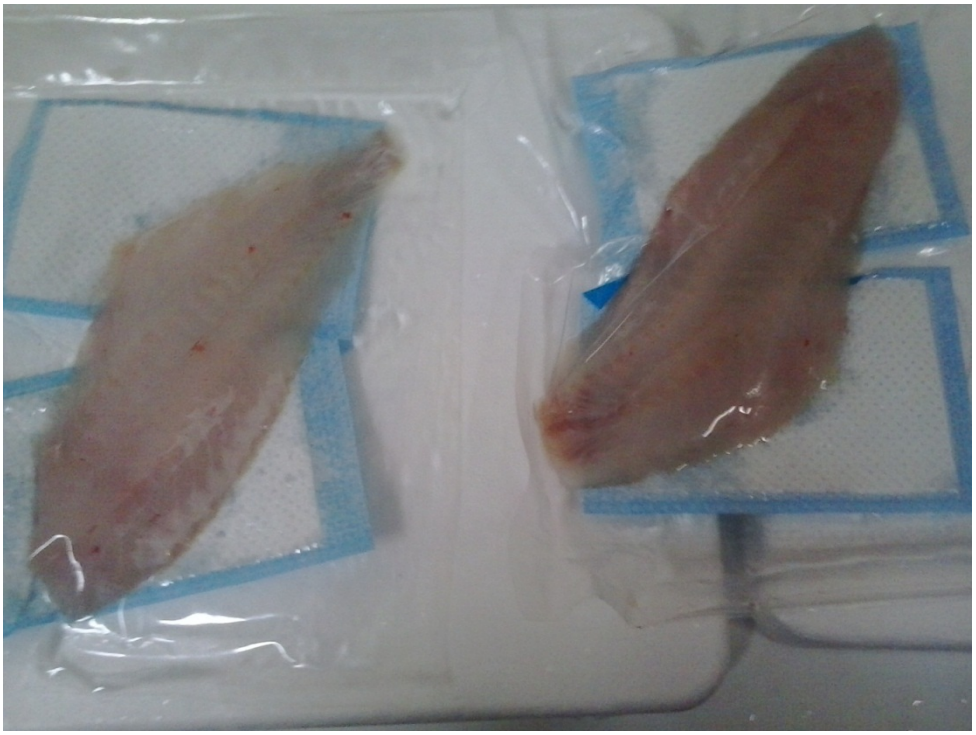


Figure 12. Fillets packed in individual vacuum bags with the addition of absorbant pads and stored for 6 days of simulated transport.

Figure 13. Fillets packed in individual vacuum bags with the addition of absorbant pads and stored for 6 days of simulated transport. The fillets were firm to the touch and still pinkish-white in colour.

Figure 14. Fillets packed in individual vacuum bags with the addition of absorbant pads and stored for 6 days of simulated transport. Handling did not produce compression marks and the yellow staining was considerably less apparent than those packed by any other method.

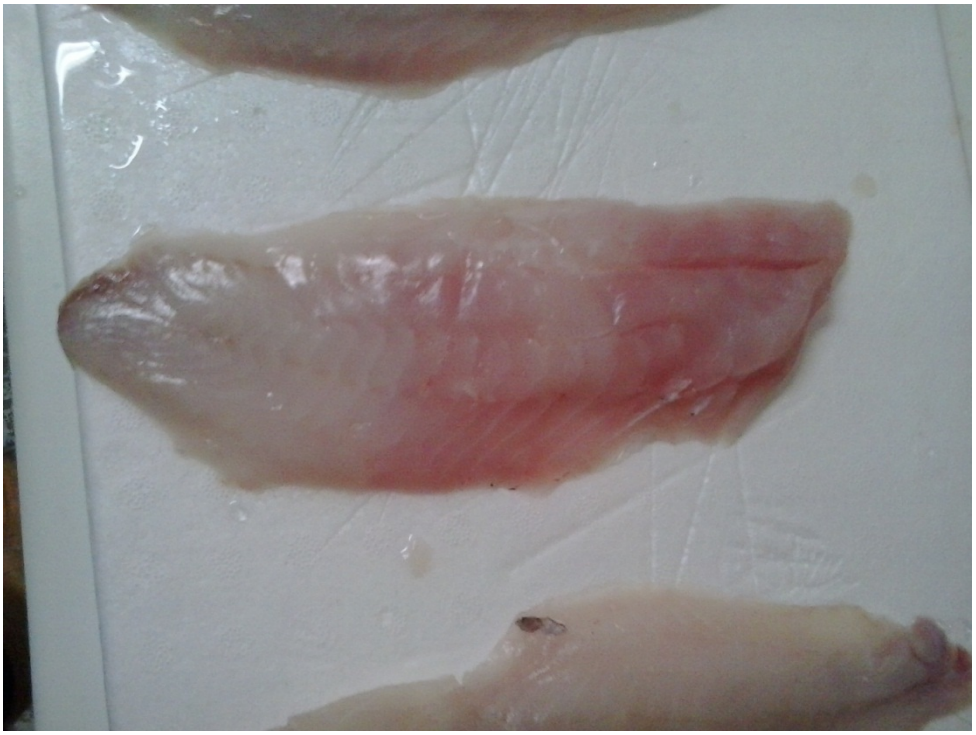


Figure 15. Fillets packed in individual vacuum bags with the addition of absorbant pads and stored for 6 days of simulated transport. The muscle was still pinkish-white in much of the fillet.

Figure 16. Fillets packed in individual vacuum bags with the addition of absorbant pads and stored for 6 days of simulated transport. As noted above, the fish packed by this method maintained much of the pinkish-white colouration observed at the freshly processed stage.

Fillets packed in individual vacuum bags with the addition of absorbant pads and carbon dioxide releasing pads.

Figure 17. Fillets packed in individual vacuum bags with the addition of both absorbant and CO_2 pads. The margins of the fillets were opaque.

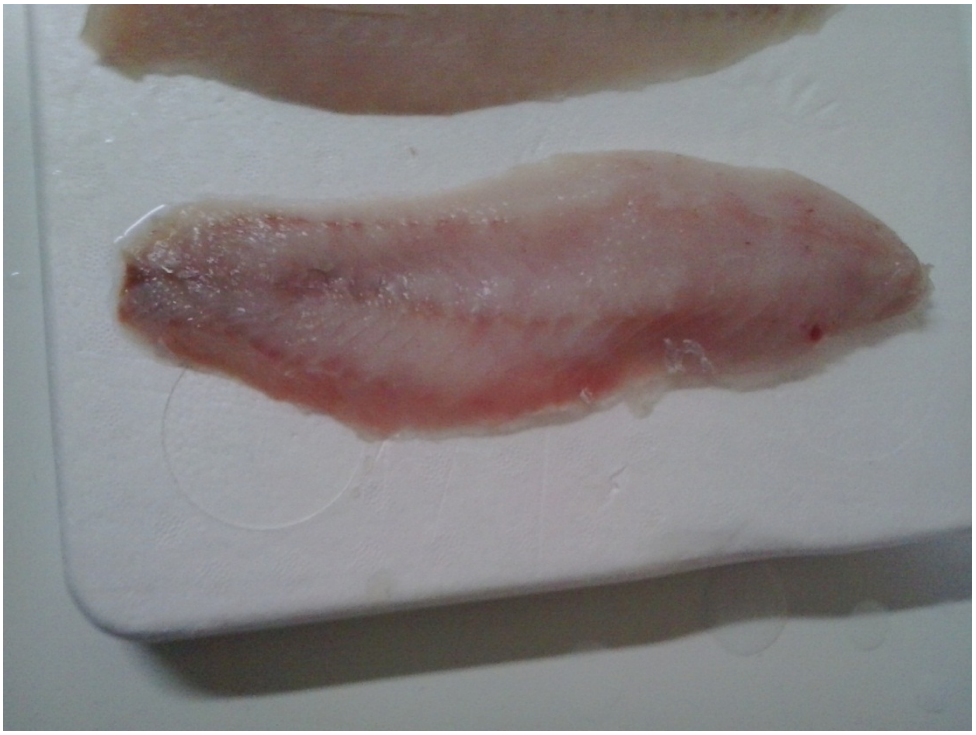


Figure 18. Fillets packed in individual vacuum bags with the addition of both absorbant and CO_2 pads. When unpacked, the fillets displayed pronounced regions of opaque white and were soft in texture.

Figure 19. Fillets packed in individual vacuum bags with the addition of both absorbant and CO_2 pads. Several fillets were completely opaque with yellow stains on the surface.

Figure 20. Fillets packed in individual vacuum bags with the addition of both absorbant and CO_2 pads. Several fillets were completely opaque with yellow stains on the surface.

Analyses

The lipid content of the fillets was somewhat variable, ranging from 2.5% through to 4%. It was a little lower in fish that had been stored using the traditional packing method (5kg in an EPS box with ice and an absorbant fish pad) but not significantly so in the samples measured. The moisture content also varied between sample groups with those that had been packed in a 5kg box with both an absorbant fish pad and carbon dioxide releasing pad being lower than all other methods. During the simulated storage period, the markers of secondary oxidation, TBARs measurement, indicated that considerable lipid oxidation took place in the box where the fillets were packed following the traditional method whilst all other packing methods appeared to provide some protection against oxidation.

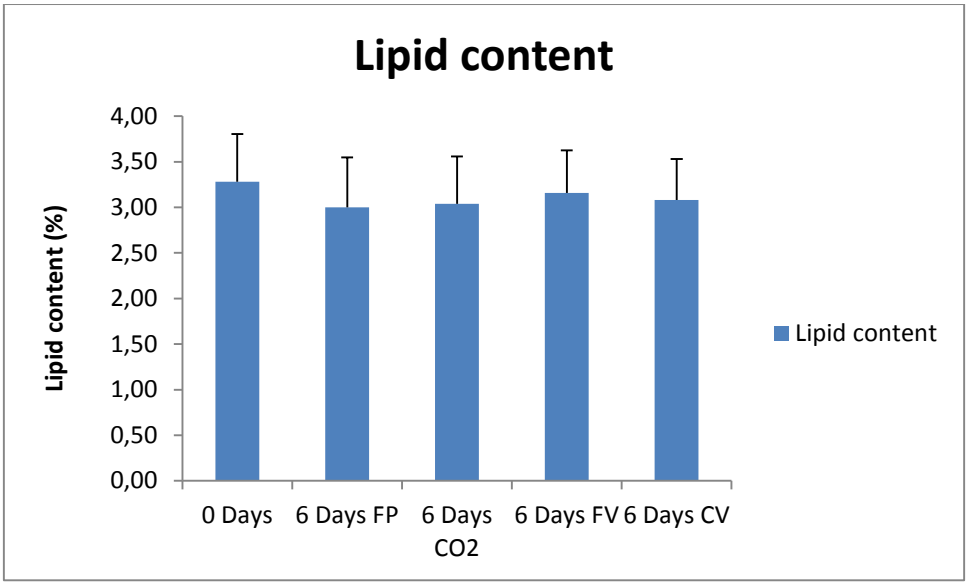


Figure 21. Average lipid content from fillets (n=5) stored for 6 days of simulated transport by various packaging methods.

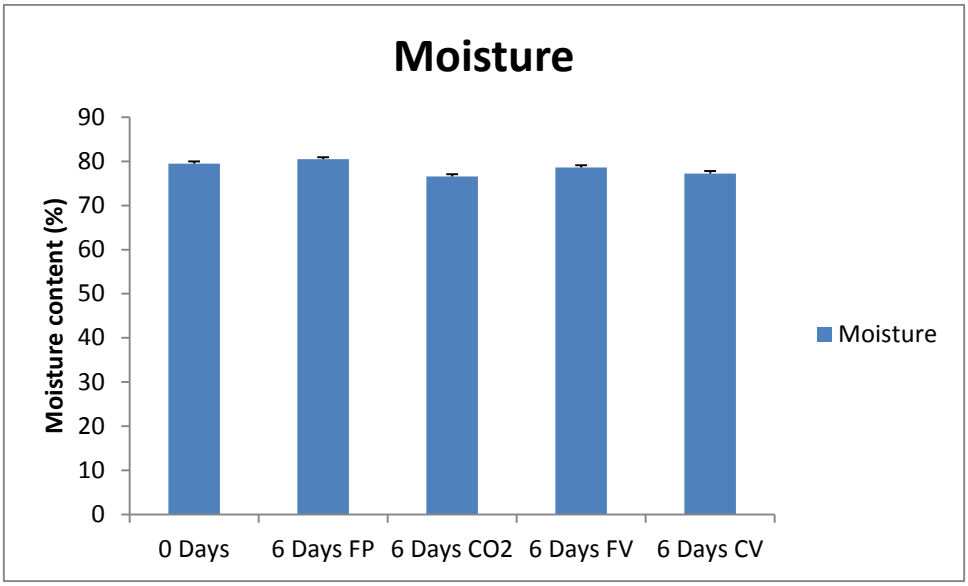


Figure 22. Averagemoisture content from fillets (n=5) stored for 6 days of simulated transport by various packaging methods.

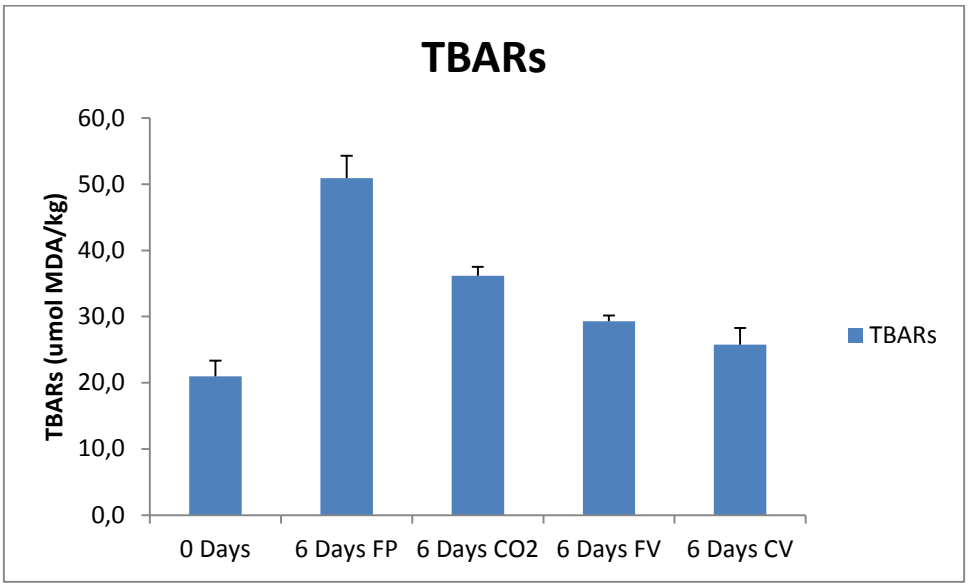
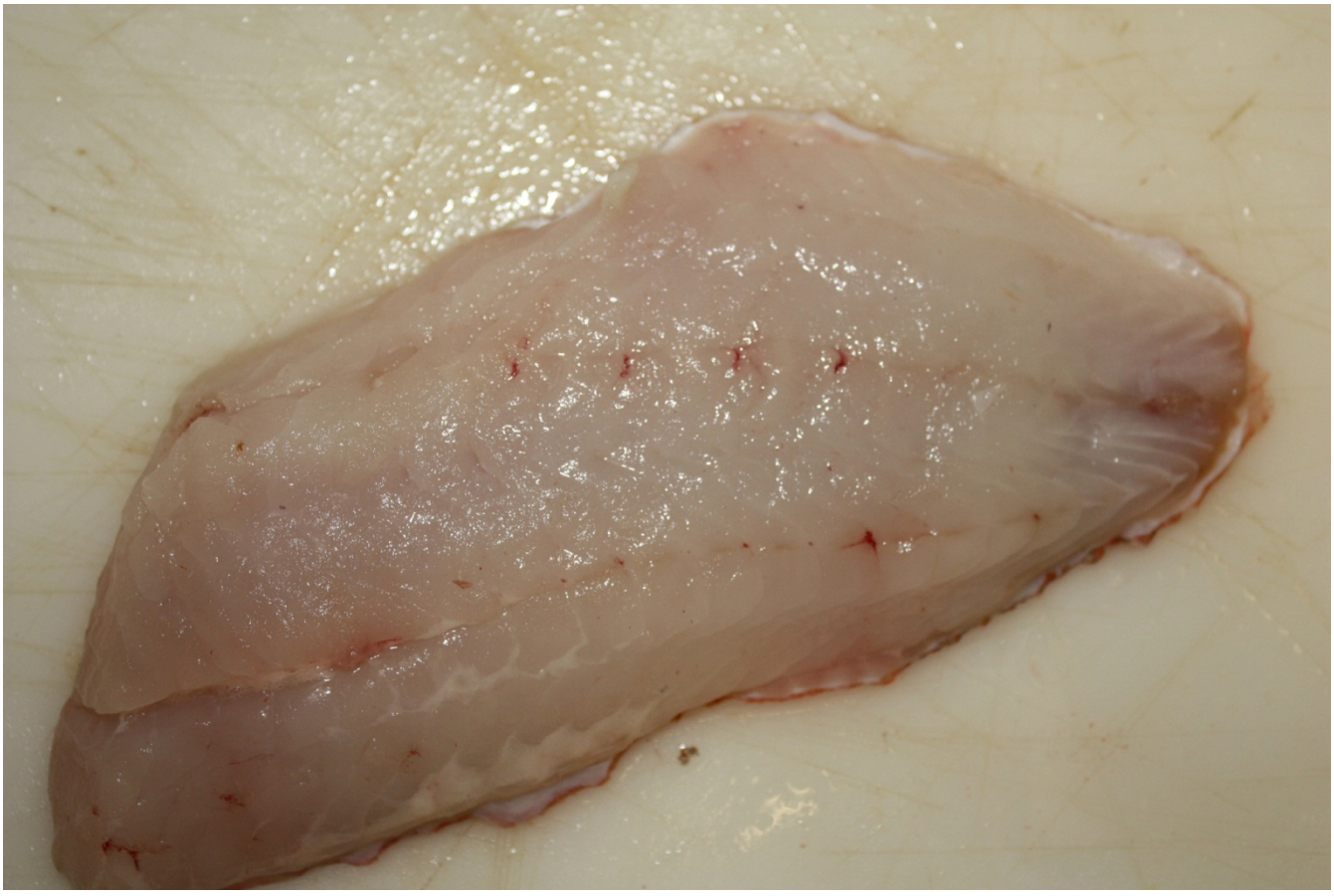
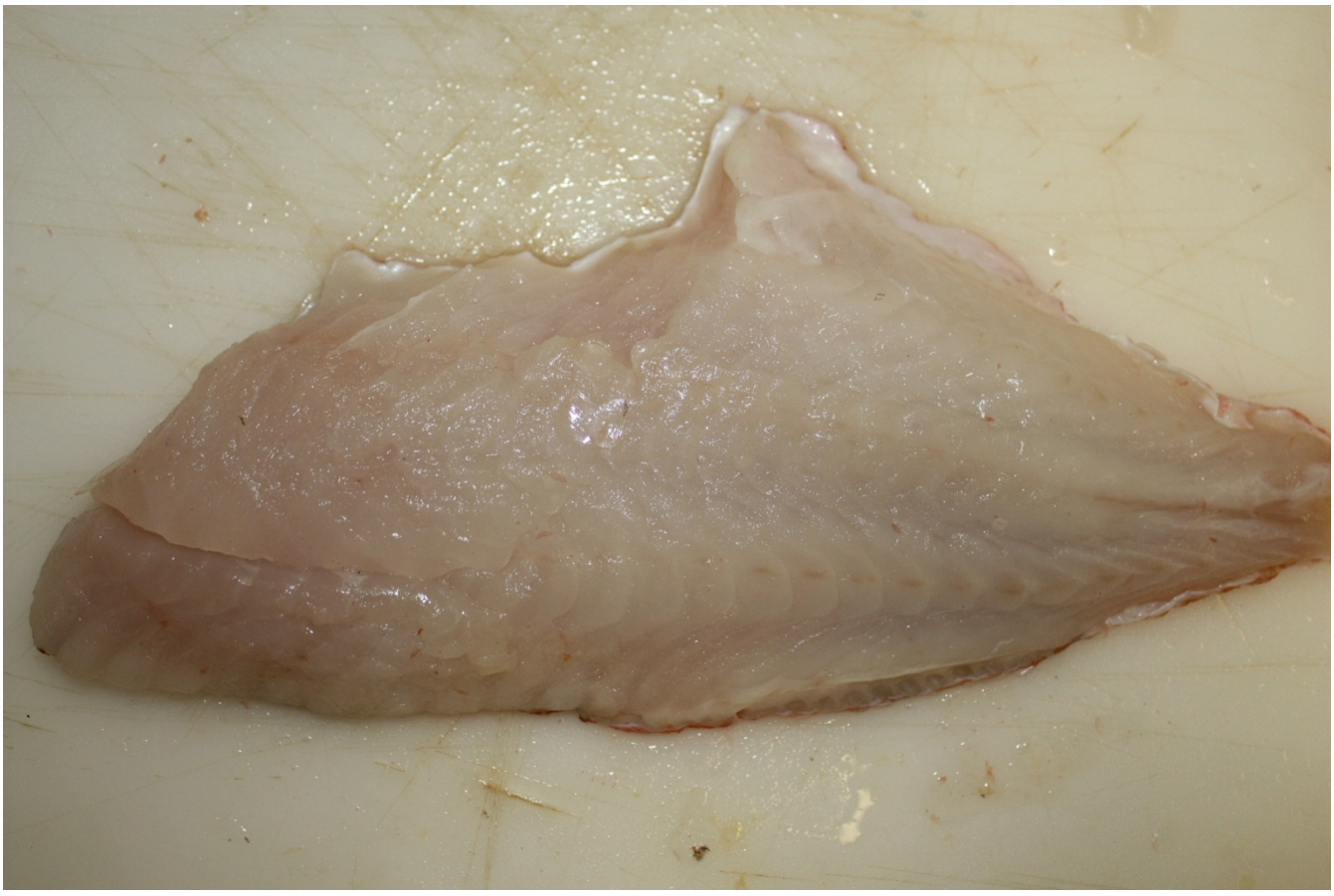


Figure 23. Average TBARs from fillets (n=5) stored for 6 days of simulated transport by various packaging methods.





Discussion

The results of this study were particularly interesting because it was revealed that oxidation of lipids is not the primary cause of the appearance of the yellow stains on the surface of the redfish fillets. This is unusual because lipid oxidation is the most common reason for the deterioration of visual quality in fish muscle, in addition to changes in flavour and texture. Further, the carotenoid content of the muscle was found to be very low and so it cannot be indicated as the factor behind the yellow stains.

Our study showed that in the first day the fillets were firm, translucent-white with a hint of pink and over the following three days they remained in this condition. However, by day 4 a hint of yellow was observed over the surface of many of the stored fillets. This became much more pronounced by day 5. During this time, the formation of secondary oxidation products, hydroperoxides given by TBARs values (umol MDA/kg) followed a prescribed course (Lauzon et al, 2010). They were low at the start of the experiment, consistent with fresh quality, and increased during the 5 day storage period. A value higher than 10 umol MDA/kg may cause noticeable rancid flavours in fish (Ke et al, 1976). Further, there were indications that lipid oxidation was retarded by the presence of the carbon dioxide releasing pads. However, despite this, the yellowish stains were still observed on those fillets indicating this is not the mechanism for the stain appearance.

In addition to this, the fillets stored in an atmosphere enriched with carbon dioxide exhibited considerably poorer overall visual appearance and texture. The fillets were more opaque, with gaping between flakes and a 'jelly-like' feel. It is possible that the formation of free fatty acids, which are known to cause textural changes, was accelerated in these fillets. The analysis was by no means comprehensive; problems with the assessment of peroxide values meant that the samples needed to be discarded and no free fatty acid analysis was undertaken. However, it is clear from even crude observation that the addition of carbon dioxide releasing pads in either the typical EPS storage box or with individually vacuum packed fillets is not a viable option for improving visual quality of stored redfish fillets.

The final test was to determine whether bleeding the fish at sea would improve on the appearance of the stains downstream. It could be seen that although the bled fish looked slightly less yellow after five days of storage, it was by no means a definitive means of

preventing the yellowing of the fillet surface. Further, after four days of storage, it was noticed that the bled fish had the same ‘jelly-like’ feel as previously described in the enriched atmosphere phase of the experiment. Further work should focus on explaining the observations of textural change, as well as continuing the search for the mechanism behind the appearance of yellow stains in redfish.

It is highly likely that the cause is related to the breakdown of iron-containing pigments such as haemoglobin and myoglobin. The field of intelligent packaging, such as modified atmosphere, has had recently produced successes such as the improved shelf life of ground beef by reducing the oxidation of such pigments. The introduction of a new, simple step in the production line, for example, adding a pad designed to release an antioxidant compound, cold dramatically increase the amount of perch that can be processed fresh in Iceland with substantial economic returns.

References

Ali-Nehari, A., Seon-Bong, K., Lee, Y-B., Lee, H. & B-S. Chun (2011) Characterization of oil including astaxanthin extracted from krill (Euphasia superba) using supercritical carbon dioxide and organic solvent as comparative method. *Korean Journal of Chemical Engineering* **8**:24-32

Choubert, G., Brisbarre, F. & M. Baccaunaud, M. (2011) Impact of dietary carotenoid and packaging during frozen storage on the quality of rainbow trout (*Oncorhynchus mykiss*) fed carotenoids. *Journal of the Science of Food and Agriculture* **91(6):** 1075-1082

FAO (1989) Yield and nutritional value of the commercially more important fish species. *FAO Fisheries Technical Paper* 309

Folch, J., Lees, M. & G.H. Sloane Stanley (1956) A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**: 497-509

http://www.statice.is/Pages/444?NewsID=8773

Ke PJ, Nash DM, Ackman RG. 1976. Quality preservation in frozen mackerel. Can. Inst. Food S ci. Technol. J. 9: 135- 138

Lauzon, H., Ólafsdóttir, A., Karlsdóttir, MG, Reynisson, E., Margeirsson, B, Arason, S & E. Martinsdóttir (2010) *Effect of cooling and packaging methods on the quality deterioration of redfish fillets*. Matís, Reykjavik

Lemon, D.W. (1975) *An Improved TBARS Test for Rancidity*. New Series Circular, Halifax-Laboratory, Halifax, Nova Scotia.

Mausse, E.C.J. (2000) Shelf life of redfish stored in ice and modified atmosphere (MA) and some aspects on the development of a Quality Index Method (QIM) scheme for redfish stored in MA. *Final Report*: UNFP, Reykjavik

Shantha, N.C., and Decker, E.A. (1994) Sensitive,iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J.AssocOfficialAna.Chem.* 77, 421

Takeungwongtrakul, S., Benjakul, S. & A. H-kittikun (2012) Lipids from cephalothorax and hepatopancreas of Pacific white shrimp (Litopeneus vannamei): Compositions and deterioration as affected by iced storage. *Food Chemistry* **134**: 2066-2074