Influence of feeding state and frozen storage temperature on the lipid stability of Atlantic mackerel (Scomber scombrus)

Paulina E. Romotowska,1,2* Magnéa G. Karlsdóttir,1 María Gudjónsdóttir,2 Hordur G. Kristinsson1,3 & Sigurjon Arason1,2

1 Matís ohf./Icelandic Food and Biotech R&D, Vinlandsleid 12, Reykjavík IS-113, Iceland
2 Faculty of Food Science and Nutrition, University of Iceland, Vinlandsleid 14, Reykjavík IS-113, Iceland
3 Department of Food Science and Human Nutrition, University of Florida, 359 FSHN Building, Newell Drive, Gainesville, FL 32611, USA

(Received 28 January 2016; Accepted in revised form 13 April 2016)

Summary Lipid deterioration of mackerel caught in Icelandic waters was studied, as affected by different frozen storage temperatures (−18 °C vs. −25 °C) and seasonal variation (August vs. September). The lipid stability was investigated by analyses of hydroperoxide value (PV), thiobarbituric acid reactive substances (TBARS), free fatty acids, as well as changes in fatty acid composition. Results showed significant lipid deterioration with extended storage time, where the lower storage temperature showed significantly more protective effects. Furthermore, a higher lipid oxidation level was recorded for fish caught in September than in August, although lipid hydrolysis occurred to be greater for fish in August than in September. Moreover, results indicated a rather stable level of omega-3 fatty acid during the whole frozen storage period. The analysis indicated that both lipid oxidation and hydrolysis were affected by the frozen storage temperature and the stability differed with regard to season of catch.

Keywords Atlantic mackerel, fatty acid composition, frozen storage temperature, lipid hydrolysis, lipid oxidation, proximate composition, seasonal variation.

Introduction

The economic importance of Atlantic mackerel in Iceland has increased significantly since 2006 (Statistics Iceland 2015), when this streamlined fast-swimming pelagic fish started to migrate into Icelandic waters in order to search for feed and restore its energy. Atlantic mackerel is caught in the Icelandic fishing zone during the summer period (June–September). The fish enters Icelandic waters after its spawning and travelling period, then it starts feeding heavily (Astthorsson et al., 2012; Jansen et al., 2012). All these factors may adversely affect the biological condition of the mackerel, and further, it may affect the quality and stability of the initial raw material intended for further processing (Aubourg et al., 2007).

Mackerel is a great source of omega-3 polyunsaturated fatty acids (PUFAs), which makes it an excellent choice from a nutritional point of view. Previous studies on the marine omega-3 PUFAs have reported healthful effects with regard to coronary artery disease, cardiovascular dealtings as well as psychological health such as depression (Perica & Delas, 2011; Delgado-Lista et al., 2012). On the other hand, pelagic fish are rather unstable products due to lipid degradation, which is the main cause of shortened shelf life (Aubourg et al., 2004), muscle discoloration (Hamre et al., 2003a), changes in flavour (Erickson, 1997) and decrease in nutritional value of fatty fish (Hamre et al., 2003b).

Frozen storage is an effective method to maintain the fish quality and prolong its shelf life due to the inhibition of microbiological growth and biochemical processes, such as lipid oxidation and hydrolysis, which are responsible for seafood quality deterioration. The quality of frozen fish products may, however, be affected by several factors, such as seasonal variation of the raw material, handling technique, freezing method, packaging material, as well as the frozen storage temperature and duration (Sørensen et al., 1995). During the freezing process, the conversion of water to ice occurs while other compounds, such as proteins and solutes, are concentrated into the nonfrozen fraction. The concentration of the non-frozen fraction varies with the composition of the fish and the temperature used during freezing and frozen storage.
storage (Novikov, 1982). Accordingly, a higher amount of frozen water and a lower microbiological and enzymatic activity, responsible for food spoilage, can be obtained by lowering the freezing and frozen storage temperatures (Karlsdottir et al., 2014). In order to commercialise frozen mackerel products, it is crucial to optimise the freezing and frozen storage conditions, which have been the main preservation method for pelagic fish species caught in Icelandic waters, intended for human consumption.

The main objective of this study was to investigate the effect of two commercial frozen storage temperatures (−18 °C vs. −24 °C) on lipid deterioration of Atlantic mackerel. Furthermore, the impact of prolonged storage on the quality of frozen mackerel products was analysed. The secondary aim of the study was to conduct a comparison of mackerel caught at two different seasons to determine whether variation within the raw material might affect the final quality of frozen Atlantic mackerel.

Materials and methods

Raw material and sampling

Atlantic mackerel (Scomber Scombrus) was caught during the summer of 2012 (beginning of August and beginning of September) in the waters Southeast of Iceland (North-East Atlantic Ocean – FAO no 27) by a trawler (Börkur NK 122). Commercially available frozen blocks of mackerel (16 kg) were used in this study. One block was sampled at each sampling day. The mackerel was frozen as whole using an automatic plate freezer (Skaginn, Akranes, Iceland) and stored at −18 °C and −25 °C. Experimental analyses were performed after 0, 3, 6, 9 and 12 months of frozen storage. Prior to examination, samples were thawed at room temperature for approximately 17 h. Three individual fishes (n = 3; 300–500 g) from each block were analysed independently. Fishes were filleted by hand, minced with skin and used for all chemical analysis. Any deviations from this protocol are included in the methods description. All chemicals used during analysis of samples were of analytical grade, and were purchased from Fluka (Buchs, Switzerland) or Sigma-Aldrich (Steinheim, Germany/St. Louis, MO, USA).

Chemical composition

Water content was determined by the weight difference during drying of a 5 g minced fillets at 104 °C ± 1 °C for 4 h (ISO 1999). Results were calculated as g water per 100 g sample.

Total lipids (TL) of the fish samples were extracted according to the method of Bligh & Dyer (1959). The lipid content was determined gravimetrically. The results were expressed as g lipid per 100 g of the sample.

The phospholipid content (PL) was determined on the total lipid (TL) extracts and was measured using a colorimetric method (Stewart, 1980). This method estimates the formation of complex between phospholipids and ammonium ferrothiocyanate, by evaluation of absorbance of the resultant solution at 488 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). A standard curve was prepared with phosphatidylcholine in chloroform (5–50 μg mL⁻¹), and results were expressed as a percentage of the total lipid content (g PL/g TL)*100. Analyses were not performed on samples after 12 months of storage due to lack of availability of samples at these otherwise potential sampling occasions.

Fatty acid profile

The fatty acid profile of the samples was determined on the TL extract by gas chromatography of fatty acid methyl esters (FAMEs) (Varian 3900 GC, Varian, Inc., Walnut Creek, CA, USA), according to the AOCS method (AOCS 1998). The Varian 3900 GC was equipped with a fused silica capillary column (HP-88, 100 m × 0.25 μm film), split injector and flame ionisation detector fitted with a Galaxie Chromatography Data System (Version 1.9.3.2 software, Varian Inc.). The setting of the oven was as follows: 100 °C for 4 min, then increased to 240 °C at a rate of 3 °C min⁻¹ for 15 min. The injector and detector temperatures were 225 °C and 285 °C, respectively. Helium was used as a carrier gas at a column flow of 0.8 mL min⁻¹ and a split ratio of 200:1. The program was based on the AOAC-996.06 (2001) method.

Lipid oxidation products

Lipid hydroperoxide values

A modified ferric thiocyanate method was used to determine the lipid hydroperoxide (Shantha & Decker, 1994). Five grams of sample was mixed with 10 mL of ice-cold chloroform/methanol (1:1) solution (with addition of 500 ppm butylated hydroxytoluene (BHT), which was used to prevent peroxidation during measurements). Five millilitre of sodium chloride (0.5 m) was added to the mixture, which was then homogenised at 2400 rpm for 10–20 s (Ultra-Turrax T25 basic, IKA Labortechnik). Phase separation was facilitated by centrifugation at 5311 g for 5 min at 4 °C (TJ-25 Centrifuge, Rotor TS-5.1-500, Beckman Coulter, Brea, CA, USA). The lower chloroform layer containing the lipids was collected (100 μL) and mixed with 900 μL of chloroform/methanol (1:1) solution. For samples that had endured the longest storage time, the results only became readable (within the
parameters of the prepared standard curve) after changing the ratio of chloroform to solvent to 50:950 μL. Finally, a 5 μL mixture (1:1) of ammonium thiocyanate (4 mM) and ferrous chloride (80 mM) was added, before vortexing. After 10 min of incubation at room temperature, the absorbance was measured at 500 nm (Tecan Sunrise, Männedorf, Switzerland) on a polypropylene microplate (Eppendorf, microplate 96/F-PP). The concentration of lipid hydroperoxide was determined using a standard curve prepared from cumene hydroperoxide (60 μM). Results were expressed as μmol lipid hydroperoxide per g of sample.

Thiobarbituric acid reactive substances (TBARS)
TBARS were determined with a modified method of Lemon (1975). The sample (5 g) was homogenised (Ultra-Turrax T25 basic, IKA Labortechnik, Germany) with 10 mL of 7.5% trichloroacetic acid solution, 0.1% propyl gallate and 0.1% ethylenediaminetetraacetic acid. After centrifugation at 5000 rpm for 20 min at 4 °C (Beckman Coulter TJ-25, Rotor TS-5.1-500), the collected supernatant was filtered with a Whatman qualitative filter paper no 4. Thiobarbituric acid (0.02 mM) in an amount of 900 μL was mixed with the collected supernatant (100 μL) before heating the mixture in a water bath at 95 °C for 40 min. After heating, the mixture was immediately placed on ice for cooling and the absorbance was measured at 530 nm (Tecan Sunrise). TBARS were determined using a standard curve prepared from 1,1,3,3-tetraethoxypropane. The results were expressed as μmol malonaldehyde (MDA) diethyl acetal per g of sample.

Enzymatic lipid hydrolysis
The free fatty acid (FFA) content was determined using the method of Lowry & Tinsley (1976) with a modification as described by Bernardez et al. (2005). The absorbance of the solution was read at 710 nm (UV-1800 spectrophotometer, Shimadzu, Japan) and the amount of free fatty acids was determined, using a standard curve prepared from oleic acid in a concentration range of 2–22 μmol. Results were expressed as grams FFA per 100 g of total lipids.

Statistical analysis
Statistical analysis of data was performed using Microsoft Office Excel 2010 (Microsoft Inc. Redmond, WA, USA), NCSS (NCSS 2000, Kaysville, UT, USA) and SigmaStat 3.5 (Dundas Software Ltd., GmbH, Germany). One-way ANOVA, Duncan’s comparison test and Pearson’s correlation were applied on means (n = 3) for each group. The significance level was set at P < 0.05. A significant difference between samples affected by seasonal variation was indicated for each sampling point separately (every variable). In case where there was no significant difference between samples affected by frozen storage condition, samples were pooled across over the storage period.

A principal components analysis (PCA) was performed using Unscrambler® (Version 10.2, CAMO ASA, Trondheim, Norway) to identify the main variation between the samples and the effect of the experimental variables. The data were centred and all variables were weighed with the inverse of the standard deviation to correct for different scales of the variables. The model was fully cross-validated.

Results and discussion
Chemical composition
The influence of the frozen storage conditions and seasonal variation on water, total lipid and phospholipid content were summarised in Fig. 1. In the present study, seasonal variation had no statistically significant effect on the raw material, although fish caught in August (54.7 ± 0.9%) showed slightly lower water content than fish caught in September (59.6 ± 2.8%). Additionally, the total lipid content of the mackerel was slightly higher for fish caught in August (21.0 ± 7.1%) than for fish caught in September (20.4 ± 4.0%). In general, neither frozen storage time nor temperature conditions did affect the lipid content significantly. Simultaneously, the water content was not affected by storage time (P > 0.05). These findings may be explained by very high standard deviations of the individuals within the same treatment group (from 1% up to 6%). Seasonal variation in fat and water content of Icelandic mackerel may be affected by migration time and access to feed resources during summer time (Astthorsson et al., 2012; Jansen et al., 2012). The proximate content of the fish may vary due to seasonal variation especially in the fast-swimming fatty fish species where the fat is mainly stored in the muscle tissue (Agustinelli & Yeannes, 2015). Moreover, the small changes observed in total lipid content may be due to selective lipolysis of the triglyceride and phospholipids, and therefore, no visual impact of the frozen storage on the total lipid content was recorded. These results were in agreement with previous findings of Polvi et al. (1991), where no changes in total lipid fatty acids of Atlantic salmon were observed before and after frozen storage.

The relatively low content of phospholipids of Atlantic mackerel (from 0.7% up to 4%) may indicate that the majority of the lipids were present as triglycerides, and therefore, the initial raw material did not show significant seasonal variation in phospholipids ratio (Bandarra et al., 2001). During frozen storage, a
significant increase of phospholipids occurred after 9 months of storage for fish caught in August and after 6 months of storage for fish caught in September (Fig. 1c). These findings may be explained by increased extractability of phospholipids resulting from the protein denaturation occurring during extended frozen storage (Saoussem 2000). These results are in general agreement with previous study (Polvi et al., 1991), where researchers observed either an increase or no changes in phospholipids classes due to frozen storage of Atlantic salmon. The mechanism of lipids selection prone to hydrolysis is very complex. For instance, it has been found that free fatty acids may have been released from triglycerides and phospholipids by hydrolytic enzymes (Shewfelt, 1981). Furthermore, amongst the different lipid classes of fish muscle, there may be a variation in their subjection to lipid deterioration. Ohshima et al. (1984) observed that contents of phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SPH) and phosphatidylserine (PS) decreased during frozen storage of skipjack tuna while contents of lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE) and free fatty acids (FFAs) increased.

**Fatty acid profile**

In general, the fatty acid distribution in the studied Atlantic mackerel was dominated by polyunsaturated fatty acids (PUFA), followed by monounsaturated

---

**Figure 1** Water (a), lipid (b) and phospholipids (c) contents (%) of mackerel fillets as affected by seasonal variation (August, September), frozen storage time (0, 3, 6, 9 and 12 months) and temperature (−25 °C, −18 °C); (n = 3; mean ± standard deviation (SD)).
fatty acids (MUFA) and saturated fatty acids (SFA) (Fig. 2). The fatty acid composition analysis showed significant seasonal variation in PUFA, MUFA and SFA levels. Changes in fatty acid composition over 12 month of frozen storage are summarised in Tables 1 and 2 for fish caught in August and September, respectively. Fish caught in August reached significantly higher levels of PUFA (35.3 ± 1.3%) and SFA (24.3 ± 1.0%) in comparison with fish caught in September (33.6 ± 1.3% and 22.4 ± 0.9%, respectively). On the contrary, a higher amount of MUFA was recorded in fish caught in September (35.3 ± 1.7%) than in fish caught in August (31.9 ± 1.5%). Higher content of PUFA in fish caught in August indicates higher nutritional value than for the fish caught in September where MUFA were the predominate fatty acids. Seasonal fluctuation in SFA may be related to the fishing location and the environmental conditions such as temperature of the ocean and feed availability (Bandarra et al., 2001). Results showed a significant negative correlation between SFA and phospholipid content for fish caught in August and stored at −25 °C (r = −0.60; P < 0.05). On the contrary, fish caught in September showed slight positive correlation between SFA and phospholipids (r = 0.41; P < 0.05). It is believed that marine organisms may adapt the flexibility and permeability (by means of phospholipids) of their cell membranes according to temperature changes of the ocean (Henderson & Tocher, 1987).

Among SFAs, palmitic acid (16:0) was the predominant fatty acid observed, followed by myristic acid (C14:0) and stearic acid (18:0). Palmitic acid (C16:0) levels were significantly higher for fish from August in comparison with fish from September. Moreover, its content decreased significantly in fish from August after 6, 9 and 12 months of storage at −18 °C and after 9 months of storage at −25 °C. Myristic acid (C14:0) showed no significant difference regarding seasonal variation, but its content decreased significantly after 9 months of storage at −25 °C for fish caught in August. Additionally, the amount of myristic acid was higher for samples stored at −18 °C than at −25 °C (P < 0.05), indicating a lower saturation degree of fatty acids at the lower frozen storage temperature.

The major fatty acid among MUFAs was oleic acid (C18:1n-9), followed by erucic acid (C22:1), eicosenoic acid (C20:1n-9) and palmitoleic acid (C16:1n-7). No significant influence of seasonal variation, frozen storage time or temperature was observed on the oleic acid content. The analysis showed significant seasonal variation in erucic acid contents, where fish from September displayed a higher amount (11.4 ± 2.1%) compared to fish from August (8.9 ± 2.3%). Similar to erucic acid, eicosenoic acid was at significantly higher level in the samples from September (7.6 ± 1.2%) than in the samples from August (6.1 ± 1.3%). These changes may be explained by variation in feeding condition (Sargent, 1995).

Among the PUFAs, the predominating fatty acids were docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3), followed by stearidonic acid (C18:4n-3), α-linoleic acid (C18:3n-3) and linoleic acid (C18:2n-6). In general, the DHA level was higher for fish caught in September than fish from August (P < 0.05). There was no significant change in the amount of DHA affected by frozen storage time or temperature. EPA level was slightly higher for fish from August than fish from September, during the whole frozen storage time, while the seasonal variation was not significant. Similarly to the DHA amount, the EPA levels decreased slightly after 9 months of storage for both seasons, although these changes were not significant. The amount of stearidonic acid was observed to be higher for fish caught in August. Similar to DHA and EPA, the amount of stearidonic acid decreased slightly after
9 months of storage. Consequently, it can be assumed that the frozen storage temperature did not affect the amount of omega-3 fatty acids in Atlantic mackerel for up to 9 months of storage, indicating a high stability of the Atlantic mackerel containing its fatty acid nutritional value. This finding is in agreement with the study of Polvi et al. (1991), who recorded a constant level of omega-3 fatty acids in Atlantic salmon muscle for up to 3 months of storage at −12 °C.

### Lipid deterioration

The development of primary oxidation products in the mackerel fillets appeared to be highly affected by extended frozen storage (Fig. 3a). There was a significant constant increase of hydroperoxide (PV) formation from 6 months of storage up to 12 months of storage for fish caught in August. The fish caught in September reached a maximum PV after 9 months of storage (P < 0.05), followed by decomposition after 12 months of storage. The results therefore indicated a more progressive lipid oxidation of mackerel caught in September in comparison with mackerel caught in August.

Secondary oxidation products, as estimated by TBARS analysis, were summarised in Fig. 3b. Corresponding to the results of the primary oxidation products, TBARS showed significant variation in catching season, where the lipid deterioration was more extensive for fish caught in September (P < 0.05), while it was rather stable for fish caught in August (P > 0.05). Furthermore, TBARS results were well correlated with the PV regarding the influence of frozen storage temperatures. Significantly higher secondary oxidation level was observed for the samples stored at −18 °C than at −25 °C. These findings are in general agreement with other studies regarding the effects of storage temperature on lipid oxidation (Saeed & Howell, 2002; Aubourg et al., 2005, 2007).
Seasonal variation in stability of lipids due to lipid oxidation can be explained by the condition of the fish which comes from migration time after spawning into Icelandic waters in order to reach a suitable source of feed. The heavy feeding period for Atlantic mackerel in Icelandic waters starts early summer (June), and therefore, fish caught in August is generally well fed and thus contains high total lipid content. Furthermore, the higher lipid stability in fish caught in August may be due to the presence of natural antioxidants in the fish diet (Brannan & Erickson, 1996). However, during late summer (September), the source of feed for the mackerel starts to be limited due to changes in oceanographic conditions. As a result, the mackerel stock starts migrating from the Icelandic fishing waters back to its spawning grounds at the coasts of Norway (Astthorsson et al., 2012).

The formation of free fatty acid (FFA), a marker for enzymatic activity causing lipid hydrolysis, was summarised in Fig. 3c. As with the PV and the TBARS results, the FFA data followed a similar overall pattern between different frozen storage temperatures, where samples stored at −25 °C were observed to be more durable against lipid deterioration in comparison with samples stored at −18 °C (P < 0.05) for both seasons. This is in agreement with the observations of Aubourg et al. (2004) who stated that the lower temperature during frozen storage was believed to reduce lipid hydrolysis by inhibition of enzyme activity such as lipase, which are present in the nonfrozen phase and may be responsible for food spoilage. The lipid hydrolysis was greater in fish caught in August than in September, which may be related to the higher content of PUFA in fish from August. The PUFA is believed to be mainly present as phospholipids (Peng et al., 2003) and may therefore be highly prone to lipid hydrolysis (Polvi et al., 1991).
Multivariate data analysis

A principal components analysis (PCA) was carried out to obtain an overview of the changes in the samples and how the quality measurements (PV, TBARS, FFA, water and total lipid content, phospholipid content, SFA, MUFA, PUFA) were affected by the experimental variables (season, frozen storage time and temperature). Three PCs described 77% of the sample variation. The scores and correlation loadings from the first, second and third principal components (PC1, PC2 and PC3) are shown in Fig. 4. The first principal component, representing 29% of the total variation, described the differences in fatty acid composition (SFA, MUFA and PUFA) of the fish as affected by seasonal variation (August vs. September). The second principal component (PC2), representing 26% of the total variation, mainly described the variation of the raw material with regard to its proximate content (lipid and water) as affected by different catching season as well as frozen storage time. The third principal component (PC3), accounting for 22% of the total variation, described the effect of frozen storage (temperature and time) on lipid deterioration (PV, TBARS and FFA). According to the PC1 and PC2 (Fig. 4a and b), changes in the major fatty acid classes as well in proximate content occurred due to seasonal variation, where fish from August resulted in higher PUFA content and fish from September in higher MUFA content. According to PC3 (Fig. 4c and d), significant

![Figure 3](image-url) Development of lipid hydroperoxide value (PV; µmol g⁻¹ muscle); thiobarbituric acid reactive substances (TBARS; µmol MDA per g muscle); and free fatty acids (FFAs; g 100 g⁻¹ total lipids) of mackerel caught at different season (August, September) during frozen storage at −25 °C and −18 °C for 0, 3, 6, 9 and 12 months. Bars represent standard deviation (n = 3).
changes in quality between fish stored at different temperature occurred after 6 months of storage up to 12 months of storage. More progressive lipid oxidation (PV, TBARS) and hydrolysis (FFA) were observed for mackerel stored at $-18 \, ^\circ C$ compared to fish stored at $-25 \, ^\circ C$. Furthermore, the development of oxidation from primary oxidation products (PV) to secondary products (TBARS) occurred with an increase in storage time.

**Conclusions**

The study indicated a higher stability of lipids (formation of primary and secondary oxidation products, free fatty acid formation and stability of polyunsaturated fatty acids) at lower temperatures during frozen storage. In conclusion, it can be recommended to store frozen products of Atlantic mackerel at $-25 \, ^\circ C$ rather than at $-18 \, ^\circ C$ in order to maintain its quality during long-term storage. Moreover, this study demonstrated a relatively stable content of omega-3 fatty acid during frozen storage, which indicated a high nutritional value of the frozen Atlantic mackerel products. Furthermore, this study demonstrated the effects of seasonal variation in quality of Atlantic mackerel, where fish caught in August showed greater lipid stability in comparison with fish caught in September. Moreover, fish from early summer (August) had a higher nutritional value, as its polyunsaturated fatty acids level was greater than for fish caught in late summer (September).
Acknowledgments

The authors would like to gratefully acknowledge the financial support of AVS (The Added Value of Seafood, research programme) fund of the Ministry of Fisheries and Agriculture in Iceland (project no. R081-13), as well as Sildarvinslan hf. for providing raw fish material and processing facility for this research study.

References


