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Influence of seasonal variation and frozen storage temperature on the lipid stability of Atlantic mackerel (*Scomber scombrus*)

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Skýrsla Matís 16-15

Desember 2015
ISSN 1670-7192
DOI 10.5281/zenodo.12517564

Skýrsluágrip Matís ohf
Icelandic Food and Biotech R&D

Report summary

ISSN: 1670-7192

Titill / Title	**Influence of seasonal variation and frozen storage temperature on the lipid stability of Atlantic mackerel (*Scomber scombrus*)**		
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Skýrsla / Report no.	16-15	*Útgáfudagur / Date:*	Desember 2015
Verknr. / Project no.	20022166		
Styrktaraðilar /Funding:	AVS rannsóknarsjóður í sjávarútvegi (R 040-12)		
Ágrip á íslensku:	Áhrif geymsluhitastigs (-18 °C vs. 25 °C) og veiðitíma (ágúst vs. september) á niðurbrot fitu í Atlantshafs makríl veiddum við Íslandsstrendur voru skoðuð í þessu verkefni. Stöðugleiki fitunnar var metinn með því að mæla fyrstastigs (PV) og annarsstigs myndefni þránunar (TBARS), fríar fitusýrur (FFA) auk fitusýrusamsetningu. Niðurstöðurnar sýna marktækan mun í fituniðurbroti með langvarandi geymslu, þar sem niðurbrotið var marktækt minna þegar geymt var við -25 °C samanborið við -18 °C. Auk þessa var fiskur veiddur í september með hærri þránunargildi samanborið við fisk frá ágúst. Aftur á móti var ensímatískst fituniðurbrot meira í águst en september. Niðurstöðurnar gáfu einnig til kynna að magn ómega-3 fjölómettaðra fitusýra var nokkuð stöðugt út geymslutímann. Með öðrum orðum þá sýndu niðurstöðurnar að hitastig í frostgeymslu hafði mikil áhrif á fituniðurbrot en stöðugleikinn var háður því hvenær fiskurinn var veiddur.		
Lykilorð á íslensku:	*Atlandshafs makríll; frostgeymsla; hitastig; árstíðarbreytileiki; fituniðurbrot, þránun*		

Skýrsluágrip Matís ohf
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ISSN: 1670-7192

Summary in English:	Lipid deterioration of Atlantic mackerel (*Scomber scombrus*) 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 regards to season of catch.
English keywords:	*Atlantic mackerel; frozen storage temperature; seasonal variation; lipid oxidation; lipid hydrolysis*

Contents

1 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. Uncommonly, Atlantic mackerel is caught in the Icelandic fishing zone during the summer period (June – September). The fish is after spawning and travelling period, and during heavy feeding. All these factors may adversely affect the biological condition of the mackerel, and further may affects the quality and stability of the initial raw material intended for further processing (Overholtz, Hare & Keith, 2011; Jansen, Campbell, Kelly, Hatun & Payne, 2012; Astthorsson, Valdimarsson, Gudmundsdottir & Oskarsson, 2012; Valdimarsson, Astthorsson & Palsson, 2012). 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 regards to coronary artery disease, cardiovascular dealings as well as psychological health such as depression (Delgado-Lista, Perez-Martinez, Lopez-Miranda & Perez-Jimenez, 2012; Perica & Delas, 2011). On the other hand, pelagic fish are rather unstable products due to lipid degradation, which is the main cause of shortened shelf life (Karlsdottir, Sveinsdottir, Kristinsson, Villot, Craft & Arason, 2014a; Aubourg, Lugasi, Hovari, Pineiro, Lebovics & Jakcozi, 2004), muscle discoloration (Hamre, Lie & Sandnes, 2003a), changes in flavour (Erickson, 1997) and decrease in nutritional value of fatty fish (Aubourg, Pineiro & González, 2004).

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 (Hamre, Lie & Sandnes, 2003b; Sörensen, Brataas, Nyvold & Lauritzsen, 1995). During the freezing process, the conversion of water to ice occurs while other compounds, such as proteins and solutes, are concentrated into the non-frozen fraction. The concentration of the non-frozen fraction varies with the composition of the fish and the temperature used during

freezing and frozen 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., 2014a). In order to commercialize frozen mackerel products, it is crucial to optimize the freezing and frozen storage conditions, which have been the main preservation method for Icelandic pelagic fish species, intended for human consumption.

The main objective of the present 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.

2 Materials & Methods

2.1 Raw material and sampling

Atlantic mackerel (*Scomber Scombrus*) was caught during the summer of 2012 (beginning of August and beginning of September) in the South-East of Iceland (North-East Atlantic Ocean - FAO no 27) by trawler (Börkur NK 122). The mackerel was frozen as whole using an automatic plate freezer (Skaginn, 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 hours. Three fishes (n = 3) from each group 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).

2.2 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/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 / 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 a complex between phospholipids and ammonium ferrothiocynate, 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) and results were expressed as a percentage of the total lipid content (g PL/ g TL)*100).

2.3 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 x 0.25 µm film), split injector, and flame ionization detector fitted with a Galaxie Chromatography Data System, (Version 1.9.3.2 software, Varian Inc., Walnut Creek, CA, USA). 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 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, and a split ratio 200:1. The program was based on the AOAC-996.06 (2001) method.

The polyene index (PI) was calculated according to the fatty acid content ratio as follows (Rodrígues, 2007): PI = (C22:6+C20:5)/C16:0, where C22:6 represents docosahexaenoic acid, C20:5 eicosapentaenoic acid and C16.0 palmitic acid.

2.4 Lipid oxidation products

2.4.1 Lipid hydroperoxide values

A modified ferric thiocyanate method was used to determine the lipid hydroperoxide (Shantha & Decker, 1994). Five grams of sample were 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 mL of sodium chloride (0.5 M) were added to the mixture, which was then homogenized at 2400 rpm for 10-20 sec. (Ultra-Turrax T25 basic, IKA Labortechnik, Germany). Phase separation was facilitated by centrifugation at 5000 rpm for 5 min at 4 °C (TJ-25 Centrifuge, Rotor TS-5.1-500, Beckman Coulter, California, 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 M) 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 Sunrice, Austria) 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.

2.4.2 Thiobarbituric acid reactive substances (TBARS)

TBARS was determined with a modified method of Lemon (1975). The sample (5 g) was homogenized (Ultra-Turrax T25 basic, IKA Labortechnik, Germany) with 10 mL of 7.5% trichloroacetic acid (TCA) solution, 0.1% propyl gallate and 0.1% etylenediaminetetraacetic acid (EDTA). After centrifugation at 5000 rpm for 20 min at 4 °C (Beckman Coulter TJ-25, Rotor TS-5.1-500, USA) the collected supernatant was filtered with a Whatman qualitative filter paper no 4. Thiobarbituric acid (0.02 M) 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 Sunrice, Austria). TBARS were determined using a standard curve prepared from 1.1.3.3-tetraethoxypropane (TEP). The results were expressed as µmol malomaldehyde diethyl acetal per kg of sample.

2.5 Enzymatic lipid hydrolysis

The free fatty acid (FFA) content was determined using the method of Lowery & Tinesley (1976) with a modification as described by Bernardez, Pastoriza, Sampedro, Herrera and Cabo (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.

2.6 Statistical analysis

Statistical analysis of data was performed using Microsoft Office Excel 2010 (Microsoft Inc. Redmond, Wash, USA), NCSS (NCSS 2000, Utah, USA) and SigmaStat 3.5 (Dundas Software Ltd.,

GmbH, Germany). One-way ANOVA, General Linear Models (GLM), Duncan´s comparison test and Pearson correlation were applied on means (n = 3) for each group. The significance level was set at $p \leq 0.05$.

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 was 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.

3 Results & Discussion

3.1 Chemical composition

The influence of the frozen storage conditions and seasonal variation on water, total lipid and phospholipid content were summarised in Table 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, an increase of water content was recorded for fish from August after 6 and 9 months of storage ($p > 0.05$). 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 (Overholtz et al., 2011; Jansen et al., 2012; Astthorsson 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, Ackman, Lall and Saunders (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 bound to triglycerides and therefore the initial raw material did not show significant seasonal variation in phospholipids ratio (Bandarra, Batista, Nunes & Empis, 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 (Table 1). 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 (Karlsdottir, Sveinsdottir, Kristinsson, Villot, Craft & Arason, 2014b; Shewfelt, 1981). Furthermore, amongst the different lipid classes of fish muscle there may be a variation in their subjection to lipid deterioration. Ohshima, Wada and Koizumi (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.

3.2 Fatty acid profile

In general, the fatty acid distribution in the studied Atlantic mackerel was dominated by poly-unsaturated fatty acids (PUFA), followed by mono-unsaturated fatty acids (MUFA) and saturated fatty acids (SFA) (Figure 1). 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 summarized in Table 2a and Table 2b 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 to 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 (Celik, 2008; Osako, Yamaguchi, Kurokawa, Kuwahara, Saito & Nozaki, 2003; 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 ($r = -0.60$). On the contrary, fish caught in September showed slight positive correlation between SFA and phospholipids ($r = 0.41$). 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 **s**ignificantly higher for fish from August in comparison to 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 ($p < 0.05$).

The major fatty acid among MUFAs, was oleic acid (C18:1*n*-9), followed by erucic acid (C22:1), eicosenoic acid (C20:1*n*-9) and palmitoleic acid (C16:1*n*-7). No significant influence of seasonal variation, frozen storage time or temperature was observed on the oleic acid content. However, the oleic acid amount was slightly higher for fish caught in August than for fish caught in September ($p > 0.05$). 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%). Similarly 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%).

Amongst the PUFAs, the predominating fatty acids were docosapentaenoic acid (DHA, C22:6*n*-3) and eicosapentaenoic acid (EPA, C20:5*n*-3), followed by stearidonic acid (C18:4*n*-3), α-linoleic acid (C18:3*n*-3) and linoleic acid (C18:2*n*-6). In general, the DHA level was higher for fish caught in September than fish from August ($p < 0.05$). There was no significant changes in the amount of DHA affected by frozen storage time or temperature, although its amount decreased slightly after 6 months of storage (September) and after 9 months of storage (August). 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 ($n = 9$). Similarly 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 regarding 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.

3.3 Lipid deterioration

The development of primary oxidation products in the mackerel fillets appeared to be highly affected by extended frozen storage (Figure 2A). 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. Furthermore, the formation of PV was slightly more pronounced in the fish stored at -18 °C compared to -25 °C for both seasons ($p > 0.05$).

Secondary oxidation products, as estimated by TBARS analysis, were summarised in Figure 2B. Correspondingly 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 (Karlsdottir et al., 2014a; Aubourg, S., Lago, Sayar & González, 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 (Bragadottir, 2001). 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 summarized in Figure 2C. 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 to samples stored at -18 °C ($p < 0.05$) for both seasons. This is in agreement with the observations of Aubourg, Pineiro, 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 non-frozen 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, Larondelle, Pham, Ackman & Rollin, 2003; Bandarra et al., 2001) and may therefore be highly prone to lipid hydrolysis (Polvi et al., 1991).

3.4 Multivariate data analysis

A principal component 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 loads from the first, second and third principal components (PC1, PC2 and PC3) are shown in Figure 3. 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, representing 26% of the total variation, mainly described the variation of the raw material with regards to its proximate content (lipid and water) as affected by different catching season as well as frozen storage time. The third principal component, 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 (Figure 3A), 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 (Figure 3B), significant 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) was observed for mackerel stored at -18 °C comparing to fish stored at -25 °C. Furthermore, the development of oxidation from primary oxidation products (PV) to secondary products (TBARS) occurred with an increase in storage time.

4 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 °C rather than at -18 °C in order to maintain its quality during long term storage. Moreover, the present 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, the present study demonstrated the effects of seasonal variation in quality of Atlantic mackerel, where fish caught in August showed greater lipid stability in comparison to fish caught in September. Moreover, fish from early summer (August) had a higher nutritional value, since its polyunsaturated fatty acids level was greater than for fish caught in late summer (September).

5 Acknowledgement

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

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Figures and Tables

Table 1 Water (%), total lipid (%) and phospholipid content (g/100g lipids) 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 ± stdv.).

Storage time (months)	August		September	
	-25 ˚C	-18 ˚C	-25 ˚C	-18 ˚C
Total lipid content (%)				
0	21.0 ± 7.1		20.4 ± 4.0	
3	23.2 ± 3.1	25.5 ± 5.4	23.6 ± 2.4	21.3 ± 7.7
6	17.7 ± 3.3	22.9 ± 0.7	22.5 ± 1.9	19.7 ± 3.9
9	19.9 ± 3.7	17.8 ± 6.5	22.9 ± 2.0	23.1 ± 1.1
12	21.8 ± 3.0	23.3 ± 4.0	19.3 ± 6.4	19.8 ± 0.9
Water content (%)				
0	54.7 ± 0.9		59.6 ± 2.8	
3	56.9 ± 2.4	60.1 ± 2.5	59.8 ± 2.4	58.0 ± 1.3
6	57.0 ± 3.0	59.8 ± 0.5	60.5 ± 5.4	55.7 ± 3.0
9	57.5 ± 3.8	56.7 ± 1.5	56.3 ± 1.6	57.5 ± 2.8
12	58.3 ± 5.5	59.4 ± 3.4	57.4 ± 1.4	57.9 ± 0.5
Phospholipids (%)				
0	1.6 ± 1.4		1.3 ± 0.3	
3	1.1 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	1.3 ± 0.5
6	1.8 ± 0.1	0.8 ± 0.1	2.0 ± 0.2	2.0 ± 0.3
9	3.7 ± 1.3	3.0 ± 1.7	1.8 ± 0.3	1.5 ± 0.1
12	NA*	NA*	NA*	NA*

*NA:Not analysed

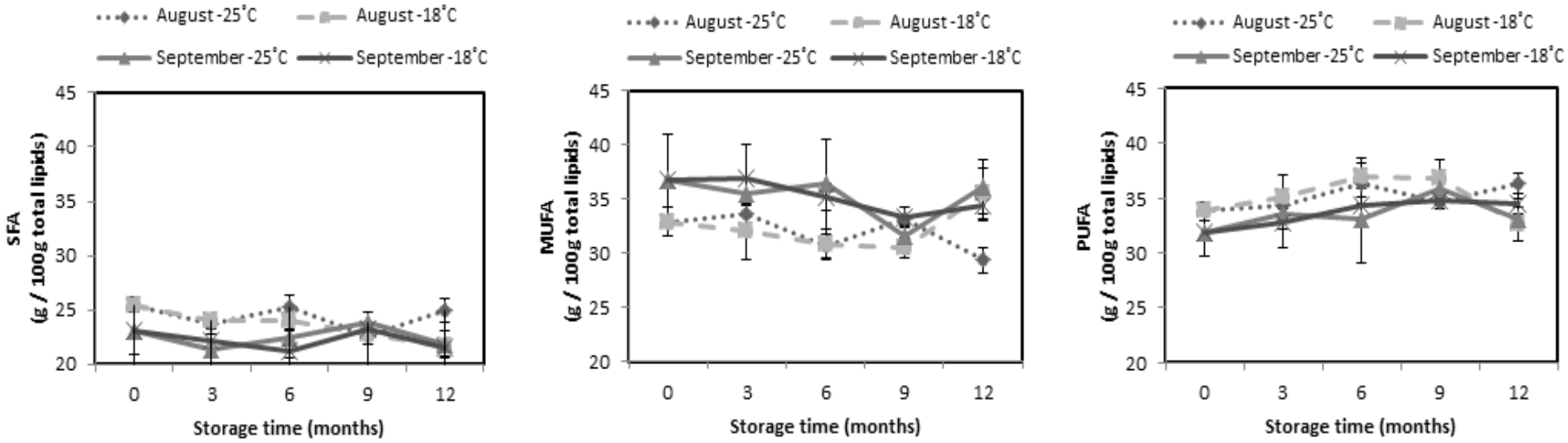

Figure 1 Changes in major fatty acid classes (g fatty acids/100 g total lipids) of mackerel caught at different season (August, September) during frozen storage at -25 ˚C and -18 ˚C for 0, 3, 6, 9 and 12 months. Bars represent standard deviation (n = 3).

Table 2a Changes in fatty acid profile (g fatty acids/100 g total lipids, mean ± SD) of mackerel caught in August, during frozen storage at -25 ˚C and -18 ˚C for 0, 3, 6, 9 and 12 months.

Fatty acids	August								
	Control	-25 °C				-18 °C			
		3 months	6 months	9 months	12 months	3 months	6 months	9 months	12 months
C14:0	7.6±0.5^{a}	7.3±0.6^{ab}	7.2±0.9^{ab}	6.4±0.3^{b}	7.5±0.7^{a}	7.0±0.7^{a}	7.7±0.2^{ab}	6.7±0.1^{ac}	8.0±0.3^{bd}
C16:0	14.8±0.3^{a}	13.7±0.2^{b}	15.0±0.5^{a}	13.1±0.6^{b} ^{c}	14.4±0.6^{ab} ^{A}	13.8±0.2^{a}	13.5±0.7^{b}	13.0±0.8^{b}	10.9±0.7^{cB}
C17:0	0.4±0.2^{a}	0.3±0.0^{aA}	0.4±0.0^{a}	0.5±0.0^{a}	0.4±0.0^{a}	0.5±0.0^{aB}	0.4±0.0^{a}	0.5±0.0^{a}	0.5±0.0^{a}
C18:0	2.4±0.1^{a}	2.1±0.2^{a}	2.4±0.3^{a}	2.3±0.4^{a}	2.2±0.1^{aA}	2.3±0.2^{a}	2.1±0.1^{ab}	2.2±0.2^{a}	1.7±0.2^{bB}
SFA	**25.5±0.7**^{a}	**23.8±0.5^{b}**	**25.3±1.0^{a}**	**22.7±0.8^{b}** ^{c}	**25.0±1.1^{ab}**	**24.0±0.7^{a}** ^{b}	**24.1±0.8^{a}** ^{b}	**22.8±0.9^{b}** ^{c}	**21.7±0.7^{c}**
C16:1n9	0.2±0.1^{a}	0.2±0.1^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}
C16:1n7	**3.3**±0.1^{a}	2.8±0.2^{a}	3.1±0.1^{a}	3.3±0.6^{a}	3.4±0.2^{a}	3.3±0.2^{a}	3.0±0.2^{a}	3.3±0.7^{a}	3.5±0.4^{a}
C18:1n11	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.1^{a}	0.4±0.1^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.4±0.0^{a}
C18:1n9	12.6±0.9^{a}	9.6±1.4^{a}	11.4±0.9^{a}	9.1±3.2^{b}	10.9±0.6^{aA}	9.9±1.9^{ab}	8.5±1.8^{b}	8.5±1.1^{b}	6.5±1.8^{bcB}
C18:1n7	1.8±0.2^{a}	1.6±0.3^{a}	1.8±0.2^{a}	1.7±0.2^{a}	1.7±0.1^{a}	1.6±0.3^{a}	1.5±0.2^{a}	1.5±0.2^{a}	1.4±0.3^{a}
C18:1n5	0.5±0.0^{a}	0.5±0.0^{ab}	0.5±0.1^{a}	0.5±0.1^{a}	0.6±0.1^{acA}	0.5±0.0^{ab}	0.4±0.1^{ab}	0.5±0.0^{a}	0.4±0.1^{bB}
C20:1n11	0.5±0.0^{a}	0.6±0.0^{b}	0.4±0.1^{ac}	0.6±0.1^{ab}	0.5±0.1^{acA}	0.6±0.0^{ab}	0.6±0.0^{ab}	0.5±0.1^{a}	0.6±0.1^{bB}
C20:1n9	5.2±0.2^{a}	6.6±0.1^{b}	4.9±0.3^{a}	6.6±1.0^{b}	4.6±0.6^{aA}	5.9±0.3^{a}	6.1±0.4^{a}	6.0±0.3^{a}	8.8±1.5^{bB}
C20:1n7	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{ab}	0.2±0.0^{b}	0.2±0.0^{aA}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{b}	0.2±0.0^{bB}
C22:1	7.6±0.8^{a}	10.4±07^{a}	6.8±1.1^{a}	9.7±2.0^{a}	6.2±1.2^{a}	8.7±0.4^{a}	9.3±0.7^{a}	8.5±0.7^{a}	12.9±3.3^{a}
C24:1	0.7±0.1^{a}	0.8±0.0^{a}	0.7±0.0^{ab}	0.7±0.1^{aA}	0.6±0.1^{bA}	0.7±0.0^{ab}	0.7±0.1^{ab}	0.6±0.1^{bB}	0.5±0.0^{cB}
MUFA	**32.9±1.4**^{a}	**33.6±1.0^{a}** ^{c}	**30.6±1.1^{a}** ^{b}	**33.1±0.4^{a}**	**29.4±1.2^{bA}**	**32.1±2.7^{a}**	**30.9±1.3^{a}**	**30.5±0.9^{a}**	**35.5±2.4^{ab}** ^{B}
C16:2n4	0.4±0.1^{a}	0.4±0.0^{ab}	0.4±0.0^{ab}	0.4±0.1^{a}	0.4±0.0^{ac}	0.4±0.0^{ab}	0.5±0.0^{ac}	0.4±0.1^{abc}	0.3±0.0^{bd}
C18:2n6	1.6±0.1^{a}	1.6±0.1^{a}	1.7±0.0^{a}	1.6±0.1^{a}	1.7±0.1^{a}	1.7±0.1^{a}	1.8±0.1^{a}	1.7±0.1^{a}	1.8±0.1^{a}
C18:3n3	2.3±0.1^{a}	2.3±0.2^{a}	2.6±0.2^{a}	2.5±0.3^{a}	2.6±0.2^{a}	2.4±0.1^{ab}	2.6±0.2^{ab}	2.7±0.3^{b}	1.8±0.2^{c}
C18:4n3	6.1±0.4^{a}	6.7±0.7^{a}	6.6±0.6^{a}	6.0±0.3^{a}	6.9±0.2^{a}	6.4±0.7^{a}	7.5±0.6^{a}	6.6±1.0^{a}	5.9±0.2^{a}
C20:2	0.2±0.0^{a}	0.3±0.0^{bA}	0.2±0.0^{aA}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{aB}	0.3±0.0^{bB}	0.2±0.0^{a}	0.3±0.0^{b}
C20:4n6	0.9±0.2^{a}	1.0±0.1^{ab} ^{A}	0.9±0.0^{abc}	0.7±0.0^{acd}	0.6±0.0^{d}	0.6±0.1^{bB}	0.9±0.0^{ac}	0.6±0.0^{b}	0.7±0.1^{ab}
C20:4n3	1.3±0.2^{a}	1.4±0.1^{a}	1.6±0.1^{a}	1.3±0.2^{a}	1.6±0.0^{a}	1.2±0.1^{ab}	1.6±0.1^{a}	2.0±0.5^{ac}	1.5±0.2^{a}
C20:5n3 (EPA)	8.7±0.4^{a}	7.7±0.4^{a}	8.8±0.6^{a}	8.5±0.8^{a}	9.2±0.7^{a}	9.0±0.6^{a}	8.4±0.2^{a}	9.1±0.6^{a}	7.9±0.8^{a}
C22:6n3 (DHA)	11.2±0.7^{a}	11.9±0.2^{a}	12.5±1.1^{a}	12.2±0.9^{a}	12.0±0.8^{a}	12.1±1.2^{a}	12.3±0.8^{a}	12.5±0.7^{a}	11.3±0.6^{a}
PUFA	**33.8±0.8**^{a}	**34.4±0.3^{a}**	**36.3±1.8^{a}**	**34.7±0.3^{a}**	**36.3±1.0^{aA}**	**35.2±2.0^{a}** ^{b}	**36.9±1.7^{b}**	**36.9±1.7^{b}**	**32.7±1.6^{aB}**
PI	**1.3±0.1**^{a}	**1.4±0.1^{a}**	**1.5±0.1^{a}**	**1.4±0.2^{b}**	**1.5±0.1^{aA}**	**1.6±0.1^{ab}**	**1.7±0.1^{ab}**	**1.5±0.1^{bc}**	**1.8±0.0^{cB}**

$^{a–d}$Different lowercase superscript letters in each raw indicate a significant difference between months of storage ($p < 0.05$).

$^{A–B}$Different upperrcase superscript letters in each raw indicate a significant difference between temperaturs at the same storage time ($p < 0.05$).

Table 2b Changes in fatty acid profile (g fatty acids/100 g total lipids, mean ± SD) of mackerel caught in September, during frozen storage at -25 ˚C and -18 ˚C for 3, 6 and 12 months.

Fatty acids	September								
	Control	-25 °C				-18 °C			
		3 months	6 months	9 months	12 months	3 months	6 months	9 months	12 months
C14:0	7.9±1.0^{a}	6.9±0.6^{a}	6.0±0.1^{a}	8.1±0.2^{a}	6.5±1.8^{a}	6.8±1.3^{a}	6.6±0.6^{a}	7.5±0.9^{a}	7.3±0.4^{a}
C16:0	12.6±1.2^{a}	11.7±1.1^{a}	13.2±0.4^{a}	13.0±0.9^{a}	12.3±1.8^{a}	12.3±0.6^{a}	11.5±0.8^{a}	12.8±0.7^{a}	11.3±1.0^{a}
C17:0	0.3±0.0^{a}	0.4±0.0^{c}	0.6±0.1^{b}	0.5±0.0^{b}	0.5±0.0^{b}	0.4±0.1^{b}	0.6±0.0^{c}	0.5±0.1^{bd}	0.6±0.0^{cd}
C18:0	1.8±0.0^{a}	1.9±0.3^{a}	2.3±0.1^{a}	1.7±0.1^{a}	2.0±0.8^{a}	2.1±0.5^{a}	2.0±0.4^{a}	2.0±0.2^{a}	1.7±0.2^{a}
SFA	**23.0±2.2^{a}**	**21.4±0.8^{a}**	**22.5±0.7^{a}**	**23.8±1.0^{a}**	**21.9±1.2^{a}**	**22.1±0.6^{a}**	**21.3±0.7^{a}**	**23.3±0.5^{a}**	**21.5±0.8^{a}**
C16:1n9	0.2±0.1^{a}	0.3±0.0^{b}	0.4±0.0^{c}	0.3±0.0^{bc}	0.2±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}	0.2±0.0^{a}
C16:1n7	3.7±0.5^{a}	3.6±0.9^{a}	3.2±0.0^{a}	3.2±0.1^{a}	3.6±0.5^{a}	3.6±0.9^{a}	2.9±0.1^{a}	3.7±0.8^{a}	3.5±0.3^{a}
C18:1n11	0.4±0.0^{a}	0.4±0.1^{a}	0.4±0.0^{a}	0.4±0.0^{a}	0.3±0.1^{a}	0.4±0.1^{a}	0.5±0.1^{a}	0.4±0.0^{a}	0.4±0.0^{a}
C18:1n9	7.1±1.5^{a}	7.4±2.0^{a}	13.2±2.1^{a}	8.0±0.8^{a}	8.9±3.6^{a}	8.5±3.7^{a}	7.5±2.0^{a}	9.0±0.9^{a}	6.4±1.3^{a}
C18:1n7	1.7±0.2^{a}	1.8±0.6^{a}	2.2±0.4^{a}	1.4±0.1^{a}	1.9±1.1^{a}	1.9±0.5^{a}	1.4±0.2^{a}	1.8±0.1^{a}	1.5±0.3^{a}
C18:1n5	0.4±0.1^{a}	0.3±0.0^{ab}	0.5±0.0^{ac}	0.5±0.1^{ac}	0.4±0.0^{abc}	0.4±0.1^{ab}	0.5±0.1^{abc}	0.5±0.0^{ac}	0.4±0.0^{abc}
C20:1n11	0.7±0.1^{a}	0.7±0.1^{a}	0.6±0.1^{a}	0.6±0.0^{a}	0.6±0.1^{a}	0.7±0.0^{a}	0.8±0.1^{a}	0.6±0.0^{a}	0.7±0.0^{a}
C20:1n9	8.4±1.9^{a}	7.9±1.5^{a}	6.4±0.5^{a}	6.6±0.6^{a}	7.8±1.5^{a}	7.8±0.3^{a}	7.9±0.8^{a}	6.6±0.3^{a}	8.4±1.4^{a}
C20:1n7	0.2±0.1^{a}	0.3±0.1^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.3±0.1^{a}	0.3±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}
C22:1	13.2±3.1^{a}	12.2±1.7^{a}	8.6±1.3^{a}	9.7±0.8^{a}	11.5±2.3^{a}	12.2±0.9^{a}	12.4±2.2^{a}	9.5±0.8^{a}	12.1±1.4^{a}
C24:1	0.7±0.1^{a}	0.7±0.1^{a}	0.6±0.0^{a}	0.6±0.0^{ab}	0.5±0.0^{b}	0.7±0.1^{ac}	0.6±0.1^{a}	0.6±0.1^{ab}	0.5±0.0^{b}
MUFA	**36.8±4.2^{a}**	**35.6±1.1^{a}**	**36.4±4.2^{a}**	**31.7±0.7^{a}**	**36.2±2.5^{a}**	**36.9±3.2^{a}**	**35.2±1.2^{a}**	**33.4±0.9^{a}**	**34.4±1.3^{a}**
C16:2n4	0.5±0.1^{a}	0.5±0.1^{a}	0.4±0.0^{b}	0.5±0.0^{a}	0.4±0.0^{b}	0.5±0.1^{a}	0.4±0.0^{ac}	0.6±0.1^{ab}	0.4±0.0^{c}
C18:2n6	1.5±0.1^{a}	1.7±0.3^{a}	1.6±0.3^{a}	1.7±0.0^{a}	1.5±0.2^{a}	1.6±0.3^{a}	1.8±0.1^{a}	1.6±0.2^{a}	1.8±0.1^{a}
C18:3n3	1.6±0.3^{a}	1.5±0.3^{a}	2.0±0.7^{ab}	2.3±0.1^{b}	1.5±0.5^{a}	1.4±0.3^{ab}	1.5±0.1^{a}	2.0±0.5^{ac}	1.8±0.0^{a}
C18:4n3	4.8±0.7^{a}	5.0±1.3^{a}	4.9±1.1^{a}	7.0±0.3^{a}	5.0±2.0^{a}	4.3±0.8^{a}	5.3±0.4^{a}	5.7±0.9^{a}	5.6±0.5^{a}
C20:2	0.3±0.0^{a}	0.3±0.0^{a}	0.2±0.0^{a}	0.3±0.0^{a}	0.2±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.2±0.0^{a}	0.3±0.0^{a}
C20:4n6	1.3±0.1^{a}	1.0±0.1^{bc}	0.8±0.2^{bd}	1.0±0.1^{b}	0.9±0.1^{b}	1.1±0.1^{b}	0.7±0.1^{c}	0.9±0.1^{bd}	0.8±0.1^{cd}
C20:4n3	1.1±0.3^{a}	1.0±0.1^{a}	1.1±0.3^{a}	1.6±0.1^{b}	1.9±0.3^{c}	1.1±0.1^{a}	1.1±0.2^{a}	1.5±0.1^{b}	1.7±0.1^{b}
C20:5n3 (EPA)	8.0±0.5^{a}	8.2±0.7^{a}	8.5±0.6^{a}	8.3±0.2^{a}	8.2±0.4^{a}	8.2±0.4^{a}	7.8±1.0^{a}	8.9±1.1^{a}	8.1±0.3^{a}
C22:6n3 (DHA)	11.6±0.6^{a}	13.3±0.4^{b}	12.4±1.2^{a}	12.2±0.2^{a}	12.0±0.7^{aA}	13.2±1.4^{b}	14.3±0.2^{bc}	12.1±0.6^{ab}	12.8±0.7^{ab}
PUFA	**31.8±2.1^{a}**	**33.6±1.4^{a}**	**33.1±3.9^{a}**	**35.9±0.5^{a}**	**33.0±1.9^{a}**	**32.8±2.3^{a}**	**34.4±1.6^{a}**	**34.8±0.8^{a}**	**34.5±0.9^{a}**
PI	**1.6±0.1^{a}**	**1.8±0.1^{a}**	**1.7±0.2^{a}**	**1.6±0.2^{a}**	**1.9±0.2^{a}**	**1.6±0.1^{a}**	**1.6±0.2^{a}**	**1.7±0.2^{a}**	**1.9±0.1^{a}**

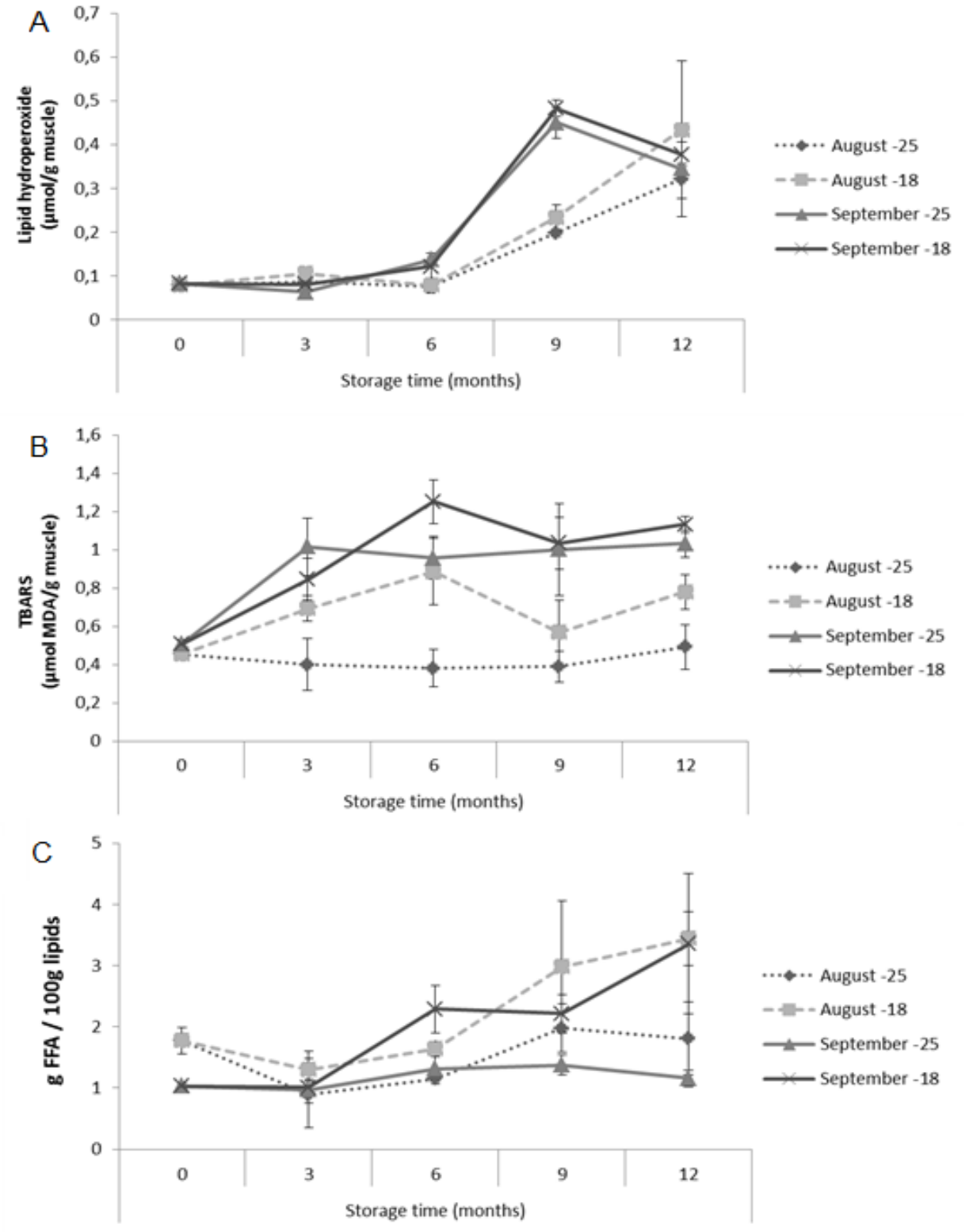

Figure 2 Development of lipid hydroperoxide value (PV; µmol/kg sample); thiobarbituric acid reactive substances (TBARS; µmol MDA/kg samples); and free fatty acids (FFAs; g /100g lipids) of mackerel caught at different season (August, September) during frozen storage at -25 ˚C and -18 ˚C for 0, 3, 6, 9 and 12 months. Bars represent standard deviation (n = 3).

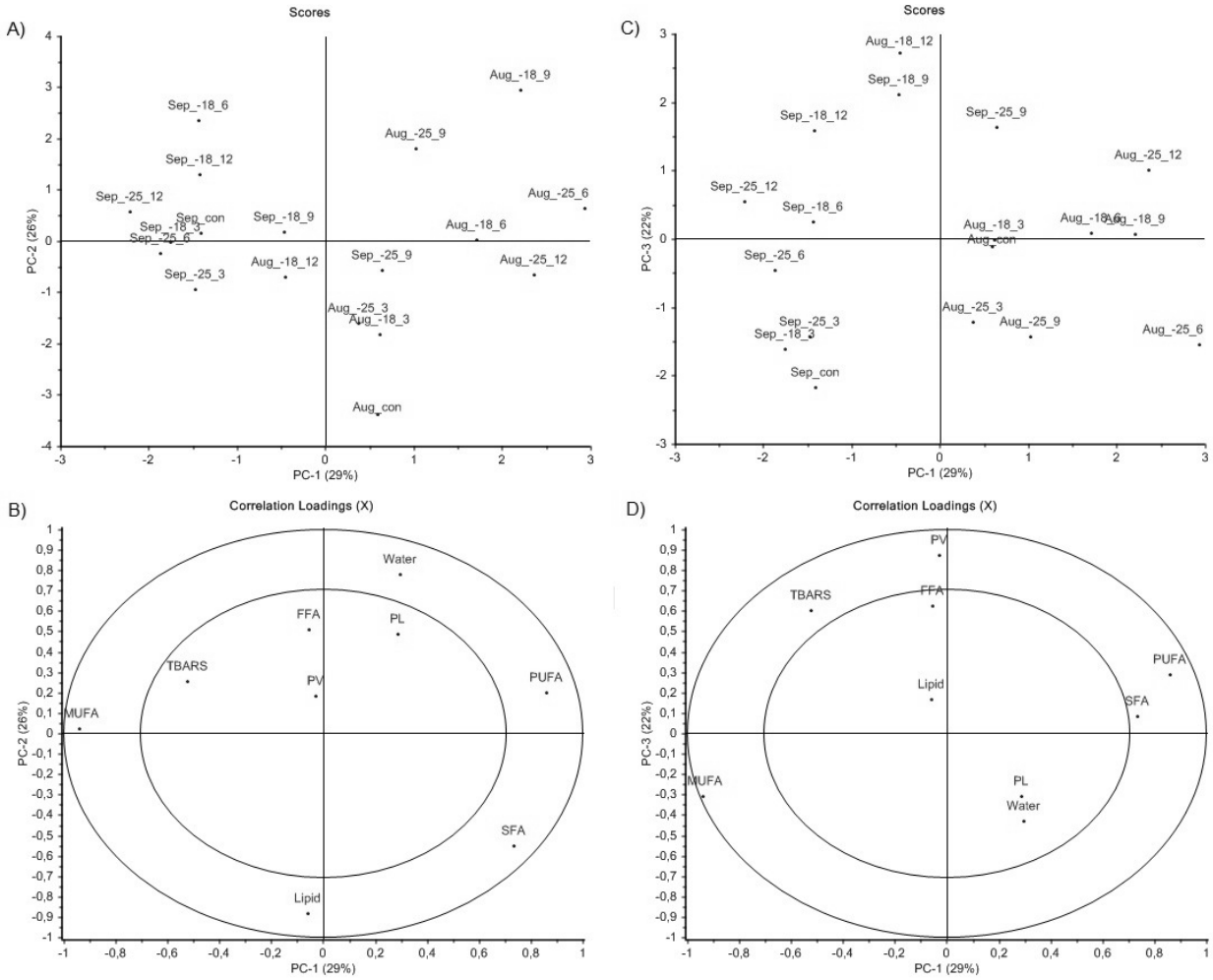

Figure 3 Scores and correlation loadings from PC1, PC2 (A and B) and PC3 (C and D) from the principal component analysis (PCA) of frozen mackerel muscles. All samples and analytical parameters were used. Aug indicates fish caught in August, and Sep fish caught in September. -18 and -25 in the sample description refer to the storage temperature (°C). The last number of the sample description indicates the storage time (months).