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Seasonal and geographical variation in chemical composition and lipid stability of Atlantic mackerel (*Scomber scombrus*) caught in Icelandic waters

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Report summary

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Ágrip á íslensku:	Á þeim tíma sem makríll er við íslandsstrendur er hann í miklu æti sem veldur því að hann snögg fitnar með þeim afleiðingum að holdið verður mjög viðkvæmt fyrir meðhöndlun. Í þessari rannsókn var makríll sem var veiddur sumarvertíðarnar 2012 og 2013 (júlí, ágúst, september) og frá mismunandi veiðisvæðum (austur, norðaustur, suður og suðaustur) skoðaður. Til þess að meta á hversu vel hráefnið hentar til vinnslu á hágæðaafurðum til manneldis, var makríllinn mældur m.t.t. vatns- og fituinnihalds, fitusýrusamsetningar, litar, þránunar og frírra fitusýra. Almennt var makríllinn sem safnað var sumarið 2012 af betri gæðum en makríll frá 2013. Niðurstöðurnar gáfu einnig til kynna breytileika á milli veiðimánaða m.t.t. fituinnihalds og framgang þránunar. Makríll sem var veiddur um miðbik vertíðarinnar hafði lægsta þránunargildið, sem gefur til kynna að sá makríll hentar best fyrir vinnslu á hágæðaafurðum til manneldis.		
Lykilorð á íslensku:	*Makríll; árstíðarbreytileiki; veiðisvæði; efnasamsetning; stöðugleiki fitu*		

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Summary in English:	Atlantic mackerel (*Scomber scombrus*) appears in Icelandic waters during its heavy feeding period, resulting in variation in mackerel products quality. Fish caught at different season during the summers of 2012 and 2013 (July, August, September) and at different sites of the Icelandic fishing area (East, Northeast, South and Southeast) were analysed. Measurements of lipid and water content, fatty acid composition, colour changes, lipid hydroperoxide (PV), thiobarbituric reactive substances (TBARS) and free fatty acid (FFA) were studied with the aim of investigating whether this raw material was suitable for the production of high quality products for human consumption. In general, samples collected during the summer of 2012 showed a better condition than fish from 2013. The results indicated seasonal variation in lipid content and rancidity development. The lowest rancidity values were observed in the middle of the Icelandic catching season, indicating that this raw material was best suited for production of high quality products. Moreover, geographical variation of the mackerel catches had an impact on the saturation of the fatty acids, and appeared as follows: East > Southeast > Northeast > South.
English keywords:	*Atlantic mackerel, seasonal variation, geographical variation, composition, lipids stability*

Contents

1 Introduction

Atlantic mackerel (*Scomber scombrus*) constitute an excellent source of omega-3 polyunsaturated fatty acids (PUFAs) which makes it a valuable specie seen from a health perspective (Orban, Di Lena, Nevigato, Masci, Casini & Caproni, 2011). Previous studies have shown beneficial roles of marine omega-3 PUFAs in regards to health conditions such as: coronary heart disease, high blood pressure, rheumatoid arthritis, and possibly diseases of central nervous system, such as depression (Delgado-Lista, Perez-Martinez, Lopez-Miranda & Perez-Jimenez, 2012; Perica & Delas, 2011).

Atlantic mackerel is a novel specie for the Icelandic fish industry. It was discovered in great quantities in 2007 within the Icelandic fishing area and has since then gained a great commercial importance. This pelagic fish, well known from its long distance migration, appears in Icelandic waters during the summer period (June – September) in order to find larger and richer feeding areas to rebuild its muscle lipids and to restore energy sources after spawning and travelling period (Astthorsson, Valdimarsson, Gudmundsdottir & Oskarsson, 2012; Overholtz, Hare & Keith, 2011; Valdimarsson, Astthorsson & Palsson, 2012). The feeding migration of Atlantic mackerel has changed in the last decade and it has been observed that its presence within the Icelandic fishing area is highly related to ocean warming (Hannesson, 2013). Mackerel was initially only discovered in the South and Southeast of Iceland, where the ocean temperature reached 10 °C - 12 °C. Mackerel has later been observed migrating further to the East of Iceland (ocean temperature around 7 °C - 9 °C), where it has been found in large quantities since. In addition, recently it has been spotted in relatively small amounts in the Northeast (5 °C - 7 °C) of Iceland (Nøttestad et al., 2015; Nøttestad et al., 2012; Nøttestad et al., 2013).

Mackerel migrations patterns are very unstable and are influenced by oceanographic conditions (Iversen, 2002). Variability of external factors such as size of the stock, ocean temperature, feed condition, feed availability and competition for feed with other species, such as herring, may negatively affect the biological condition of the mackerel, and hence affect the quality and stability of the initial raw material intended for further processing. Moreover, the heavy feeding

period and the variation in muscle lipid content, as well as variation in biological conditions may lead to great fluctuations in the quality of the Icelandic mackerel. There is limited information on how the seasonal and geographical variation may affect the lipid characteristics and fatty acid distribution in Atlantic mackerel. Therefore, little is known about the characteristics and processability of Atlantic mackerel, caught during the summer months, and whether this raw material is suitable for the production of high quality products for human consumption. Thus in-depth analyses are required.

The main emphasis of the present study was to investigate the impact of different catching grounds (East, Northeast, South, and Southeast of Iceland) and seasons (middle and end of July, beginning, middle and end of August, and beginning of September) on the composition and lipid stability of Atlantic mackerel caught in Icelandic waters. The variation in the quality of mackerel between different years of catch (2012, 2013) was also studied.

2 Materials & Methods

2.1 Raw material and sampling

Atlantic mackerel (*Scomber Scombrus*) was caught during the summer time (July – September) in the years 2012 and 2013. Collection of the samples was carried out approximately every 10 days. Correspondingly, samples collected between the 1st and the 10th day of the month are referred to as fish from the *beginning of each particular month* (e.g. beginning August, beginning September); fish sampled during the 11th to the 20th day of the month are referred to as fish caught in the *middle of the month* (e.g. middle July, middle August); and fish caught during the 21st and the 31st day are referred to as fish caught at the end of the month (e.g. end July, end August). Additionally, samples were collected at different sites in the Icelandic waters (Northeast Atlantic Ocean - FAO no 27) to give an indication of geographical differences on the condition of the mackerel.

Samples collected at the end of July 2012 were from the East and the Northeast fishing areas around Iceland. Fish caught in the beginning and end of August 2012 were only from the East, while samples collected in the beginning of September 2012 were only from the Northeast.

Correspondingly, samples collected in 2013 during the middle of July and beginning of August were both from the East and Northeast of Iceland, while samples from the middle of August 2013 were only from the Southeast. Samples from the end of August 2013 were then only from the South. Information of year, season and area of catch is displayed in all figures and tables.

The mackerel was caught by trawls and frozen as whole using an air-box freezing method. Analyses of the samples were performed within one week from the time of catch. All samples were thawed at room temperature for approximately 17 hours prior to further processing. 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 analyses were of analytical grade, and were purchased from Fluka (Buchs, Switzerland) or Sigma-Aldrich (Steinheim, Germany / St. Louis, MO, USA).

2.2 Water and total lipid content

The water content of the mackerel samples was determined by the weight difference during drying of a 5 g minced fillets at 104 ± 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 and Dyer (1959). The lipid content was determined gravimetrically and the results were expressed as g lipid / 100 g of the sample.

2.3 Fatty acid profile

The fatty acid profile of the samples was determined on the TL extracts by gas chromatography (Varian 3900 GC, Varian, Inc., Walnut Creek, CA, USA) of fatty acid methyl esters (FAMEs), 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), a 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.

Analyses were neither performed on samples from the middle of July 2013 (East/Northeast of Iceland), nor at the beginning of August 2013 (East of Iceland) due to lack of availability of samples at these otherwise potential sampling occasions.

2.4 Colour analysis

The colour intensity was measured on the muscle surface of the fillets by use of a Minolta CR-300 chromameter (Minolta Camera Co., Ltd; Japan) using the Lab* system (CIE, 1976) with a

CIE Illuminant C. The instrument recorded the L* value (lightness), a* value (redness) and b* value (yellowness) on the CIELAB colour scale. The colour was measured at three positions of the light muscle surface of fillets (2x loin, 1x tail) and the average L*, a* and b* values of the measurements for each fish were used to calculate the mean and standard deviation.

2.5 Lipid oxidation products

2.5.1 Lipid hydroperoxide values

A modified ferric thiocyanate method was used to determine the lipid hydroperoxide (Shantha & Decker, 1994). Five grams of samples 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) and 5 mL of sodium chloride (0.5 M) was added to the mixture, which was homogenized at 2400 rpm for 10-20 sec. (Ultra-Turrax T25 basic, IKA Labortechnik, Germany). Phase separation was facilitated by centrifugation at 5100 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 a chloroform: methanol (1:1) solution. 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 Sunrise, Austria) in 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.

2.5.2 Thiobarbituric acid reactive substances (TBARS)

TBARS was determined with a modified method of Lemon (1975). The sample (5g) 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 5100 rpm for 20 min at 4 °C (Beckman Coulter TJ-25, Rotor TS-5.1-500, USA) the collected supernatant was filtrated with Whatman qualitative filter paper no 4. Thiobarbituric acid (0.02M) in amount of 900 µL was mixed with the collected supernatant (100

µl) before heating in a water bath at 95 °C for 40 min. After heating, the mixture was immediately placed on ice for cooling and the absorbance measured at 530 nm (Tecan Sunrise, 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.

2.6 Enzymatic lipid hydrolysis

Free fatty acid (FFA) content was determined using the method of Lowery and Tinesley (1976) with a modification according to Bernardez, Pastoriza, Sampedro, Herrera and Cabo (2005). The absorbance was read at 710 nm (UV-1800 spectrophotometer, Shimadzu, Japan) and the amount of free fatty acids 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.

2.7 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 individual samples (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

3.1.1 Water and lipid content

The water and lipid contents of Atlantic mackerel caught at different seasons and at different location within the Icelandic fishing area is presented in **Figure 1**. Variations in lipid and water content of the Atlantic mackerel were highly associated with its catching time, both catching year and season. The geographical location, i.e. the fishing area, also affected the chemical composition of the collected Atlantic mackerel.

Significant difference in lipid content was observed between the different years where fish caught in 2012 reached a higher content (26.5 ± 7.4%) in comparison to the fish caught in 2013 (20.3 ± 4.5%). On the contrary, the water content was at a significantly higher level in fish caught in 2013 (59.0 ± 3.7%) than in 2012 (55.0 ± 3.7%). This might be related to the ocean temperature changes as well as size of the mackerel stock present around Iceland during the summers of 2012 and 2013. As reported by Nøttestad et al. (2013); 2012) the total count for Atlantic mackerel in Northeast Atlantic ocean was higher in 2013 than in 2012 which resulted in increased competition for feed.

The water content in the samples from 2012 showed a significant increase from the end of July (53.0 ± 2.3%), until end of August (59.6 ± 2.8%), followed by a slight decrease in the beginning of September (55.5 ± 2.9%). However, results obtained from samples collected in 2013 showed no significant seasonal variation in water content, although similar seasonal patterns were observed as for 2012. The lipid content did not vary significantly with different season of catch, although a reverse pattern to the water content pattern was observed, where greater values were recorded in the second month of the Icelandic catching season (end of July 2012 / middle of July 2013), followed by a drop in lipid content at the end of August (both years), where it slightly increased again in the beginning of September for both years. These results are in line with the fact that the Atlantic mackerel starts to appear in Icelandic water in June when the heavy feeding takes place

after a starvation period (spawning, migration) (Brix, Apablaza, Baker, Taxt & Gruner, 2009). In July, the mackerel is able to obtain sufficient feed to supply energy and restore lost lipids, which appeared in the highest peak of the lipid content in this month. Further in August and September, feed sources are increasingly limited due to decrease of the biomass and increased competition for feed, especially with herring (Astthorsson et al., 2012; Overholtz et al., 2011; Valdimarsson et al., 2012). Accordingly, a slight drop of the total lipid content could be observed at the end of summer.

The lipid content was affected by the different location of catch during the summer of 2012 and was significantly higher for samples collected Northeast of Iceland (29.7 ± 7.2%) in comparison to samples collected East of Iceland (22.1 ± 5.2%). The lipid content of fish caught in 2013 did not show significant geographical variation. In general, the mackerel stock distribution was more abundant in the East than in Northeast. The present findings may be associated with ocean temperature, feed condition and availability, and competition for feed with other species (Hannesson, 2013). Furthermore, catches of mackerel in 2012 in the Northeast of Iceland were conducted more off-shore, where there is less competition for feed and richer feeding grounds. Therefore fish from that region may be fattier (Oskarsson, 2013).

3.1.2 Fatty acid profile

The fatty acid composition of the Atlantic mackerel was relatively stable throughout the catching seasons at both years (2012, 2013), although some variation was observed between the different fishing location **(Table 1).** The fatty acid profile of the mackerel was characterised by high amounts of monounsaturated fatty acids (MUFA; 35.4 ± 1.8%) and polyunsaturated fatty acids (PUFA; 32.8 ± 1.1%), along with minor proportion of saturated fatty acids (SFA; 22.5 ± 1.4%). The Atlantic mackerel was shown to be a good source of omega 3 fatty acids (29.5 ± 1.2%) and displayed a high n-3/n-6 PUFA ratio**.** These results are in agreement with previous studies on horse mackerel (Orban et al., 2011).

No significant seasonal variation in the major fatty acid classes was recorded. However, the present results indicated a higher degree of saturation of the fatty acids in the beginning of the summer and slightly lower in the end of the catching season. Regarding the impact of different catching grounds on the fatty acid composition, a significant variation was observed in the SFA and PUFA levels, where higher values were recorded for samples collected East of Iceland (25.5 ± 0.7%, 33.8 ± 0.8%) in comparison to the Northeast of Iceland (21.6 ± 0.6%, 32.2 ± 1.0%) at the same catching season (end of July 2012), respectively. At the same time, the MUFA level showed an opposite geographical pattern with higher values recorded Northeast (36.8 ± 1.2%) in comparison to the East (32.9 ± 1.4%). The degree of unsaturation of the mackerel fatty acids might be related to the seasonal variation in lipid content due to difference in the environmental conditions, such as ocean temperature and feed availability/composition (Bandarra, Batista, Nunes & Empis, 2001; Celik, 2008; Kainz, Arts & Mazumder, 2004; Osako, Yamaguchi, Kurokawa, Kuwahara, Saito & Nozaki, 2003). Further, the ocean temperature, affects the fluidity and permeability of the cell membranes (Henderson & Tocher, 1987). As reported before, the highest temperatures were recorded in the South of Iceland and the lowest in the Northeast (Nøttestad et al., 2012; Nøttestad et al., 2013). Therefore, it can be assumed that the fatty acids saturation rate decreases with warmer ocean temperature (Henderson et al., 1987; Orban et al., 2011).

Among the SFA, palmitic acid (16:0) was the predominant fatty acid, followed by myristic acid (C14:0) and stearic acid (18:0). The **s**tearic acid level was higher in the summer of 2012 than in

2013 ($p < 0.05$). Analysis of the geographical location of the catch showed a higher level of the palmitic acid in the East and lower in the Northeast ($p < 0.05$).

The major fatty acid among the MUFA, 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). The oleic acid amount was significantly higher in the fish caught in 2012 in comparison to year 2013.

Furthermore, docosapentaenoic acid (DHA, C22:6*n*-3) was the predominant fatty acid amongst the PUFAs, followed by eicosapentaenoic acid (EPA, C20:5*n*-3) and stearidonic acid (C18:4*n*-3), with a minor amount of α-linoleic acid (C18:3*n*-3) and linoleic acid (C18:2*n*-6). DHA levels in fish from 2012 significantly increased from the end of July until the beginning of August, then decreased once more at the end of August, followed by a significant increase in the beginning of September. No significant changes were recorded in 2013 between different seasons of catch, which may be related to the lack of the results from the beginning of the catching season. Correspondingly, EPA level was observed to be significantly higher in 2012 (8.2 ± 0.5%) than in 2013 (7.5 ± 1.3%). No significant seasonal variation, but similar seasonal patterns to DHA levels were observed. Furthermore, a significant difference was observed in EPA levels between different locations of catch, where fish caught in the East showed the highest values (8.7 ± 0.5%) in comparison to the fish from the Northeast (7.7 ± 0.2%) where the EPA amount was the lowest (end July 2012).

In order to maximize the value of Atlantic mackerel, a proper utilization method are required depending on the nature of the raw material. In this manner, it is recommended to utilize fish characterized by high nutritional value (dominant PUFA, omega-3 fatty acids) and great fat content for human consumption with use of freezing techniques and/or smoking methods. Fish characterized by dominated level of MUFA and high water content is better suited for canning and fishmeal production (Keay, 2001; Murray & Burt, 2001; Sveinbjörnsson, Guðmundsdóttir et al. 2008).

A high n-3/n-6 PUFA ratio, which constitutes an index for lipid quality, indicted a high nutritional importance of the Atlantic mackerel in the study. These results are in agreement with previous researches on horse mackerel (Orban et al., 2011) and cod (Nguyen, Thorarinsdottir, Thorkelsson, Gudmundsdottir & Arason, 2012).

The polyene index (*PI*) may provide information regarding the stability of the fish lipids by indication of the oxidative rancidity rate (Bragadottir, 2001). Thus, results from the mackerel caught in 2012 showed a significant negative correlation between *PI* and FFA (r = -0.75), as well as with SFA (r = -0.87) (Table 2), whereas positive correlation between *PI* and peritoneum deterioration (r = 0.84; data not shown). Fish from year 2013 also displayed a significant negative correlation between *PI* and SFA (r = -0.88), whereas a positive correlation was observed between PI and PUFA levels (r = 0.77). The fatty acid analysis of the samples from 2012 showed a significant negative correlation of PUFA with MUFA (r = -0.90), as well as between MUFA and SFA (r = -0.74). However, samples from 2013 showed a negative correlation between PUFA and SFA (r = -0.79). Additionally, mackerel caught in 2013 showed a significant positive correlation between lipid content and SFA (r = 0.97) and negative with PUFA (r = -0.82), while samples collected in 2012 did not show any correlation between the lipid content and fatty acid composition. Consequently, the analysis of the polyene index of Atlantic mackerel indicated better stability of the lipid in the fish caught in 2012 in comparison to the fish caught in 2013. Furthermore, seasonal variation in the fish from 2012 was observed ($p < 0.05$), where the highest polyene index was recorded for fish caught in the beginning of August and the lowest in the end of July and August.

3.2 Physical properties

3.2.1 Colour

Variation in the colour of Atlantic mackerel fillets, as affected by catching year, season and location appears in **Figure 2**. Significant difference between years of catch was displayed in the yellowness analysis, where fish from 2012 was recorded with higher b* values than fish from 2013. Redness of the mackerel fillets did not vary significantly between years of catch, although each year showed a different seasonal pattern. A constant decrease of muscle redness during the summer time was observed in fish from 2012, whereas fish from 2013 showed a decrease of redness from the middle of July until the middle of August, followed by a significant increase from the end August until the beginning of September. A similar seasonal pattern was seen in yellowness, although these changes were not significant. Furthermore, colour results showed interesting variation in the yellowness between different locations of catch. In the middle of July

2013 the yellowness of the fish muscle showed higher b* values ($p < 0.05$) for the fish caught in the East (6.3 ± 0.1) than in the Northeast (4.9 ± 0.8). Moreover, results of the redness showed a reverse geographical pattern, where fish from end July 2012 showed higher a* values for the fish from the East (5.8 ± 4.3) and lower from the Northeast (3.4 ± 1.2). Thus in the middle of July 2013 higher a* values were also observed in the Northeast (2.5 ± 1.2) than in the East (1.8 ± 0.6). A significant positive correlation between redness and FFA ($r = 0.79$) and negative correlation between redness and peritoneum deterioration ($r = -0.78$; data not shown) was observed in fish from 2012, which may have indicated a higher enzymatic activity, accelerated by a higher nonheme iron amount present from myoglobin (Chaijan, Benjakul, Visessanguan & Faustman, 2005; Rawdkuen, Jongjareonrak, Benjakul & Chaijan, 2008). Variation in redness may be related to the migration time, and thus higher muscle activity of the particular fish. Studies conducted on sardine and mackerel, reported high amount of the dark muscle fibres, due to high levels of lipids and myoglobin. It is also well known that myoglobin, as a heme protein, among other functions provides the colour of the fish muscle, which may vary due to concentration, as well as on the interaction of myoglobin with other compounds (Chaijan et al., 2005). Redness of fish from 2013 showed significant positive correlation with MUFA ($r = 0.94$) and TBARS ($r = 0.83$) which may be due to a high rate of lipid oxidation observed. Additionally, a significant positive correlation was recoded for b* value and MUFA ($r = 0.81$), while a negative correlation was observed to PUFA ($r = -0.79$) in fish from 2013. These results are in general agreement with previous findings on frozen herring fillets (Hamre, Lie & Sandnes, 2003) and cuttlefish (Thanonkaew, Benjakul, Visessanguan & Decker, 2006), which indicated that muscle discoloration may be due to formation of yellow fluorescent pigments as a product of lipid oxidation.

3.3 Lipid deterioration

Primary (Figure 3A) and secondary oxidation products (Figure 3B), as well as free fatty acids formation (Figure 3C), with regard to different catching year, season and location, are summarized and presented in **Figure 3**. Both PV and TBARS results indicated higher lipid deterioration in the fish caught in 2013 in comparison to the fish from 2012 ($p < 0.05$). Further, the lipid stability varied with season of catch. Primary and secondary oxidation products, as well as free fatty acid were at a high level in the beginning of the catching period (end of July 2012 / middle of July, beginning of August 2013), but then decreased in the middle of the summer time (beginning of August / middle of August 2013). Furthermore, in the end of the catching season (end of August and beginning of September) in both years, an increase of oxidation products could be observed once more. These changes may have been related to the total lipid content variation due to differences in accessibility of the feed source. It is well known that fattier fish is highly susceptible to the lipid oxidation and the present findings are in general agreement with previous studies (Aubourg, Rodríguez & Gallardo, 2005). It can be also assumed that the phospholipid content of the mackerel was not constant during the catching time and may have been at a higher level in the season corresponding to higher total lipid content (Bandarra et al., 2001). Furthermore, fish collected in 2012 showed significant negative correlation between FFA and lightness ($r = -0.79$) and polyene index ($r = -0.75$), as well as a positive correlation with redness ($r = 0.79$) and SFA ($r = 0.83$). These findings may indicate that MUFA concentrations may be affected by lipid hydrolysis and therefore to enzymatic activity. On the other hand, samples collected in 2013 showed negative correlation of FFA with SFA ($r = -0.69$), and a positive correlation with PUFA ($r = 0.84$) and the polyene index ($r = 0.75$).

Variation in the lipid stability seemed to be related also to the geographical location of the catch. Lipid oxidation products, as well as lipid hydrolysis products, were observed to be higher in the samples collected in the East coast of Iceland in comparison to the Northeast region, at the same catching period. Furthermore, slightly lower lipid deterioration was recorded in fish caught in the South and further in the Southeast, but these results may have been affected by the seasonal changes and not only with the location of the catch.

3.4 Multivariate data analysis

A principal component analysis (PCA) was carried out to obtain a summary of the changes in the samples and how the quality measurements (PV, TBARS, FFA, water and total lipid content, SFA, MUFA, PUFA, b* value, a* value) were affected by the experimental variables (catching year, season and location). Two PCs described 61% of the sample variation. The scores and correlation loads from the first and second principal components (PC1, PC2) are shown in **Figure 4.**

The first principal component, representing 34% of the total variation, described the differences between the samples caught at different year with regard to the proximate content (water vs. lipid) and fatty acid composition (PUFA vs. MUFA). Furthermore, PC1 showed close correlation between the fatty acid compositions towards increased lipid oxidation and changes in physical properties. Accordingly, b* value displayed strong correlation with TBARS in samples from 2012 ($r = 0.74$), and correlations towards MUFA ($r = 0.81$) and PUFA ($r = -0.79$) in samples from 2013 (**Table 2**). The second principal component (PC2), representing 27% of the total variation, described catching season and place of catch as the main contributors for the variation between the samples. The differences in PC2 were due to the stability factors, especially lipid deterioration (PV, TBARS, FFA) and fatty acid saturation degree (SFA vs. MUFA+PUFA unsaturated fatty acids ratio), as well as changes in changes in colour (redness).

According to PC1, samples collected during the summer of 2012 showed better quality and higher nutritional value than fish from 2013. Furthermore, changes in the major fatty acid classes, as well in proximate content, were dependent on seasonal variation (especially in samples from 2012) and the differences in geographical location. Differences in the total lipid content of the mackerel muscle were mainly due to variation in feed availability and competition for feed at each catching season. Geographical variation of the mackerel catches had an impact on the saturation of the fatty acids, and appeared as follows: East > Northeast. These changes were related to the ocean temperature (Nøttestad et al., 2012; Nøttestad et al., 2013), as well as feed availability and its composition (Kainz et al., 2004). According to PC2, more progressive lipid oxidation (PV, TBARS) and hydrolysis (FFA) was observed for mackerel in the beginning of summer followed by a decrease in the middle of the summer and increase once again in the end of catching season.

Furthermore, the development of secondary oxidation products (TBARS), and lipid hydrolysis products (FFA) displayed an increase in redness. Correspondingly, a* value gave positive correlation between FFA ($r = 0.79$) in samples from 2012, and between TBARS ($r = 0.83$) in samples from 2013.

4 Conclusions

Mackerel as a novel specie in Iceland required an in-depth analysis on how the seasonal and geographical variation may affect physicochemical properties of mackerel as a raw material for further processing was investigated. Atlantic mackerel from the Icelandic waters was characterized with a high nutritional value due to high amount of omega-3 PUFA, as well as a high n-3/n-6 ratio at a stable level during the whole catching season. The present results indicated a high commercial value of the Icelandic mackerel. On the other hand, high seasonal variation in the total lipid content and lipid deterioration was observed. Stability of the lipid was highly correlated to the total lipid content, where fattier fish was more prone to lipid oxidation and hydrolysis. Differences in the total lipid content of the mackerel muscle were mainly due to variation in feed availability and competition for feed at each catching season. Furthermore, the geographical location of the mackerel catches had an impact on the unsaturation of the fatty acids. These changes were related to the ocean temperature, where the highest temperatures were recorded in the East (high saturation degree) of Iceland and the lowest in the Northeast (lower saturation degree).

5 Acknowledgement

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7 Figures and Tables Caption

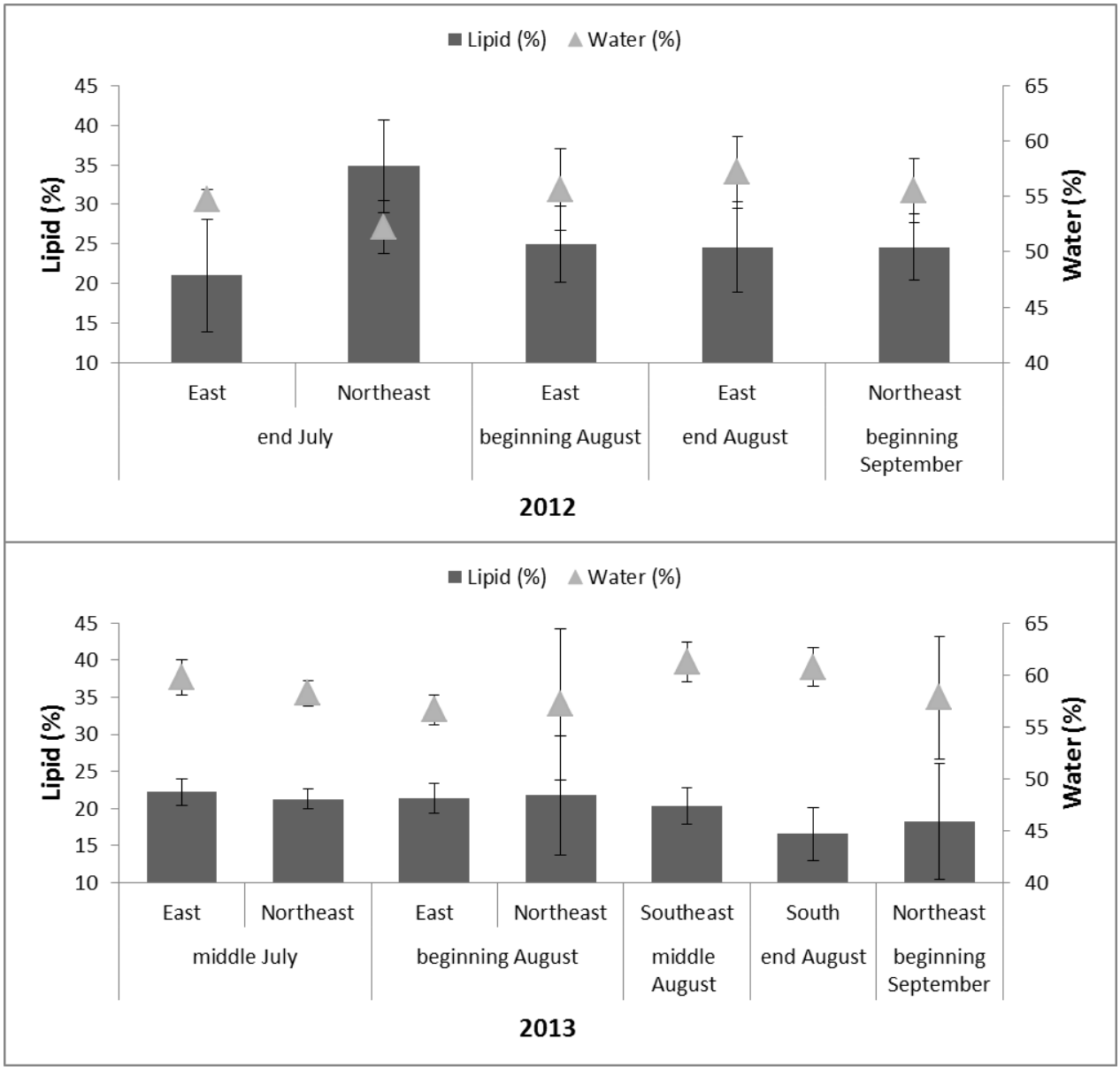


Figure 1 Lipid and water content (g/ 100g samples) of the Atlantic mackerel caught at different season during summer of 2012 (end of July, beginning and end of August, beginning of September) and 2013 (middle of July, beginning, middle and end of August, beginning of September). Fish was collected at different sites of the Icelandic fishing area (East, Northeast, South and Southeast). (mean ± stdv.).

Table 1 Fatty acids profile (g/100g of total lipids) of the Atlantic mackerel caught at different season during summer of 2012 (end of July, beginning and end of August, beginning of September) and 2013 (middle of July, beginning, middle and end of August, beginning of September). Fish was collected at different sites of the Icelandic fishing area (East, Northeast, South and Southeast). (n = 3; mean ± stdv.).

Fatty acids	2012					2013			
	end July		beginning Aug	end Aug	beginning Sep	beginning Aug	middle Aug	end Aug	beginning Sep
	East	Northeast	East	East	Northeast	Northeast	Southeast	South	Northeast
C14:0	7.6±0.4^{a}	7.5±0.2^{a}	7.1±0.9^{a}	7.3±1.0^{a}	7.5±0.6^{a}	8.1±0.1^{a}	7.5±0.5^{a}	7.4±0.2^{a}	7.4±0.6^{a}
C16:0	14.8±0.3^{a}	11.1±0.3^{b}	11.6±1.0^{b}	12.6±1.2^{b}	12.3±0.7^{b}	11.6±0.5^{a}	12.1±0.6^{a}	11.4±0.2^{a}	11.7±0.6^{a}
C16:1n9	0.2±0.1^{a}	0.3±0.1^{a}	0.3±0.0^{a}	0.2±0.1^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.2±0.0^{a}	0.3±0.0^{a}
C16:1n7	3.3±0.1^{a}	3.3±0.2^{a}	3.3±0.4^{a}	3.7±0.5^{a}	3.5±0.3^{a}	4.0±0.9^{a}	3.9±0.4^{a}	2.9±0.2^{a}	3.6±0.5^{a}
C17:0	0.4±0.2^{a}	0.5±0.1^{a}	0.7±0.0^{b}	0.3±0.0^{a}	0.6±0.1^{b}	0.5±0.1^{a}	0.5±0.1^{a}	0.5±0.1^{a}	0.4±0.0^{a}
C16:2n4	0.4±0.1^{a}	0.5±0.0^{a}	0.4±0.0^{a}	0.5±0.1^{a}	0.4±0.0^{a}	0.5±0.1^{a}	0.5±0.1^{ab}	0.3±0.0^{b}	0.4±0.0^{ab}
C18:0	2.4±0.1^{a}	1.9±0.1^{a}	2.0±0.4^{a}	1.8±0.0^{a}	2.0±0.2^{a}	1.8±0.2^{a}	1.9±0.1^{a}	1.6±0.0^{a}	1.6±0.1^{a}
C16:3n4	0.2±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.2±0.0^{a}	0.1±0.0^{a}	0.2±0.1^{a}	0.2±0.1^{a}	0.1±0.0^{a}	0.2±0.0^{a}
C18:1n11	0.3±0.0^{a}	0.4±0.0^{b}	0.4±0.0^{b}	0.4±0.0^{b}	0.4±0.0^{b}	0.3±0.1^{b}	0.3±0.0^{b}	0.5±0.1^{a}	0.3±0.0^{b}
C18:1n9	12.6±0.9^{a}	6.9±1.1^{b}	6.4±1.5^{b}	7.1±1.5^{b}	8.0±1.5^{b}	6.0±0.4^{a}	6.6±0.5^{a}	6.0±0.0^{a}	6.0±0.9^{a}
C18:1n7	1.8±0.2^{a}	1.5±0.2^{a}	1.5±0.3^{a}	1.7±0.2^{a}	1.6±0.2^{a}	1.4±0.2^{a}	1.6±0.2^{a}	1.3±0.0^{a}	1.5±0.2^{a}
C18:1n5	0.5±0.0^{a}	0.4±0.0^{a}	0.4±0.0^{a}	0.4±0.1^{a}	0.5±0.0^{a}	0.4±0.0^{a}	0.5±0.0^{a}	0.4±0.0^{a}	0.5±0.0^{a}
C18:2n6	1.6±0.1^{a}	1.8±0.1^{a}	1.7±0.1^{a}	1.5±0.1^{a}	1.7±0.2^{a}	1.7±0.1^{a}	1.6±0.2^{a}	1.6±0.1^{a}	1.5±0.1^{a}
C18:3n3	2.3±0.1^{a}	1.5±0.1^{b}	1.5±0.1^{b}	1.6±0.3^{b}	1.8±0.4^{b}	1.9±0.1^{a}	1.8±0.2^{a}	1.6±0.1^{a}	1.8±0.1^{a}
C20:1n11	0.5±0.0^{a}	0.7±0.0^{b}	0.7±0.1^{b}	0.7±0.1^{b}	0.6±0.1^{ab}	0.7±0.0^{a}	0.6±0.0^{a}	0.7±0.0^{a}	0.6±0.1^{a}
C20:1n9	5.2±0.2^{a}	8.9±0.4^{b}	8.3±1.1^{b}	8.4±1.9^{b}	7.9±0.7^{b}	8.4±0.4^{a}	8.2±0.0^{a}	8.3±0.3^{a}	8.6±0.5^{a}
C20:1n7	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.1^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}
C18:4n3	6.1±0.3^{a}	5.4±0.3^{a}	5.0±0.5^{a}	4.8±0.7^{a}	5.1±0.6^{a}	6.4±0.4^{a}	5.7±0.9^{a}	6.0±0.0^{a}	6.1±0.3^{a}
C20:2n6	0.2±0.0^{a}	0.3±0.0^{a}	0.2±0.0^{a}	0.3±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{a}
C22:1	7.6±0.8^{a}	13.5±0.9^{b}	12.6±1.4^{b}	13.2±3.1^{b}	11.5±1.2^{b}	12.3±0.8^{a}	11.6±0.3^{a}	14.0±0.4^{a}	13.6±1.2^{a}
C20:4n6	0.9±0.2^{a}	0.9±0.3^{a}	0.7±0.0^{a}	1.3±0.1^{a}	0.8±0.1^{a}	0.7±0.0^{a}	0.8±0.1^{a}	0.9±0.0^{a}	1.1±0.0^{a}
C20:4n3	1.3±0.2^{a}	0.9±0.2^{b}	0.9±0.0^{b}	1.1±0.3^{b}	1.0±0.1^{ab}	1.3±0.2^{a}	1.2±0.1^{a}	2.5±0.3^{b}	1.3±0.3^{a}
C20:5n3 (EPA)	8.7±0.4^{a}	7.7±0.3^{a}	8.3±0.5^{a}	8.0±0.5^{a}	8.2±0.3^{a}	7.4±1.3^{a}	7.7±1.0^{a}	7.1±0.3^{a}	7.9±0.2^{a}
C24:1	0.7±0.0^{a}	0.7±0.1^{a}	0.7±0.1^{a}	0.7±0.1^{a}	0.7±0.1^{a}	0.6±0.1^{a}	0.6±0.1^{a}	0.5±0.1^{a}	0.6±0.0^{a}
C22:5n3	1.0±0.1^{a}	1.1±0.1^{a}	1.1±0.1^{a}	1.0±0.1^{a}	1.1±0.1^{a}	1.0±0.1^{a}	1.0±0.1^{a}	0.9±0.1^{a}	1.0±0.1^{a}
C22:6n3 (DHA)	11.2±0.7^{a}	12.0±0.4^{ab}	13.7±0.1^{c}	11.6±0.6^{ab}	12.5±0.6^{b}	11.0±1.5^{a}	11.8±0.4^{a}	12.3±0.1^{a}	11.1±0.8^{a}
∑SFA	25.5±0.7^{a}	21.6±0.6^{b}	22.0±0.5^{b}	23.0±2.2^{b}	22.9±0.8^{b}	22.6±0.6^{a}	22.6±0.7^{a}	21.4±0.4^{a}	21.7±1.0^{a}
∑MUFA	32.9±1.4^{a}	36.8±1.2^{b}	34.9±0.7^{c}	36.8±4.2^{b}	35.3±1.8^{d}	34.7±0.7^{a}	34.6±0.7^{a}	35.2±0.6^{a}	36.0±0.5^{a}
∑PUFA	33.8±0.8^{a}	32.2±1.0^{a}	33.7±0.4^{a}	31.8±2.1^{a}	32.9±1.1^{a}	32.5±1.0^{a}	32.5±0.6^{a}	33.6±0.6^{a}	32.6±0.7^{a}
n-3 PUFA	30.6±1.0^{a}	28.7±0.2^{a}	30.5±0.4^{a}	28.1±2.2^{a}	29.6±1.1^{a}	29.0±1.0^{a}	29.2±0.5^{a}	30.4±0.6^{a}	29.2±0.8^{a}

n-3/n-6	11.6±1.0[a]	9.7±0.8[b]	11.5±0.8[a]	9.2±0.6[b]	10.9±0.6[ab]	10.6±0.3[a]	11.2±0.5[a]	11.0±0.3[a]	10.3±0.4[a]
PI	1.3±0.1[a]	1.8±0.1[b]	1.9±0.1[c]	1.6±0.1[d]	1.7±0.1[bd]	1.6±0.1[a]	1.6±0.1[a]	1.7±0.1[a]	1.6±0.1[a]

Abbreviations: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), n-3/n-6 (ratio of omega-3 and omega-6 fatty acids), *PI* (polyene index), n-3 PUFA (omega-3 among polyunsaturated fatty acids).

[a–d] Different lowercase superscript letters in each raw indicate a significant difference between the samples from the same year ($p < 0.05$).

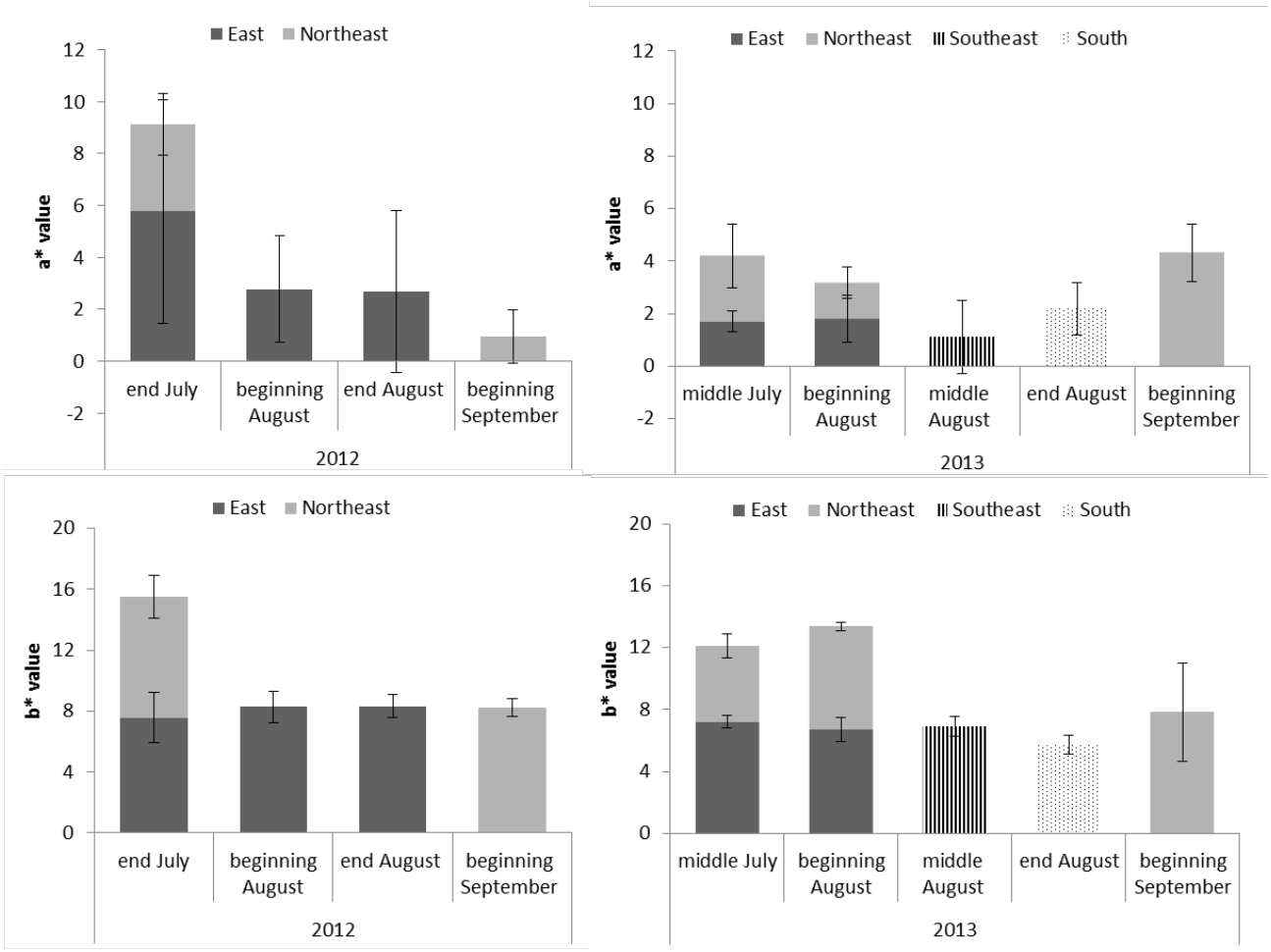
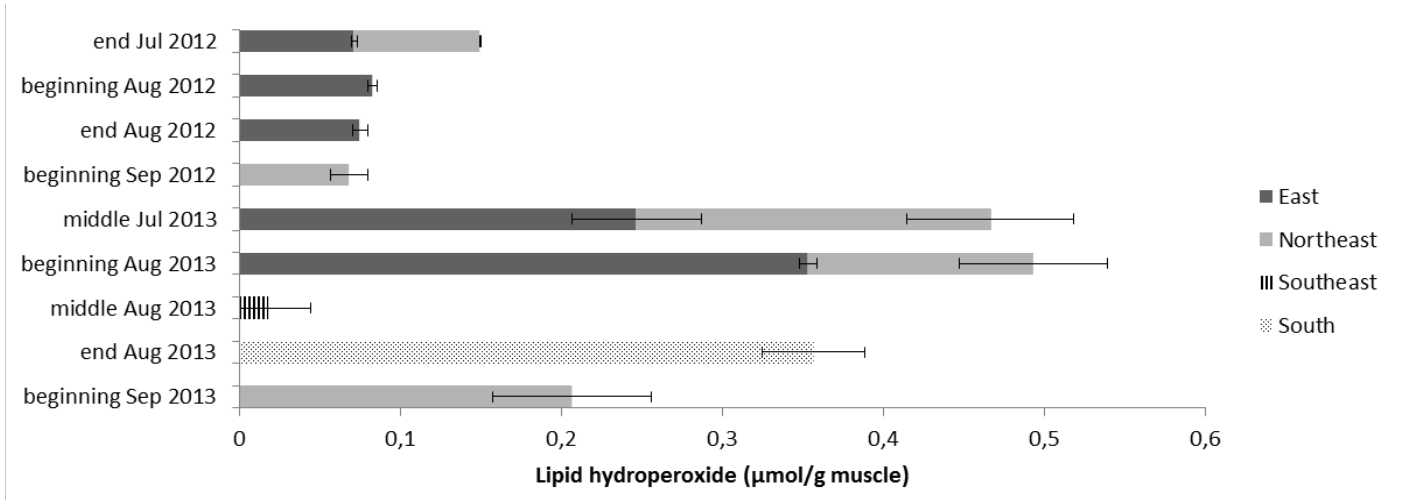
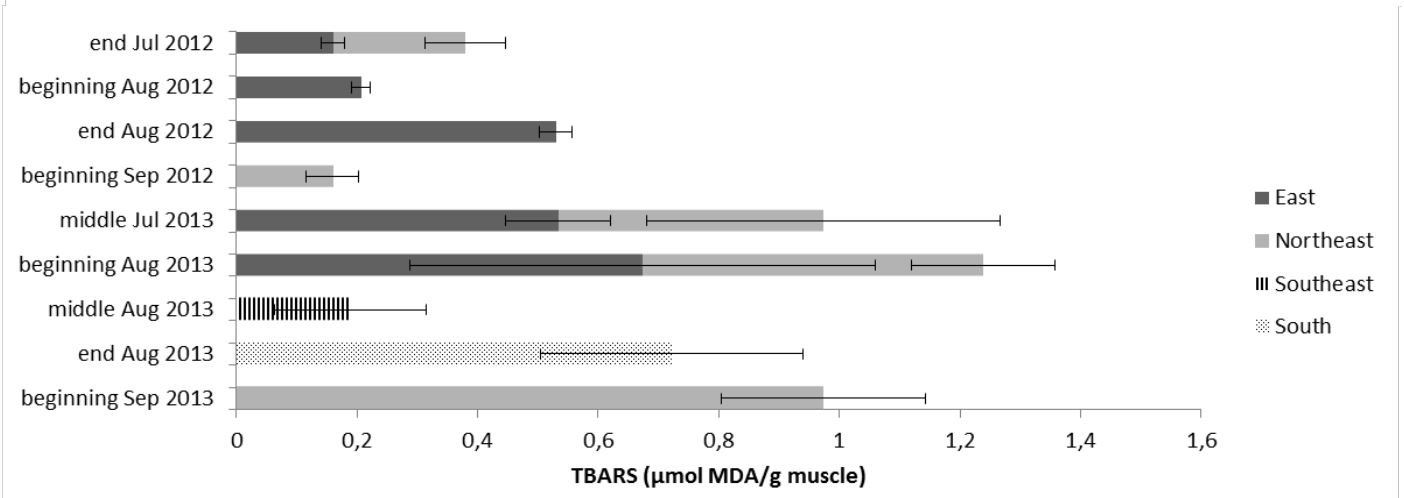


Figure 2 Changes of redness (a value) and yellowness (b* value) of the Atlantic mackerel caught at different seasons during the summer of 2012 (end of July, beginning and end of August, beginning of September) and 2013 (middle of July, beginning, middle and end of August, beginning of September). Fish was collected at different sites of the Icelandic fishing area (East, Northeast, South and Southeast). (n = 3; mean ± stdv.).*





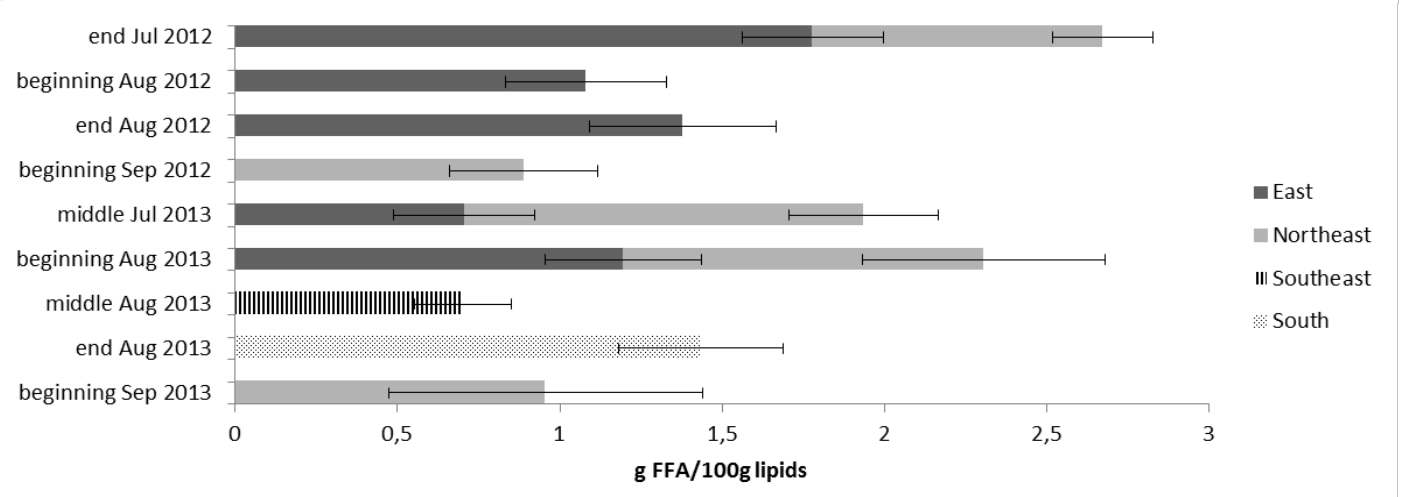


Figure 3 Formation of lipid hydroperoxide value (PV; µmol/g muscle), thiobarbituric acid reactive substances (TBARS; µmol MDA/g muscle) and free fatty acid (g FFA/100g lipids) of the Atlantic mackerel caught at different seasons during the summer of 2012 (end of July, beginning and end of August, beginning of September) and 2013 (middle of July, beginning, middle and end of August, beginning of September). Fish was collected at different sites of the Icelandic fishing area (East, Northeast, South and Southeast). (n = 3; mean ± stdv.).

Table 2a Correlation (Pearson´s) matrix for different parameters[a] evaluated on the Atlantic mackerel caught at different seasons during summer of 2012 (end of July, beginning and end of August, beginning of September). Fish was collected at different sites of the Icelandic fishing zone (East, Northeast, South and Southeast)[b].

	Water	PV	TBARS	FFA	a*	b*	SFA	MUFA	PUFA	*PI*
Lipid	**-0.94**	0.44	0.07	-0.20	0.35	-0.28	-0.37	0.26	-0.18	-0.04
Water		-0.20	0.17	0.32	-0.16	0.32	0.38	-0.17	0.11	0.00
PV			0.51	0.15	0.62	-0.08	-0.16	0.07	0.11	-0.02
TBARS				0.00	0.29	**0.74**	-0.14	-0.09	0.35	0.28
FFA					**0.79**	-0.37	**0.83**	-0.50	0.23	**-0.75**
a*						-0.32	0.51	-0.39	0.29	-0.62
b*							-0.24	-0.16	0.43	0.55
SFA								**-0.74**	0.42	**-0.87**
MUFA									**-0.90**	0.52
PUFA										-0.15

[a]Abbreviations: Lipid (total lipid content), Water (water content), PV (peroxide value), TBARS (thiobarbituric acid reactive substance), FFA (free fatty acids), a* (redness), b* (yellowness), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), *PI* (polyene index).

[b]Bold-type denotes statistical significance at ($p < 0.05$).

Table 2b Correlation (Pearson´s) matrix for different parameters[a] evaluated on the Atlantic mackerel caught at different season during summer of 2013 (middle of July, beginning of August, middle of August, end of August, beginning of September). Fish was collected at different sites of the Icelandic fishing area (East, Northeast, South and Southeast)[b].

	Water	PV	TBARS	FFA	a*	b*	SFA	MUFA	PUFA	*PI*
Lipid	-0.29	-0.32	-0.53	-0.47	-0.50	-0.12	**0.97**	-0.26	**-0.82**	-0.29
Water		-0.37	-0.53	-0.36	-0.46	-0.07	0.02	-0.52	0.32	-0.26
PV			0.61	0.66	0.39	-0.43	**-0.92**	0.11	**0.86**	**0.95**
TBARS				0.39	**0.83**	0.35	**-0.76**	**0.74**	0.28	0.63
FFA					0.20	-0.54	-0.69	-0.20	**0.84**	0.44
a*						0.30	-0.65	**0.94**	0.05	0.35
b*							0.25	**0.81**	**-0.79**	-0.47
SFA								-0.36	**-0.79**	**-0.88**
MUFA									-0.30	0.20
PUFA										**0.77**

[a]Abbreviations: Lipid (total lipid content), Water (water content), PV (peroxide value), TBARS (thiobarbituric acid reactive substance), FFA (free fatty acids), a* (redness), b* (yellowness), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), *PI* (polyene index).

[b]Bold-type denotes statistical significance at ($p < 0.05$).

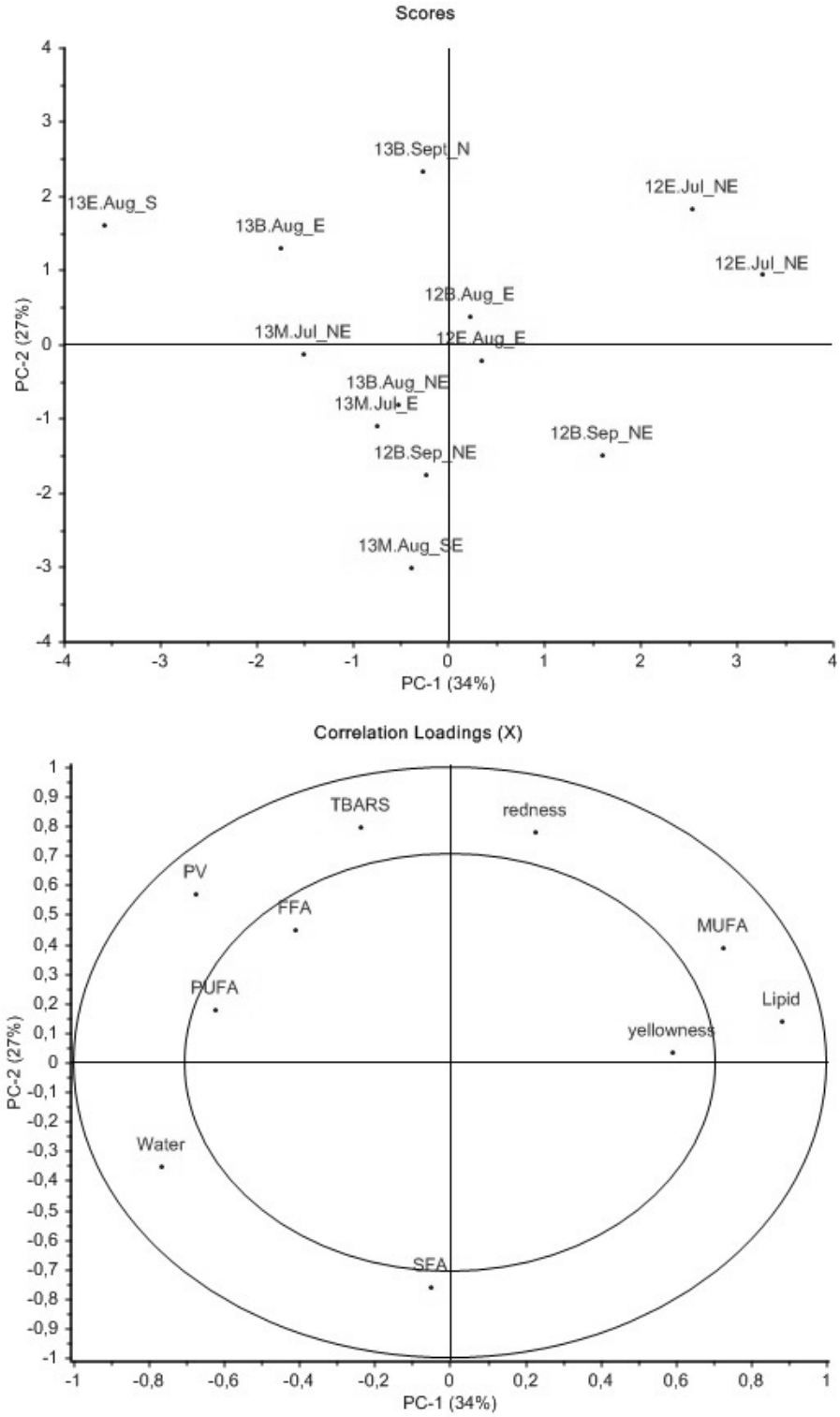


Figure 4 Scores and correlation loadings from PC1and PC2 from the principal component analysis (PCA) of mackerel muscles. All samples and analytical parameters were used. Fish caught at different year was analysed, were the number 12 in the sample naming indicates the summers of 2012 and the number 13 fish caught in 2013. The abbreviations Jul, Aug, Sept represent the month in which the fish was caught, and the letters B, M and E stand for fish caught in the beginning, middle and end of each months, respectively. The last letter(s) of the sample description indicates different sites of the Icelandic fishing area: E indicates East, NE - Northeast, S- South and SE-Southeast.