Increased quality and stability of frozen herring products

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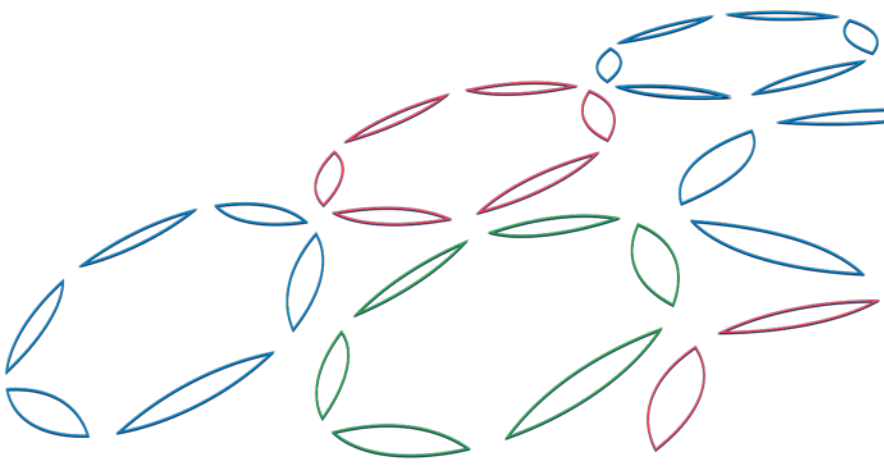

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Report summary

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Ágrip á íslensku:	Frysting og frostgeymsla er skilvirk aðferð til að viðhalda gæðum og lengja geymsluþol sjávarafurða. Framleiðsla á frosnum afurðum jafnar framboð afurða þar sem veiðar eru árstíðabundnar. Það eru margir þættir sem geta haft áhrif á gæði og stöðugleika frosinna afurða. Þar má meðal annars nefna ástand hráefnis, vinnsluaðferðir og skilyrði við geymslu og flutning svo fátt eitt sé nefnt. Markmið rannsóknarinnar var að kanna þær breytingar sem eiga sér stað á efna- og eðliseiginleikum frosinna síldarflaka m.t.t. ástands hráefnis við vinnslu og aðstæður í frostgeymslu. Atlantshafssíld var unnin fyrir og eftir dauðastirðnun, og flökin geymd annars vegar við stöðugar geymsluaðstæður (-25 °C) og hins vegar við óstöðugar aðstæður (við -25 °C í 2 mánuði, svo -12 °C í mánuð og svo aftur við -25 °C út geymslutímann). Til að rannsaka stöðugleika og eðliseiginleika afurðanna var m.a. mælt vatnstap (drip), suðunýting og litur, auk þess sem mæld voru bæði í ljósum og dökkum fiskvöðva vatnsheldni, pH, efnasamsetning, fitusýrusamsetning, ensímvirkni og þránun. Rannsóknin sýndi að það er mikilvægt fyrir sjávarútveginn að tryggja samræmda og rétta hitastýringu þegar afurðir eru geymdar í frosti. Vinnsla og frysting fyrir dauðastirðnun, samhliða stöðugum geymsluaðstæðum, hefur jákvæð áhrif á gæði og stöðugleika síldarfalka. Auk þessa, þá staðfesti rannsóknin að fituríki vöðvi síldarinnar, oft nefndur dökki vöðvinn, er mjög viðkvæmur fyrir þránun. Til þess að lengja geymsluþol frosinna síldarflaka er mælt með því að þessi vöðvi sé fjarlægður samhliða roðflettingu (e. *deep skinning*).		
Lykilorð á íslensku:	*Síld, frostgeymsla, hitastigssveiflur, gæðarýrnun, dauðastirðnun*		

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Summary in English:	Freezing and frozen storage has proven to be an effective method to preserve and prolong the storage life of seafood products. Production of frozen products provides all year around product availability although the catching is seasonal. There are several factors that can affect the quality and stability of frozen fish products, including the state of the raw material, processing methods and storage conditions. The aim of the study was to explore how physicochemical properties of frozen herring fillets are affected in regard to the state of the raw material during processing as well as storage conditions. Atlantic herring was processed and frozen pre- and post-rigor and stored at stable (-25 °C) and abused storage conditions. To investigate the storage stability and physical properties of the fillets, thawing drip, cooking yield and colour were evaluated, as well as proximate composition, fatty acid composition, pH and lipid degradation of the light and the dark muscle. The study demonstrated the importance of stable and controlled temperature during storage and transportation of frozen herring products. Processing and freezing pre-rigor, in combination with stable storage conditions, was shown to be beneficial in terms of preventing lipid oxidation, as well as reducing thawing loss and maintaining the cooking yield of the herring fillets.
English keywords:	*Atlantic herring, frozen storage, temperature abuse, quality degradation, rigor mortis*

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1 INTRODUCTION

1.1 Atlantic herring

Atlantic herring (*Clupea harengus*) is a pelagic fish and found on both sides of the North Atlantic Ocean. In the Northeast Atlantic Ocean, it occurs from the Bay of Biscay in the south to Spitzbergen and Novaya Zemlya in the north, while in the Northwest Atlantic Ocean it occurs from the coast of Maine northwards (Stroud, 2001). Herring is a popular fatty fish and is usually frozen before being processed. Herring is not normally gutted at sea, since it is impractical to handle the large numbers of small fish coming aboard in a short time; chilling or freezing soon after capture is therefore of great important to prevent spoilage (Stroud, 2001).

The chemical composition of herring varies considerably with season, catching ground, sexual maturation and feeding pattern (Nielsen *et al*., 2005). The lipid content of herring can be less than 1% immediately after spawning, and more than 20% as spawning time approaches again. The herring as a food has a high energy value since most of the lipids are stored in the muscles (Stroud, 2001). The herring muscle is very rich of valuable omega-3 fatty acids and antioxidants (Hølmer 1993; Hamre *et al*., 2003 and Ackman 1980), which are beneficial for human health. On the other hand, polyunsaturated fatty acids are susceptible towards oxidation and therefore represent a risk of developing rancid off-flavours (Hølmer 1993; Hultin 1994; Undeland *et al.*, 1998, 1999).

1.2 Effects of temperature abuse on fish quality

Freezing is one of the most common procedures applied to preserve physicochemical properties and to prolong storage life of fish products, especially fatty fish due to the high contents of unsaturated fatty acids present. The main purpose of freezing is therefore to prevent or slow down bacterial spoilage, enzyme activity and oxidation reactions.

Quality changes of frozen fish during storage can be influenced by several factors including fish species, the biological status of fish at catch, handling on board, temperature and storage time before freezing, freezing rate, frozen storage temperature, temperature fluctuations, thawing procedure and protection from light and oxygen (Nielsen and Jørgensen, 2004). Optimal handling and transport conditions can be used to ensure high quality of the final fish products, which arrive on the market (Ólafsdóttir, 2005). However, temperature fluctuations and abuse through the production and distribution chain can affect the fish quality and safety. These fluctuations mainly occur during

handover from one party/function to the next in the value chain (Moureh and Derens, 2000). Studies have shown that unstable temperature accelerates the growth of specific spoilage organisms as well as pathogens (Rediers *et al.*, 2009), which can cause quality and safety problems and moreover economic losses. Furthermore, extracellular formation of ice crystals is accelerated during temperature fluctuations and hence cellular disruption is increased (Hagyard *et al.*, 1993; Bak *et al.*, 1999). Unstable temperature can therefore cause formation of large ice crystals within the fish muscle which have negative effects on its overall quality. Therefore, controlled temperature throughout the whole value chain is necessary to ensure product quality and stability. The effects of the temperature fluctuations and abuse on the product quality are depending on the temperature range. For example, fluctuations around -18 °C has proven to cause worse effects on the product quality than fluctuations around -25 °C due to the fact that the freezing point of sodium chloride is -21.1 °C. For sensitive food products, such as fish, even short periods of temperature abuse can lead to significant loss of quality (WFLO, 2008). Temperature fluctuations can lead to an increase in the amount of unfrozen water in the product and changes in the structure of ice crystals and recrystallization. Thus, physicochemical deterioration increases as a result of enzymatic activity, lipid oxidation and breakdown of the physical structure (Nesvadba, 2008; Benjakul and Bauer, 2001; Karlsdottir *et al.*, 2014; Zaritzky, 2008).

1.3 Effect of rigor mortis on fish quality

Rigor mortis is a process that takes place post-mortem and is responsible for transforming muscle into meat (Kiessling *et al.*, 2006). This transformation is critical for the quality of the final product and is directly affected by both pre- and post-mortem factors (Stien *et al.*, 2006). A growing demand for quality assurance from consumer organizations and retailers makes measuring and documenting the rigor process more and more important (Erikson, 2000).

The onset and development of rigor mortis depends on the fish species, temperature and handling before catching, pre-slaughter stress, the biological status of the fish and temperature of pre-rigor storage (Azam *et al.,* 1989; Jerrett *et al.*, 1998; Skjervold *et al*., 2001). The stress prior to slaughtering and high storage temperature result in faster and stronger rigor contraction (Skjervold *et al.*, 2001).

The rigor process consists of an initial contractile phase (Tornberg *et al.,* 2000), during which the muscle fibres contract, and a second stiff phase that a permanent binding of the contractile proteins myosin and actin happens. This process can be influenced by post-mortem temperature. In wild-caught fish, in which pre-slaughter stress and temperature at death are difficult to control, rigor takes usually place

within short time after catching. Storage temperature is accepted as being a major factor affecting the rate of quality loss and storage life (Dawood *et al.,* 1986). Hence, reducing the fish temperature after catching as soon as possible is an effective way to delay the onset of rigor mortis and to prolong the rigor process.

2 STUDY OBJECTIVES

The aim of the study was twofold. Firstly, to explore the influence of time and temperature during frozen storage on lipid deterioration of herring by comparing the effect of temperature fluctuations and abuse during frozen storage, as can be expected during transportation, on the physicochemical characteristics and lipid stability of herring fillets. Secondly, to investigate the effect of pre- and post-rigor processing on the quality and storage stability of frozen herring.

This study forms part of PhD. research by Houng Thi Thu Dang and the results have been published in peer reviewed scientific papers. Hence, only the most relevant results and observations are summarized here. More detailed results can be observed in the before mentioned papers (Dang *et al*., 2017 and Dang *et al.*, 2018).

3 MATERIALS AND METHODS

3.1 Raw material and experimental design

Atlantic herring (*Clupea harengus*) was caught off the Southwest coast of Iceland in November 2014. The fish processed pre-rigor was caught by freezing trawler. After being caught and pumped into the hold, the herring was quickly cooled down to -1.5 °C in a tank with refrigerated seawater and within four hours from catch they were graded, headed, gutted, filleted, packed and frozen without glazing. The fish which was processed post-rigor was caught by fresh fish trawler. After catching and pumping into the hold, the herring was stored in tanks with refrigerated seawater at -1.5 °C for 2-3 days. The fish was then transported and pumped to the factory for processing and freezing. Both groups were frozen into 16 kg blocks in an automatic plate freezer (Skaginn, Akranes, Iceland), and stored at -25 °C.

Upon arrival at the laboratory, the blocks were divided into four equal sized parts, packed in plastic bags and put into waxed carton boxes. The raw materials were then distributed into two treatments: the first was stored at stressed/abused temperature conditions (-12 °C) for 1 month, followed by stable storage at -25 °C for the remaining storage period; the second was stored at a stable temperature of -25 °C for up to 14 months (Figure 1).

These conditions were set up to imitate the increased temperatures which are a common problem during loading and unloading processes during frozen storage and transportation, especially during intercontinental transport. The temperature is also often not uniform within the container, and the temperature can go up to temperatures of -12 °C or even higher, on chosen locations in poorly temperature controlled containers. Also, even though processors have temperature monitoring systems, few companies use them to actively control the storage conditions in real time. Moreover, temperature stress warmer than -12 °C can lead to significant loss quality of fish (WFLO, 2008). For these reasons, the stressed temperature conditions were designed to simulate these common conditions which frozen products are often exposed to during transportation and storage.

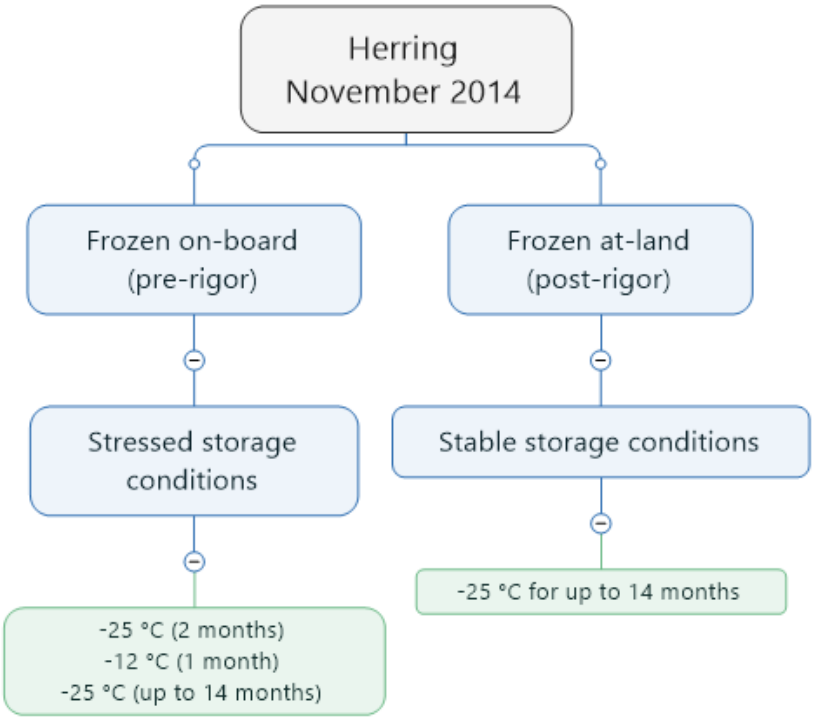

Figure 1. Experimental design.

Prior to analysis, samples were thawed at refrigerated temperatures (4±1 °C) for 24h. After thawing, the light and the dark muscles were manually separated, minced and used for all analyses. As a result of the potential for sample variation in the fish block, at each sampling point, each sample pack was divided into three groups, each group containing 15 fillets (the average weight per fillet was 40-60g). Chemical analyses were performed separately on the light and the dark muscle in triplicate (n=3) per group for lipid hydroperoxides and thiobarbituric acid reactive substances; duplicates (n=2) per group were analysed for water content, total lipids content, pH, phospholipids content, free fatty acid content and fatty acid composition.

3.2 Water, total lipids and phospholipid content

Water content was determined by drying 5 g of minced sample at 102-104 °C for 4 h (ISO, 1999). The results were calculated as the weight loss during drying as a percentage of the wet muscle.

Total lipids (TL) of the samples were extracted from 25 g of samples (the weight was adjusted according to the water content of each samples) with methanol/chloroform/0.88% KCl (at 1/1/0.5, v/v/v) according to the method of Bligh and Dyer (1959). The lipid content was determined gravimetrically, and results were expressed as a percentage of the wet muscle.

Phospholipids (PL) content of the fish muscles was determined on the TL extracts by using a colorimetric method (Stewart, 1980). Phosphatidylcholine in chloroform (1 mg/mL) was prepared for a standard curve, and results were expressed as g phospholipids/100 g TL.

3.3 Fatty acid composition

The fatty acid composition of the sample was determined on the TL extracts by gas chromatography (Varian 3900 GC, Varian, Inc., Walnut Creek, CA, USA). The methylation of fatty acids was carried out according to the AOAC Ce 1b-89 (1998) method. The programme was based on the AOAC 996.06 (2001) method. Results were expressed as a percentage of TL.

3.4 Free fatty acid content

Free fatty acids (FFA) content was determined by the Lowry and Tinsley (1976) method with modifications described by Bernardez *et al.* (2005). The absorbance of the solution was read at 710 nm (UV-1800 spectrophotometer, Shimadzu, Japan) and compared to a standard curve prepared from oleic acid in a concentration range of 2–14 µmol. Results were expressed as g FFA per 100 g TL.

3.5 Lipid oxidation

Lipid hydroperoxides (PV), a primary oxidation product, was determined by the ferric thiocyanate method (Shantha & Decker, 1994) with modifications according to Romotowska *et al.* (2016), except that the lower chloroform layer containing lipids was collected (0.2 mL for the dark and 0.5 mL for the light muscle) and mixed with 0.8 and 0.5 mL of the chloroform:methanol (1:1) solution for the dark and light muscle, respectively. The results were expressed as µmol lipid hydroperoxide per kg of the sample (µmol/kg muscle).

Thiobarbituric acid reactive substances (TBARS), secondary oxidation products, were determined by the method of Lemon (1975) with modifications as described by Romotowska *et al*. (2016), except that an amount of 0.8 and 0.5 mL thiobarbituric acid (0.02 M) were mixed with 0.2 mL collected supernatant for the dark and 0.5 mL for the light muscle, respectively. The results were expressed as µmol malondialdehyde diethyl acetal per kg of wet muscle (µmol MDA/kg muscle).

3.6 pH, thawing loss and cooking yield

The muscle pH was measured by inserting the pH probe (Radiometer PHM80 Portable pH meter, Denmark) directly into the muscle samples.

Thawing losses were calculated as the ratio (%) of liquid lost during thawing to the weight of the individual frozen blocks.

Cooking yield (CY) was calculated as the ratio (%) of the sample weight after cooking to the weight of the sample before cooking. About 35 g of each fillet (n = 5 from each group) were weighed and heated in a steaming oven (Convotherm OGS 6.10 Combi convection steam oven, Elektrogeräte GmbH, Eglfing, Germany) at 100 °C for 10 minutes. Samples were drained for 10 minutes prior to being weighed again.

3.7 Statistical analysis

Data summaries and statistical analyses were carried out and figures were drawn using the STATISTICA software (Version 10.0, StatSoft, OK 74104 USA), and Microsoft Office Excel 2013 (Microsoft Inc. Redmond, WA, USA). One-way ANOVA, Tukey HSD's test and Student t test for independent samples were performed on the means of each variable. Pearson correlation analysis was performed to find the correlations between variables. Significance of difference was defined at $p<0.05$ for all statistical analyses. Principal components analysis (PCA) was performed using Unscrambler ® (Version 10.2, CAMO ASA, Trondheim, Norway) to identify similarities and differences between samples. All variables were weighed with the inverse of the standard deviation to correct for different scales of the variables.

4 RESULTS AND DISCUSSION

In present study, the effects of rigor state and storage conditions on the physicochemical stability of herring during frozen storage were investigated. The results have, as mentioned before, been published in peer reviewed scientific papers (Dang *et al*., 2017 and Dang *et al.*, 2018). Hence, only the most relevant results and observations are summarized here.

The rigor state of the herring muscle when processed and frozen, significantly affected its physical and chemical properties during frozen storage. Pre-rigor freezing effectively reduced thawing loss and maintained the cooking yield of herring fillets throughout frozen storage (Figure 2). The differences observed between the pre- and post-rigor herring fillets in regard to thawing loss and cooking yield was most likely due to lower level of protein denaturation in the fish frozen pre-rigor than post-rigor. Previous study on frozen cod showed that the intracellular ice crystals in cod frozen pre-rigor were smaller than those in comparable post-rigor cod (Love, 2001). Hence, pre-rigor frozen cod were found to exhibit less denatured proteins than post-rigor material under the same storage conditions (fish were stored at temperature in the range from -35 to -14 °C) (Love, 1962). Furthermore, in present study, herring frozen pre-rigor on-board the vessel was only pumped once into the hold, while the herring frozen post-rigor on-land was pumped twice (first into the hold and then again during landing). Thus, the post-rigor fish were likely subjected to more physical strain during processing.

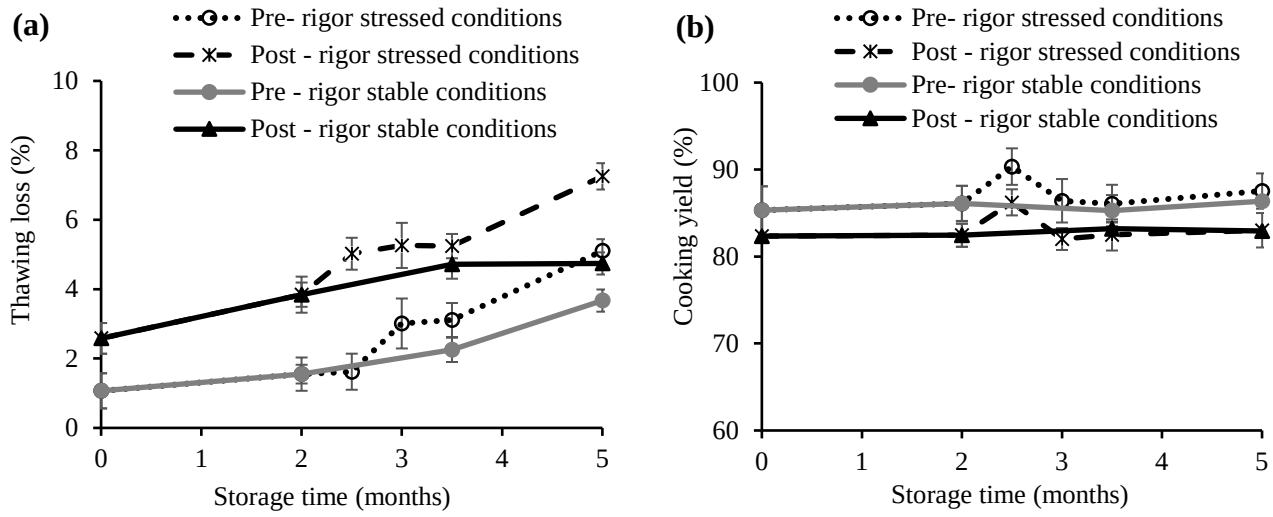

Figure 2. Thawing loss (%) (a), and cooking yield (%) (b) of herring fillets as affected by the state of rigor (pre-rigor or post-rigor) when frozen, at 0, 2, 2.5, 3, 3.5 and 5 months at stressed temperature conditions (samples were stored at -25 °C for 2 months, then stressed at -12 °C for one month, followed by storage at -25°C for the remaining storage duration) and at 0, 2, 3.5 and 5 months at stable conditions (-25 °C) (n = 3, mean ± SD). (Dang et al., 2018).

Processing and freezing of the herring in pre-rigor state were shown to be beneficial in terms of preventing lipid oxidation since the PV and TBARS content in the pre-rigor frozen herring was generally lower than in the post-rigor frozen fillets (Figure 3). However, the state of rigor did not seem to affect lipid hydrolysis since no difference was observed in the free fatty acid (FFA) content between the pre- and post-rigor frozen samples (data not shown). The lower lipid oxidation of the pre-rigor frozen fish compared to the post-rigor fish was in agreement with previous findings of Jacobsen and Timm (2001) which suggested that low pH promotes oxidation. Lower pH and higher oxidation were observed in the post-rigor frozen fish in present study.

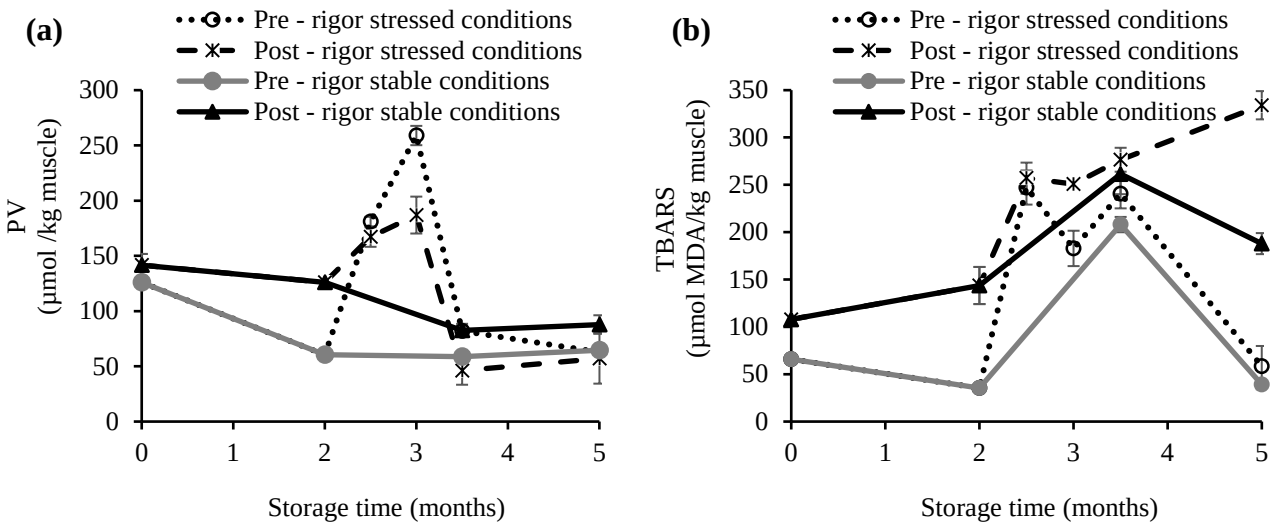

Figure 3. Peroxide value (PV; µmol/kg muscle) (a) and thiobarbituric reactive acid substances (TBARS; µmol MDA/kg muscle) (b) in the dark herring muscle as affected by state of rigor (pre-rigor or post-rigor) when frozen, at 0, 2, 2.5, 3, 3.5 and 5 months at stressed temperature conditions (samples were stored at -25 °C for 2 months, then stressed at -12 °C for one month, followed by storage at -25 °C for the remaining storage duration) and at 0, 2, 3.5 and 5 months at stable conditions (-25 °C) (n = 3, mean ± SD). (Dang et al., 2018).

Ensuring a stable and uniform temperature in a frozen storage facility or a container during transport can be challenging. In this study, herring were stored at abused conditions (-12 °C) for one month, and then restored at stable conditions (-25 °C) to simulate temperature abuses, which are common during loading and unloading processes during frozen storage and transportation. The temperature abuse and instability significantly affected the physicochemical properties of the herring muscle including thawing loss, cooking yield and lipid degradation.

Previous studies have demonstrated that unstable temperature can affect the muscle structure, causing recrystallization and an increase in size of ice crystals within the fish muscle (Pham and

Mawson, 1997). Large crystals particularly needle shaped ones, rupture muscle cells, which in turn leads to increased liquid loss during thawing (Pham and Mawson, 1997; Delgado and Sun, 2001; Nesvadba, 2008). Furthermore, temperature abuse during frozen storage accelerate protein denaturation (Kadim and Mahgoub, 2007), leading to increased thawing and cooking loss.

The temperature stress accelerated lipid degradation in present study (Figure 4), but increased temperature is one of the main factors that accelerate lipid oxidation (Shahidi & Zhong, 2010; Karlsdottir *et a.,* 2014). Lipid hydrolysis was also accelerated by temperature abuse. It is known that the action of lipases and phospholipases cause hydrolysis of phospholipids and triglycerides, resulting in accumulation of FFA in the fish muscle (Sista *et al*., 1997). Therefore, to avoid consequences of lipolysis, ice recrystallization should be minimized by keeping frozen products under stable conditions at all time (Pham and Mawson, 1997).

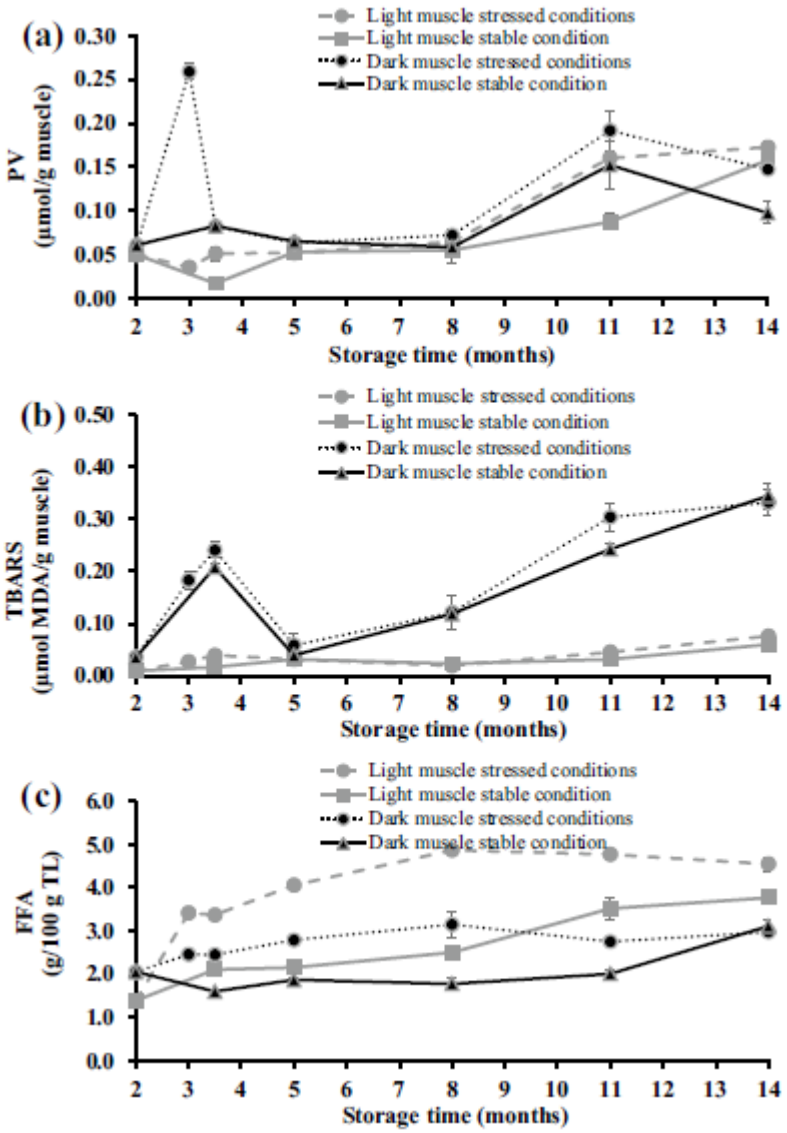

Figure 4. Changes in lipid hydroperoxide content (PV; µmol/g muscle) (a), thiobarbituric acid reactive substances content (TBARS; µmol MDA/g muscle) (b) and free fatty acids content (g FFA/100 g TL) (c) in the light and dark herring muscle after 2, 3, 3.5, 5, 8, 11 and 14 months at stressed temperature conditions (samples were stored at -25 °C for 2 months, then stressed at -12 °C for 1 month, followed by storage at -25 °C for the remaining storage duration) and after 2, 3.5, 5, 8, 11 and 14 months at stable conditions (samples were stored at a stable temperature of -25 °C for up to 14 months) (n = 3, mean ± standard deviation). (Dang et al., 2017).

A principal component analysis (PCA) of the measured variables (water, total lipids and phospholipids content, fatty acid composition and lipid deterioration) revealed mainly the difference between the dark and the light muscle of the herring, as well as the effects of storage time and conditions (Figure 5). The light muscle was characterized by a higher water content and amount of PUFA, whereas the dark muscle had a higher lipid content and amount of MUFA. The highest quantity of PUFA in both muscle types was associated with n-3 fatty acids, with DHA being the most abundant, followed by EPA. The light muscle had a significantly higher amount of DHA and EPA compared to those of the dark muscle, indicating a high stability of the n-3 fatty acids in the light muscle of herring. The present work

showed that Atlantic herring caught in Icelandic waters had high amount of MUFA and PUFA, and is a good source of DHA for human consumption.

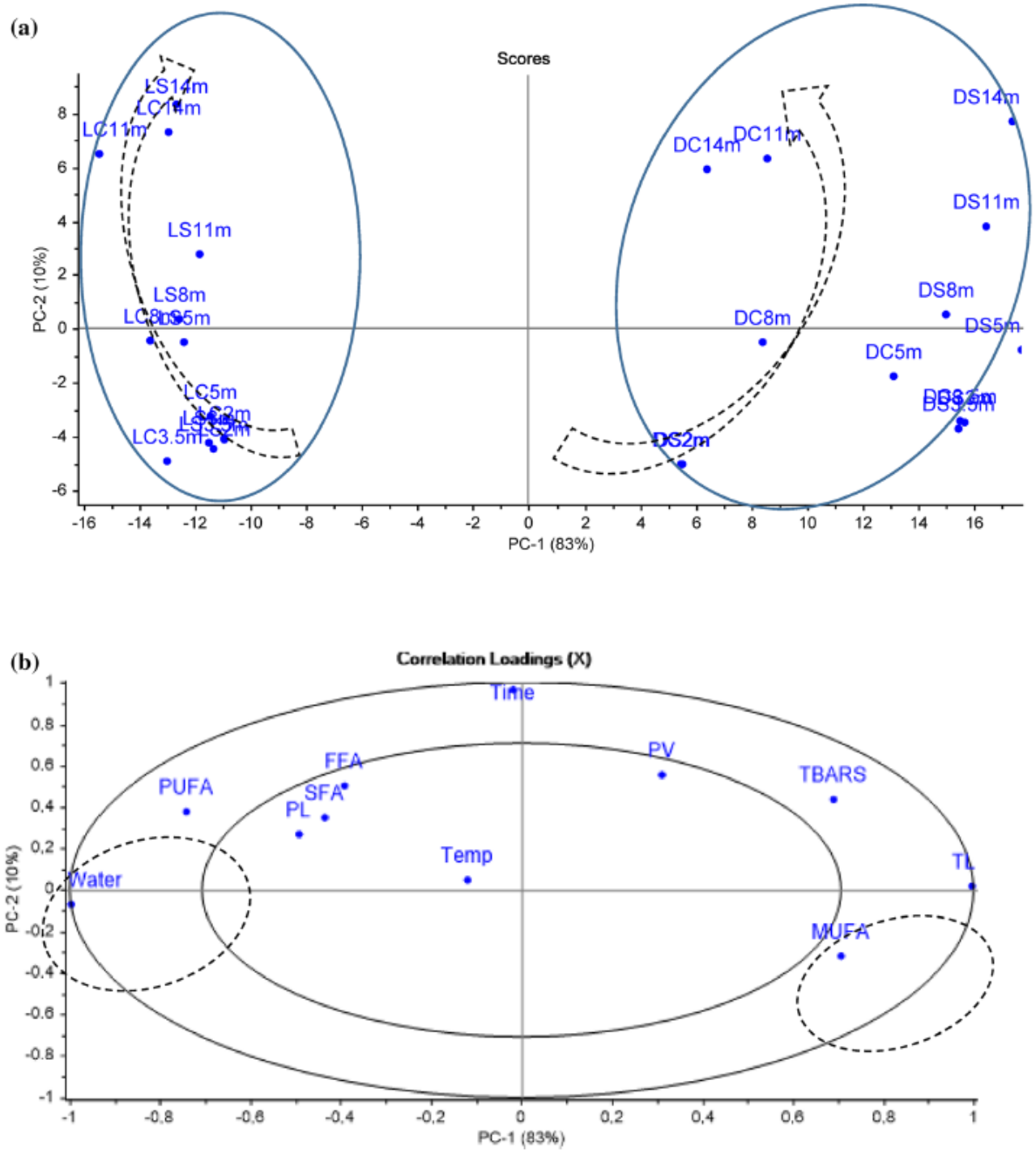

Figure 5. Scores (a) and correlation loadings (b) from PC1 and PC2 from the principal components analysis (PCA) of light and dark muscles of herring. All samples and analytical parameters were used. The first letter D or L indicates the dark and light muscle, respectively. The second letter S or C indicates the storage conditions (stressed and constant temperature, respectively). The number and the last letter (m) of the sample description indicate the storage time in months. (Dang et al., 2017).

5 CONCLUSIONS

According to present study, storage conditions is a key factor regarding quality deterioration of herring fillets during prolonged frozen storage. Pre- and post-rigor processing and freezing, in combination with stable or unstable storage conditions significantly affected the physicochemical characteristics of herring fillets during frozen storage. Pre-rigor freezing effectively reduced thawing loss and maintained the cooking yield of herring fillets throughout frozen storage. Present results demonstrate that temperatures should be strictly controlled during storage and transportation of fatty fish. By reducing the temperature to a stable and low temperature of -25 °C, thawing loss was reduced, and lipid oxidation and hydrolysis were minimized. It is therefore important to prevent temperature fluctuation during frozen storage and transportation to extend the storage life of herring fillets. Furthermore, pre-rigor processing and freezing of herring was shown to be beneficial in terms of preventing lipid oxidation, as well as reducing thawing loss and maintaining the cooking yield of the herring fillets.

6 ACKNOWLEDGEMENTS

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