

## **Effects of temperature fluctuations on the physicochemical properties of Atlantic mackerel (*Scomber scombrus*) and redfish (*Sebastes marinus*) during frozen storage**

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### **ABSTRACT**

The effects of temperature fluctuations during frozen storage on storage stability and quality of Atlantic mackerel (*Scomber scombrus*) and Redfish (*Sebastes marinus*) was studied. The products were stored at stable temperature (-25 °C) for 6 weeks and were compared to samples stored at fluctuating temperatures (-12°C to -18°C). Analyses included proximate composition, cooking yield, colour, and lipid degradation. The result showed that lower and stable temperature can effectively reduce quality loss of Atlantic mackerel and redfish, compared with fluctuating temperature (-12°C to -18°C).

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## 1 INTRODUCTION

### 1.1 Fisheries in Myanmar

Myanmar is endowed with abundant fishery resources with a long coastline of 2,800 km along the Bay of Bengal, Gulf of *Mottama* (Martaban), and the *Andaman* Sea, and 8.2 million ha of inland water bodies. Myanmar has 0.5 million ha of swamp areas along the coast with mangroves forests, coral reefs, numerous offshore islands and sand beaches. The two major sources of fish production are marine fisheries and freshwater fisheries. Marine fisheries include inshore and offshore fisheries. Freshwater fisheries consist of aquaculture, leasable, open fisheries. Fish is an essential part of the Myanmar diet, second only to rice. Households spend nearly as much on fish (14% of food expenditure) as on rice (19% of food expenditure). Fisheries and aquaculture are labour-intensive economic activities and create jobs, directly and indirectly, for a number of rural and urban households (Win, 2013).

Postharvest handling and processing of the catches, such as freezing, drying, smoking, salting and the preparation of fermented fish, fish paste and sauce products, provides important employment opportunities for rural populations, particularly women. Smoking, fermentation, and production of fish paste and fish sauce are produced for local consumption. Fermented fish and rice is a popular traditional food in Myanmar.

Freezing is most common for the export market. Dried and salted of fish are also exported but in lower quantities. In 2015-2016, the production of freshwater fish was 46% of the total fish production, and the production of marine fish was 54% of the total production of fish. The export amount was 7% of the production of fish in Myanmar in this period (figure 1) (Department of Fisheries, 2016).



Figure 1. Proportion of locally consumed and exported fish in Myanmar in 2015-2016

### 1.2 Rationale

Myanmar exports fish and fishery products to 42 countries including China, Japan, Korea, Thailand, Malaysia, Vietnam, Bangladesh, Middle East countries, Australia, U.S.A and EU member countries. A total of 116 fishery establishments in Myanmar are enforced to comply with food safety requirements and importing countries' requirements such as EU, USA, Canada and China by the Department of Fisheries (Department of Fisheries, 2016). Most of the

establishments produce frozen raw fishery products (for example frozen whole fish, frozen block shrimp), although some processing establishments produce value-added products such as frozen crumbed prawn cutlet, frozen raw prawn twisters, etc. Therefore, frozen products are the most important for export product of Myanmar.

The temperature is the most important factor affecting the quality of frozen fish. Fluctuations in the temperature adversely affect the quality and reduce the storage life of a food. Some of the causes of fluctuations are using the storage room for freezing, overloading refrigeration equipment, power failure, equipment breakdown, improper setting of control devices, transferring frozen products from one storage room to another, and transporting frozen products from producer to consumer.

The aim of this study is to evaluate quality changes of frozen products during storage time of marine fish and through this project, the study and knowledge will help Myanmar processors to process high quality frozen fish by keeping temperature stable during storage and thus produce frozen products that meet the quality standards of the EU market.

### **1.3 Research Objective**

The research objective is to evaluate the effect of fluctuating temperature on quality changes in frozen mackerel and redfish during storage and compare it to stable storage temperature. More specifically the objectives are;

- To measure the physical changes of frozen fish due to fluctuating storage temperature.
- To measure the effects of different frozen storage conditions on lipid degradation of frozen mackerel and redfish.

## 2 LITERATURE REVIEW

### 2.1 Atlantic mackerel (*Scomber scombrus*)

The mackerel (Figure 2) is found in the north east Atlantic from Norway to Morocco and the Canary Islands, and in the Mediterranean and Black Seas. The mackerel is a fatty fish, and the fat and water content vary with season. Fat content is 14-33%, water content is 56-74% and protein content 18-20% (Matis, 2010). Mackerel for freezing should be chilled immediately after capture and frozen within 24 hours. Frozen mackerel, properly glazed and kept in cold storage at -30°C, will keep in good condition for at least 6 months (FAO, 2014).

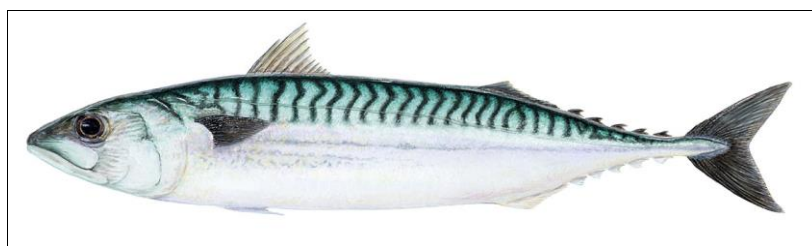


Figure 2. Atlantic mackerel (*Scomber scombrus*) (Pelagic Iceland, 2013)

### 2.2 Redfish (*Sebastes marinus*)

The redfish (Figure 3) is a slow growing, long lived and a deep-water species. They are common in the North Atlantic. Generally, the adult red fish is most found in deep-water at temperature 4°C to 13°C. The female redfish spawns from April to August (Pikanowski, Morse, Berrien, Johnson, & McMillan, 1999). Redfish is a semi-fatty fish. Redfish consists of 1.4 - 6.8% fat and is 15.7-18.8% protein (Matis, 2008).

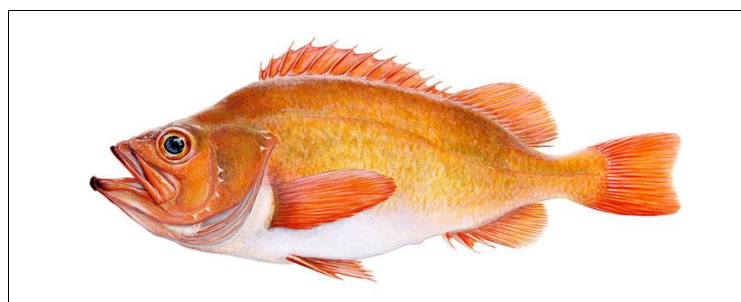


Figure 3. Redfish (*Sebastes marinus*) (Brim, 2015)

### 2.3 Freezing of fish

Freezing is one of the most common methods for food preservation. The three basic methods of freezing fish are (1) blowing a continuous stream of cold air over the fish – air blast freezers, (2) directed contact between the fish and refrigerated surface – contact or plate freezers, (3) immersion in or spraying with a refrigerated liquid – immersion or spray freezers (Johnston, Nicholson, Roger, & Stroud, 1994). Plate freezer and contact freezer are the most used methods for freezing fish in Myanmar.

Freezing is a common practice in the meat, fish and other animal protein-based industries. Freezing is more popular than other preservation method because a decrease in enzymatic and microbial activity is acquired without the application of heating or preservatives (Luan, et al., 2017). The main purpose of freezing seafood is to slow down the physical and chemical changes of fresh products (Baygar, Alparslan, & Cakh, 2012). However, quality can still deteriorate in frozen fish products. Even when frozen, fish may, for example, lose flavour, become discoloured, and lipids oxidize, and proteins can deteriorate. In lean fish, lipid oxidation is less of a problem than in fatty fish because lean fish has low lipid contents in the muscle (Karlsdottir, et al., 2014). The quality of frozen fish and shelf life depend on fish species, handling of the fish, packaging, storage temperature and temperature fluctuations (Erikson, Sigholt, Rustad, Einarsdottir, & Jorgensen, 1999; Pigott & Tucker, 1987).

Freezing slows chemical reactions but does not stop them. Chemical reaction in unfrozen phase are main factors responsible for changes of enzymatic and non-enzymatic reactions during freezing. The formation of ice crystals can affect the product during freezing and storage which is leading to quality deterioration (Nollet & Leo, 2012). Surface colour of food and ice crystal size are dependent on the freezing rate. Colour of food is an important quality parameter which is attributed to chemical properties. All the same, the fast freezing is observed to cause lighter colouration of food with less red colour (Kono, Kon, Araki, & Sagara, 2017).

## **2.4 Quality changes during freezing and storage**

### *2.4.1 Lipid oxidation*

Fish contains mostly protein, water and lipid or fat. The amount of lipid or fat content in the muscle of fish varies from species to species, sex, diet, seasonal fluctuations and tissue. The lipid content is divided into phospholipids and triglycerides. The phospholipids make up the structure of cell membranes, but triglycerides are deposited in fat cells and distributed over the whole body such as belly flap, muscle, fins, tail, liver, abdominal cavity, under the skin and in the connective tissue. Categorization of fish into lean species and fatty species is based on the way lipids are deposited differently in a species. Lean species accumulate lipids in the liver and fatty species in the fat cells. Fish have a high content of polyunsaturated fatty acid (PUFA), which are susceptible to lipid oxidation. Lipid oxidation can occur during frozen storage is an important cause of quality deterioration in seafood. Lipid oxidation is one factor responsible for shortening the shelf-life of fish products. Phospholipids oxidation in lean fish can occur in cold storage and effect the flavour. Triglycerides oxidation in fatty species can cause rancid taste and odour (H & Serap, 2008 ; Jette & Flemming, 2012 ; Frankel E. N., 1996 ; Burgaard & Jorgensen, 2010).

Rancidity is a problem in oily fish associated with the frozen and dried storage. The extent of rancidity development depends on lipid level and fatty acid composition of the lipids. The lipid level depends on species, ranging from lean fish (<2% total lipid) and fatty fish (8-20% total lipid). Good freezing practices can reduce rancidity during storage by reducing ice crystal damage. Although lower temperature can decrease the chemical reaction, oxidation in meat and fish lipid is increased below freezing point, with a maximum around -10°C. The increase of rancidity is related to ice formation, especially in the oily region of fish (Bremner, 2002). Peroxide value (PV) and the 2-thiobarbituric acid-reactive substances (TBARS) are



commonly used chemical methods for the evolution of oxidation (Iglesias, Bianchi, Careri, Mangia, & Musci, 2009).

Lipid hydrolysis reactions can occur during storage (Adawiyah, Soekarto, & Hariyadi, 2012). Free fatty acids are derived from both triglycerides and phospholipids hydrolysis. Enzymatic degradation produces free fatty acids. Free fatty acids are not only important for oxidation product but also report to have a direct sensory impact. Hydrolytic rancidity is identifiable in terms of increasing oily, bitter and metal tastes (Ashton, Unilever, & Sharnbrook, 2005).

#### 2.4.2 Color Measurement

Changes in fish freshness can be related to changes in colour. The decrease in the freshness of fish is mainly related to structure and color. Instrumental colour measurements have become important in quality control in the food in industry (Olafsdottir, et al., 1997).

### 2.5 Storage temperature

Freezing and frozen storage is an important method in fish processing. Long-term storage can cause quality changes in fish muscle, fibres, proteins, lipids and textural properties (Mazrouh, 2015). Types of packaging maintenance of proper storage temperature and freezing properties of different species also influence quality changes. The storage temperature for all fishery products in the UK is recommended at  $-30^{\circ}\text{C}$ . At this temperature, bacterial activity is does not exist and chemical changes are greatly reduced. The International Institute of Refrigeration recommends storage temperature of  $-18^{\circ}\text{C}$  for lean fish (eg: cod and haddock) and  $-24^{\circ}\text{C}$  for fatty species (eg: herring and mackerel). Lean fish intended for frozen storage for over a year, should be kept at  $-30^{\circ}\text{C}$ . Storage life is extended with lower temperature (Table 1). Lowering the temperature for  $-18^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  will more than double shelf life.

Table 1. Practical storages lives (PLS) of fish products (FAO, 2014)

Product	Storage life in months		
	$-18^{\circ}\text{C}$	$-24^{\circ}\text{C}$	$-30^{\circ}\text{C}$
Fatty fish(glazed)	5	9	>12
Lean fish (fillet)	9	12	24
Flatfish	10	18	>24
Shrimp (cooked/peeled)	5	9	12

The lower storage temperature glass transition ( $-86^{\circ}\text{C}$ ) is nearly complete suppression of deteriorative reactive reactions. However, this temperature can damage the fish structure. A temperature of  $-40^{\circ}\text{C}$  is enough to stabilize the proteins, inhibit the formation of Trimethylamine N-Oxide (TMAO) in fish tissues and maintain good textural properties of lean fish. The effective temperature for high-quality long-term storage of fish is approximately  $-35^{\circ}\text{C}$  (Tolstorebrov, Eikevik, & Bantle, 2016).

### 2.6 Effect of temperature fluctuations during storage

Temperature fluctuation can cause loss of shelf life. It has a cumulative effect on food quality and proportion of practical storage life (PSL). Time-temperature tolerance (TTT) and product-

processing-packaging (PPP) concepts are used to monitor and control the effects of temperature fluctuations on frozen food quality during production, distribution, and storage (Fellows, 2009). Temperature fluctuation can occur during frozen storage. It may affect texture (dehydration, gaping), taste and flavour of the product due to the size of ice crystals formed in the fish muscle. Additionally, frozen fish quality may be decreased due to a higher amount of unfrozen water in the product, thus increasing the risk of spoilage due to enzymatic activity (lipid hydrolysis) and lipid oxidation (Romotowska, Gudjonsdottir, Karlsdottir, Kristinsson, & Arason, 2017).

### 3 MATERIALS AND METHODS

#### 3.1 Materials

Frozen Atlantic mackerel (*Scomber scombrus*) and redfish fillets (*Sebastes marinus*) were used in this study. The materials were transferred directly to the cold storage at MATIS laboratories from the producer HB Grandi Ltd (Reykjavík, Iceland). The experiments were carried out at MATIS laboratories in Reykjavik, Iceland.

#### 3.2 Experimental design

Flowchart of the experimental design is described in Figure 4. Frozen fish were received and divided into two groups. The first group was stored at a stable temperature (-25°C) and the second group was stored under fluctuating temperature (-18°C and -12°C) for 6 weeks. Samples of the product for temperature abuse were subjected to two temperature regimes for a 6 weeks period, i.e. (i) storage at -18°C, referred to frozen temperature abuse; and (ii) storage at -12°C, referred to as loading time and transporting of products.

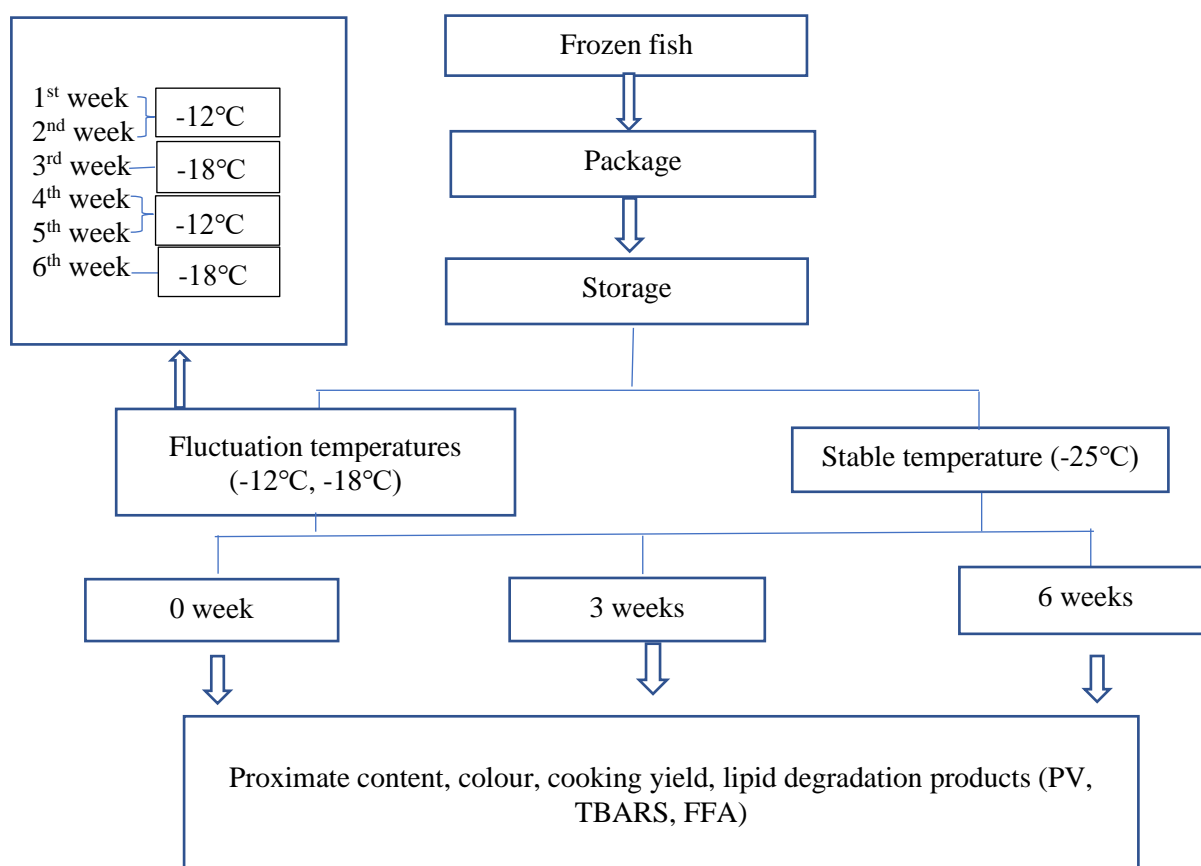


Figure 4. The Flow chart of experiment design

#### 3.3 Sampling

Samples were collected from the initial frozen fish materials and after 0, 3, 6 weeks of storage for evaluation of peroxide value (PV), thiobarbituric acid reactive substances (TBARS), free fatty acid (FFA), water and protein content and colour measurement. At each sampling time,

15 fillets from each experimental group were placed in trays and covered with plastic sheets to prevent dehydration and then transferred to thawing room and thawed at  $4\text{ }^{\circ}\text{C}\pm 1^{\circ}\text{C}$  for 24 hours.

### 3.4 Temperature Measurements

Temperature of each box was recorded in 10-minute intervals by temperature data loggers (i Button® type DS1922L, Maxim Integrated Products, Sunnyvale, CA, USA), which were placed in the middle of each box and on the outside of the boxes.

### 3.5 Chemical properties

#### 3.5.1 Protein and water content

Protein content was determined by the Kjeldahl method in accordance with ISO 5983-2 (2005).

The water content was determined according ISO 6496 (1999). The fillets were minced in a grinder for 15-20 seconds. Approximately 5.0 g of the minced sample was weighed and spread in a thin layer on a porcelain dish and then left to dry for more than 4 hours in oven at  $103\pm 2^{\circ}\text{C}$ . Then, the dishes were removed from the oven and cooled down in a desiccator at ambient temperature for 30 minutes before they were weighed again. The water content was calculated as follows:

$$W = \frac{m_1 - m_2}{m_1} \times 100 (\%)$$

Where: W is water content of sample (%)

$m_1$  is the mass of the sample (g)

$m_2$  is the mass of the sample after dried (g)

#### 3.5.2 Peroxide value (PV)

Lipid hydroperoxides were determined by the ferric thiocyanate method (Santha & Decker, 1994). Total lipids were extracted from 5.0 g of samples with 10 mL ice cold chloroform: methanol (1:1) solution, containing 500 ppm BHT to prevent further peroxidation during the extraction process. Sodium chloride (0.5 M) was added (5.0 mL) into the mixture and homogenized for 10 seconds (Ultra-Turrax T-25 basic, IKA, Germany) before centrifugation at 5100 rpm for 5 minutes (TJ-25 Centrifuge, Beckmann Coulter, USA). The chloroform layer was collected 50  $\mu\text{L}$  and completed with 950  $\mu\text{L}$  chloroform: methanol solution. A total amount of 5  $\mu\text{L}$  of ammonium thiocyanate (4 M) and ferrous chloride (80 mM) mixture (1:1) was finally added. The samples were incubated at room temperature for 10 minutes and measured at 500 nm in a microplate reader (Tecan Sunrise, Austria). A standard curve was prepared using cumene hydroperoxides. The results were expressed as mmol lipid hydroperoxides/kg of wet muscle.

#### 3.5.3 Thiobarbituric acid reactive substances (TBARS)

TBARS was measured by a modified method of Lemon (1996). A sample (5.0 g) was homogenized with 5.0 mL of trichloroacetic acid (TCA) extraction solution (7.5% TCA, 0.1% propyl gallate and 0.1% ethylenediaminetetraacetic acid mixture was prepared in ultrapure water) using a homogenizer at maximum speed for 10 seconds (Ultra-Turrax T-10 basic, IKA,

Germany). The homogenized samples were completed with 5.0 mL TCA extraction solution and centrifuged at 5100 rpm for 20 minutes (TJ-25 Centrifuge, Beckmann Coulter, USA) and filtrated with filtrate paper. Supernatant 50  $\mu$ L was collected and mixed with 950  $\mu$ L of thiobarbituric acid (0.02 M) and heated in a water bath at 95 °C for 40 minutes. The samples were cooled down on ice and immediately loaded into 96-wells microplates (NUNC A/S Thermo Fisher Scientific, Roskilde, Denmark) for reading at 530 nm (Tecan Sunrise, Austria). A standard curve was prepared using tetraethoxypropane. The results were expressed as  $\mu$ mol of malomaldehyde diethylacetal per kg of samples.

#### 3.5.4 Lipid content and free fatty acid (FFA)

Total lipid extraction was carried out based on the Bligh & Dyer (1974) method with some adaptations. To a 250-mL centrifuge bottle containing 25 g of sample, 50 mL methanol (MeOH) and 25 mL chloroform ( $\text{CHCl}_3$ ) were added and the mixture was homogenized for 2 minutes. 25 mL  $\text{CHCl}_3$  was added a second time and the mixture was homogenized for 1 minute. 25 mL 0.88% KCl was added and mixed for 1 minute. The final mixture was centrifuged 2500 rpm for 20 minutes at 4 °C. The total lipid exact (lower layer) was filtrated on a glass microfiber under suction. The suction flask content was poured into a 50 mL volumetric flask. Every trace of the upper phase (aqueous phase) was removed and the 50 mL volumetric flask was filled with chloroform. The lipid content was determined gravimetrically, and results was expressed as grams lipid/100 wet muscle.

Free fatty acid (FFA) content was determined on the total lipid extract according to the method of Lowery & Tinsley (1976), with modification by Bernardez et. al (2005). The FFA concentration was calculated as  $\mu$ molar quantities of oleic acid based on a standard curve in 2–22  $\mu$ mol range. Results were expressed as grams FFA/100 g of lipids.

### 3.6 Physical properties

#### 3.6.1 Cooking yield

Fillets were cut into not more than 100 g slices and weighed before and after cooking. They were cooked 6 minutes on a grill pan in a pre-warmed oven (Convotherm Elektrogerate GmbH, Eglfing, Germany) at 100 °C with air circulation and steam, then left for 15 minutes at room temperature and weighed for cooking determination. Result were expressed as g/g%.

The equation for calculating cooking yield is;

$$\text{Yield (\%)} = 100 \times (W_{ch} / W_{cr})$$

Where;  $W_{cr}$  = the cooked sample's raw weight

$W_{ch}$  = the cooked sample's cooked weight

#### 3.6.2 Colour

The intensity of the colour was measured with a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) using the CIE Lab system. The instrument recorded the L value, brightness on the scale of 0 to 100 from black to white; a value from -60 to 60, a>0 represents red component

and  $a < 0$  represents green component;  $b$  value from  $-60$  to  $60$ , (+) stands for yellow component and (-) stand for blue component (Figure 5).

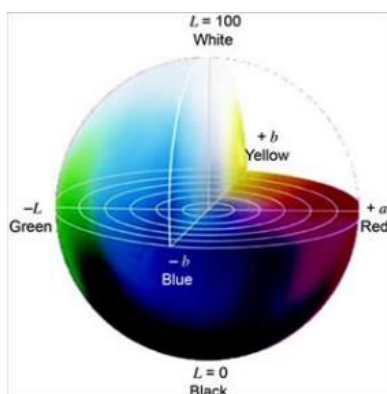


Figure 5. CIE Lab colour space

### 3.7 Statistical analysis

Statistical analysis was carried out by Microsoft Office Excel 2016 (Microsoft Inc, Redmond, Wash, USA). One-way ANOVA and Duncan's test were performed on the means ( $n = 4$  to  $6$ ) of the values obtained. The significance level was set at 95% ( $p < 0.05$ ) for all samples.

## 4 RESULTS

### 4.1 Water content

The water content of the mackerel samples ranged from 40.89% to 54.11% throughout the storage time (Figure 6A). The water content decreased significantly after 6 weeks of storage compared to the initial raw material regardless of storage conditions. No significant differences were observed regarding storage conditions at all sampling points.

The water content of the redfish samples ranged from 68.16% to 79.61% during the storage. As for the mackerel samples, the water content was significantly lower after 6 weeks of storage compared to the initial raw material. No significant differences in water content were observed regarding storage conditions at the end of the storage period.

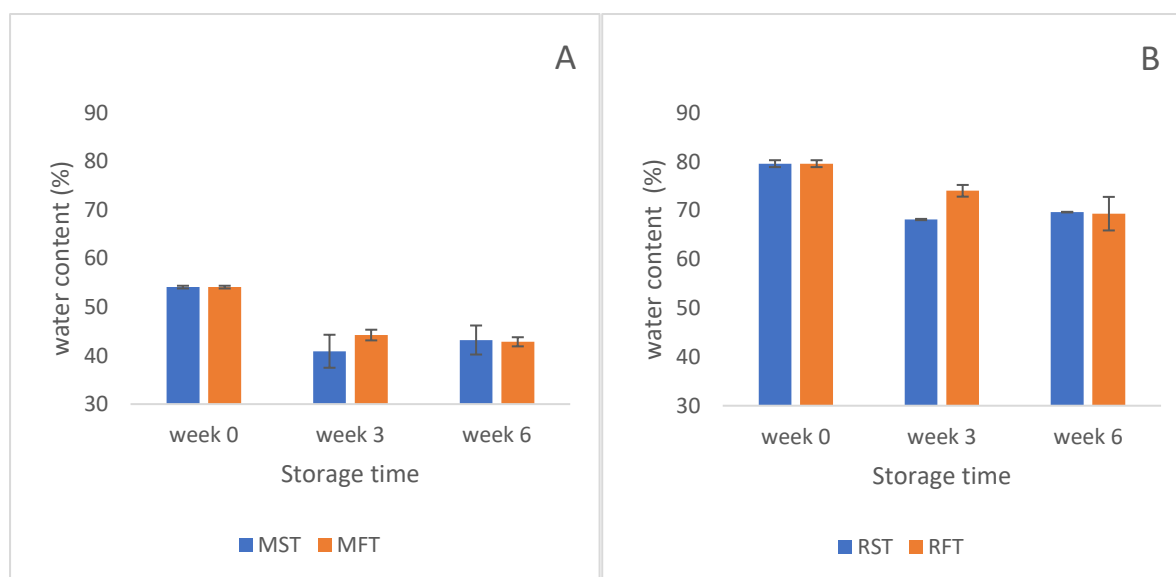


Figure 6. Water content of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at a stable temperature (ST; -25 °C) and fluctuating temperature (FT; -12 °C to -18 °C)

### 4.2 Lipid Content

The lipid content of the mackerel and redfish fillets ranged from 25.6% to 28.7% and 3.0% to 6.3%, respectively (Figure 7). The lipid content of both species was rather stable throughout the storage period, regardless of storage condition.

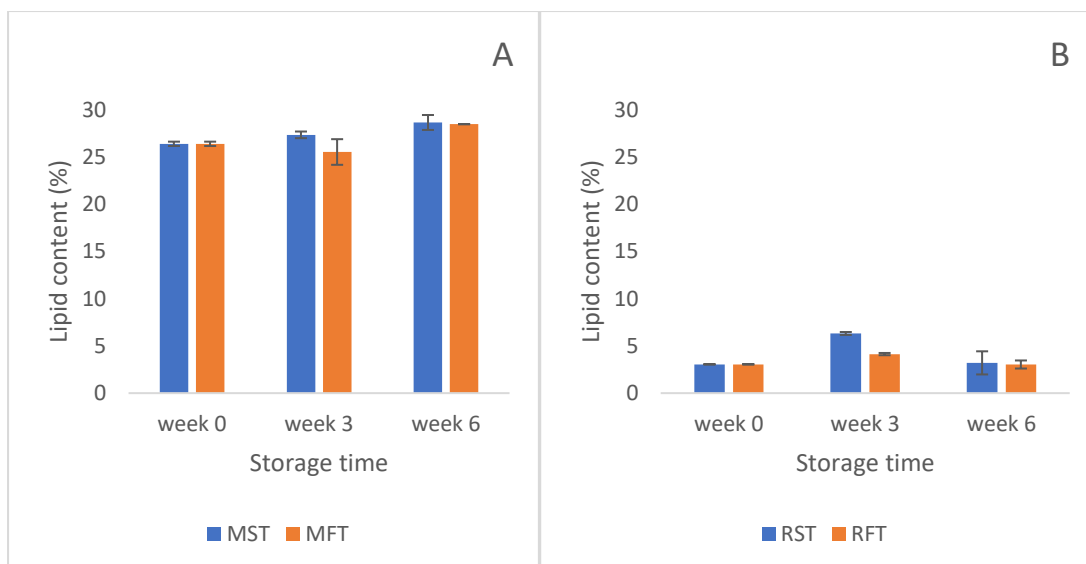


Figure 7. Lipid content (%) of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at stable temperature (ST:  $-25^{\circ}\text{C}$ ) and fluctuating temperature (FT;  $-12^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ )

### 4.3 Colour changes

The lightness (L value) of the mackerel and redfish fillets are shown in Figure 8. The lightness of the mackerel samples decreased with prolonged storage time, regardless of storage condition, but the changes were not significant (Figure 8 A).

The lightness of the redfish samples increased during the storage at both storage conditions, but the changes were not significant.

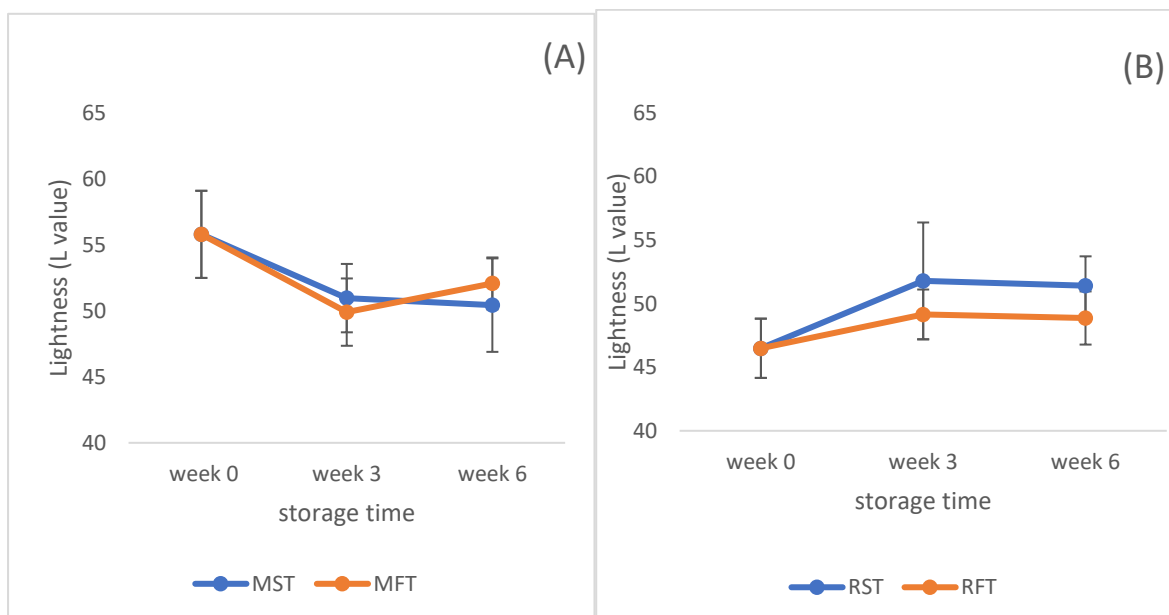


Figure 8. Lightness (L value) of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at stable temperature (ST;  $-25^{\circ}\text{C}$ ) and fluctuating temperature (FT;  $-12^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ )



The redness (a value) of the mackerel and redfish fillets ranged from 5.5 to 7.6 and -0.1 to 1.0, respectively. Storage conditions and storage time had no effect on the redness of all samples.

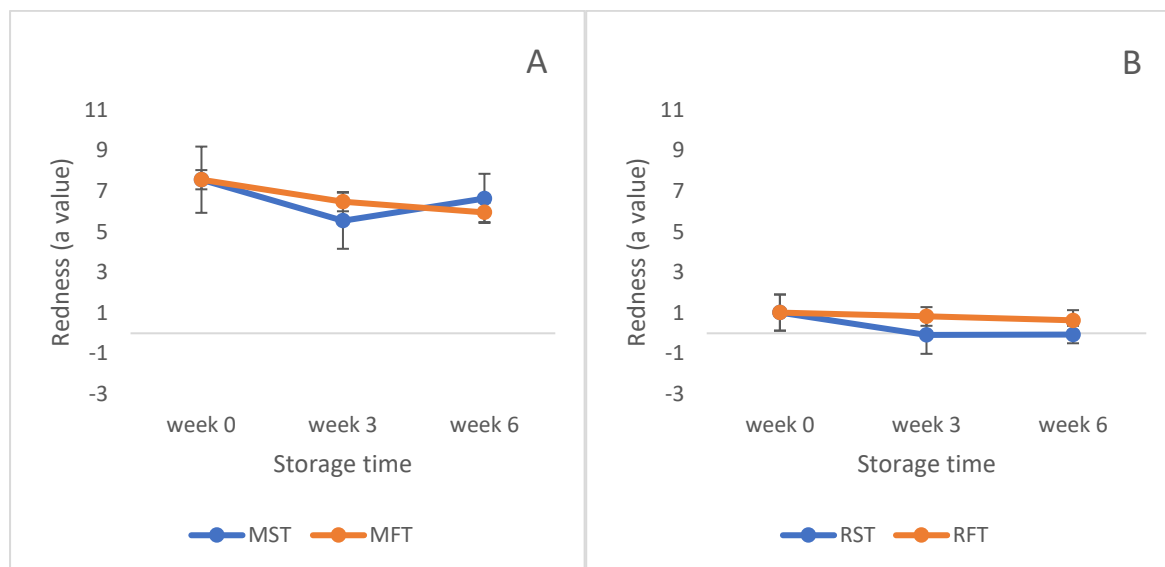


Figure 9. Redness (a value) of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at stable temperature (ST;  $-25^{\circ}\text{C}$ ) and fluctuating temperature (FT;  $-12^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ )

The yellowness (b-value) of the mackerel and redfish fillets ranged from 5.6 to 9.9 and -2.9 and -0.1, respectively. Storage conditions and storage time had no significant effect on the yellowness of all samples.

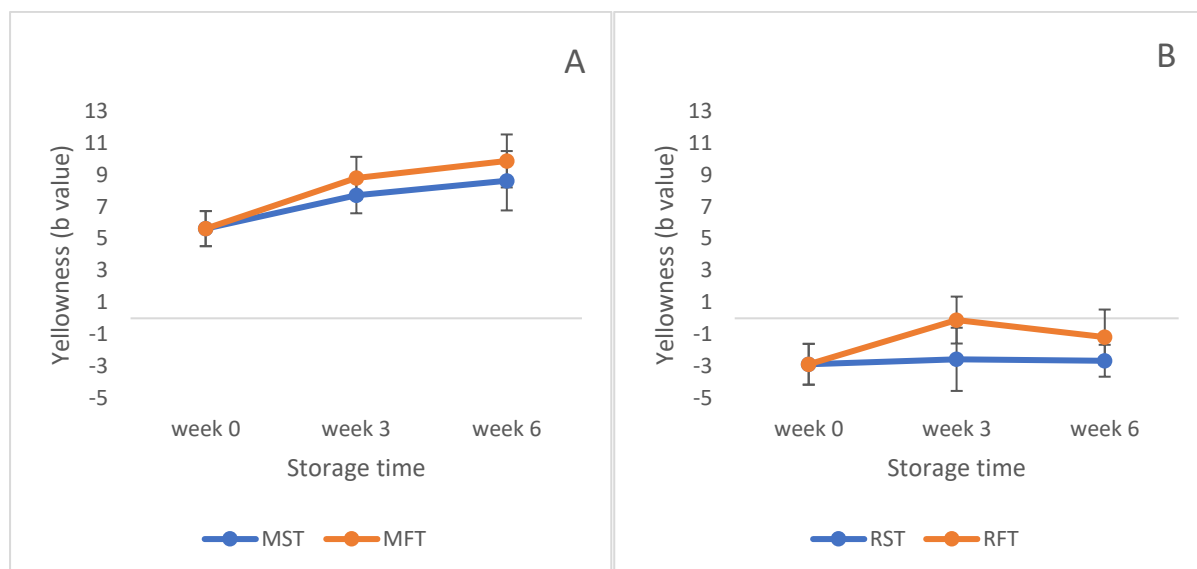


Figure 10. Yellowness (b value) of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at stable temperature (ST;  $-25^{\circ}\text{C}$ ) and fluctuating temperature (FT;  $-12^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ )

#### 4.4 Cooking yield

The cooking yield of the mackerel and redfish fillets are shown in Figure 11. The cooking yield of the mackerel fillets ranged from 89.9% to 93.4%. After 6 weeks of storage, the cooking yield

of the mackerel had decreased significantly compared to the initial raw material, regardless of storage conditions.

On the other hand, the cooking yield of the redfish fillets increased at week 6 compared to week 0, but the difference was not significant. The storage conditions had no significant effects on the cooking yield of both the mackerel and redfish fillets.

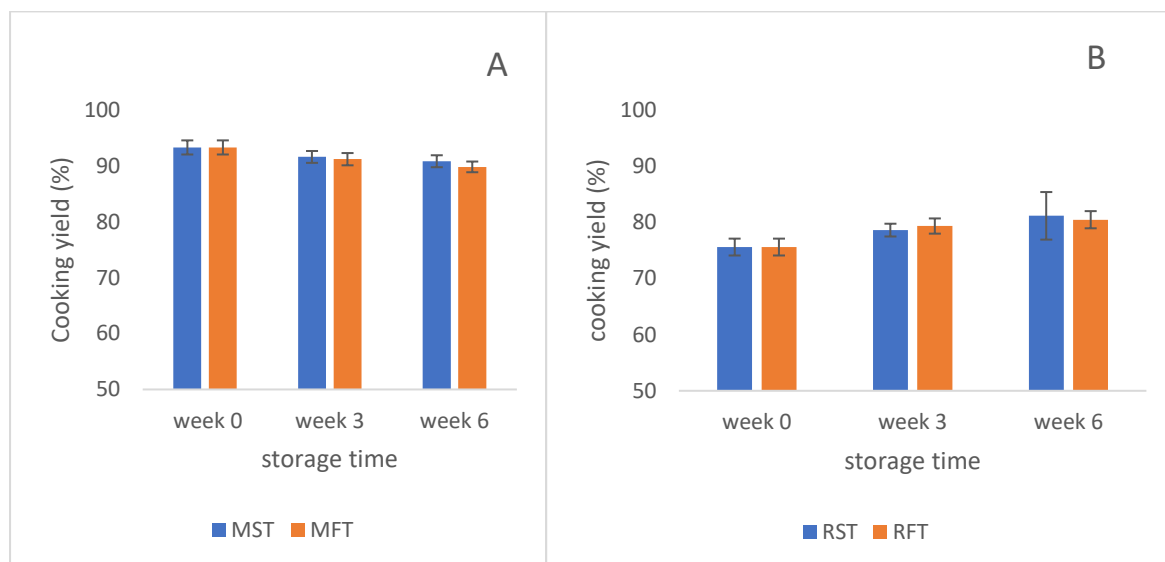


Figure 11. Cooking yield of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at stable temperature (ST;  $-25^{\circ}\text{C}$ ) and fluctuating temperature (FT;  $-12^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ )

#### 4.5 Peroxide value (PV)

Peroxide value (PV) is a primary oxidation product. The PV of both the mackerel and redfish samples stored at fluctuating temperature were significantly higher after 3 and 6 weeks of storage than the samples stored at stable temperature, as shown in Figure 12. No significant difference in PV was observed for mackerel samples stored at stable temperature for 6 weeks. The result showed that lower and stable storage temperature had stronger effects in inhibiting the lipid oxidation process in mackerel.

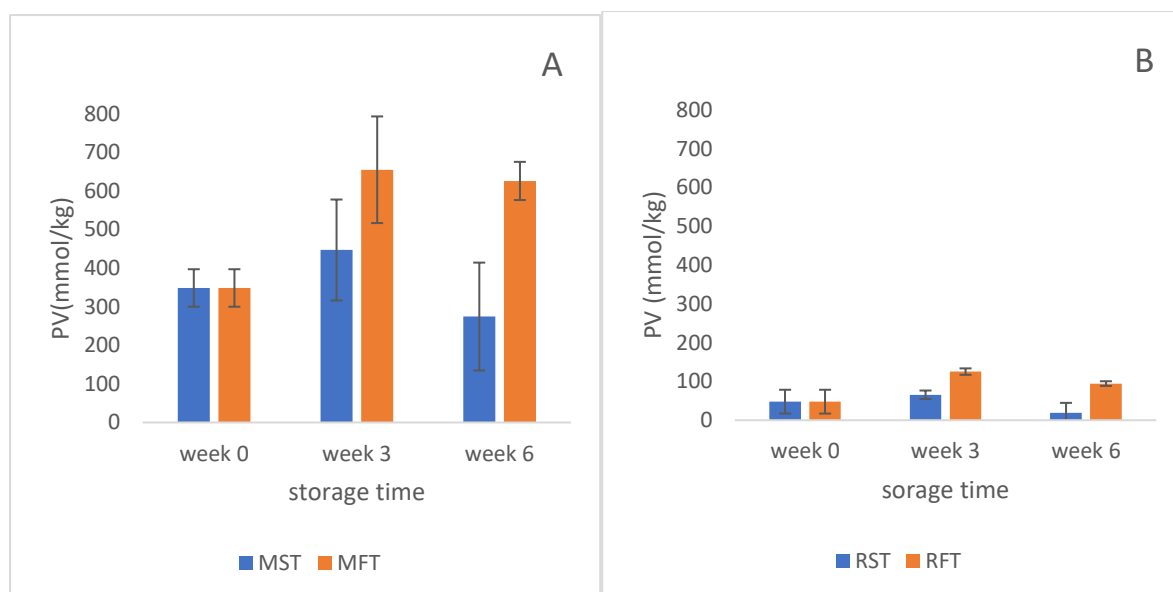


Figure 12. PV of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at stable temperature (ST;  $-25^{\circ}\text{C}$ ) and fluctuating temperature (FT;  $-12^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ )

#### 4.6 Thiobarbituric acid reactive substances (TBARS)

The results for TBARS analysis, a marker for secondary lipid oxidation products, appear in Figure 13. The TBARS of the mackerel samples increased significantly at week 6 compared to week 0. After 6 weeks of storage, the mackerel samples stored at stable temperature ( $-25^{\circ}\text{C}$ ) had significantly lower TBARS compared to samples stored at fluctuated temperature.

No significant changes in TBARS were observed for the redfish samples stored at stable temperature. However, when stored at fluctuating temperature, the TBARS of the redfish fillets was higher after 6 weeks of storage compared to the initial raw material ( $p < 0.05$ ).

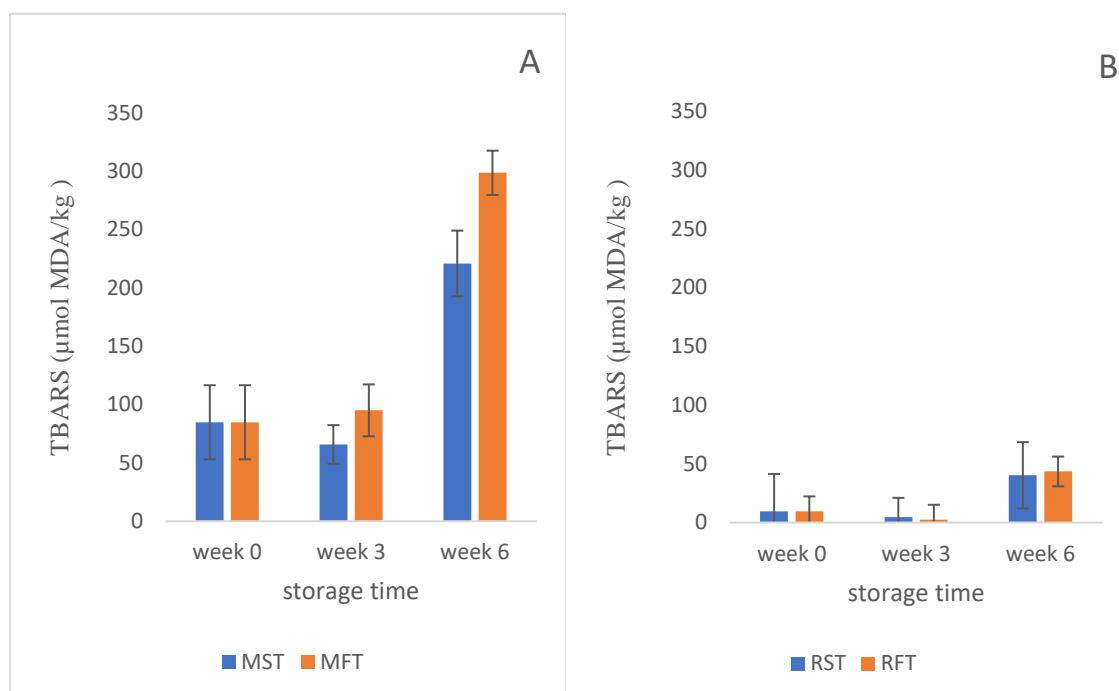


Figure 13. TBARS of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at stable temperature (ST;  $-25^{\circ}\text{C}$ ) and fluctuating temperature (FT;  $-12^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ )

#### 4.7 Free fatty -acid (FFA)

Free fatty acid of the mackerel and redfish fillets are shown in Figure 14. The FFA content of mackerel and redfish samples stored at stable ( $-25^{\circ}\text{C}$ ) temperature was rather stable during the 6 weeks storage period. However, the FFA content of samples stored at fluctuating temperature, increased significantly for both species.

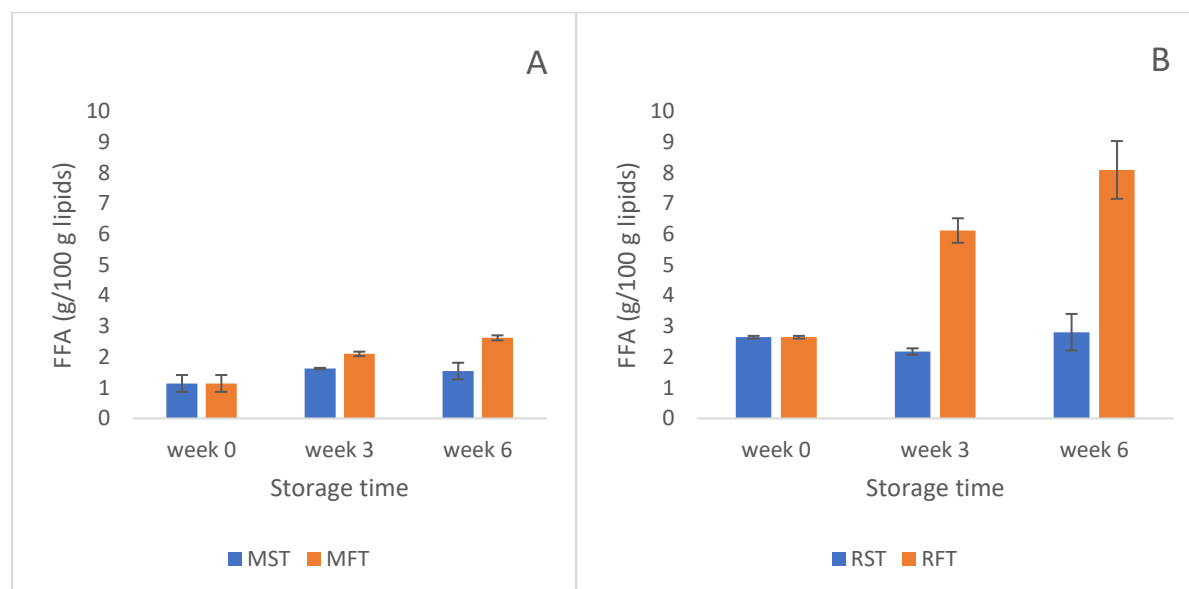


Figure 14. FFA of frozen (A) Atlantic mackerel and (B) redfish during 6 weeks of storage at stable temperature (ST;  $-25^{\circ}\text{C}$ ) and fluctuating temperature (FT;  $-12^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ )

## 5 DISCUSSION

### 5.1 Water and lipid contents

In the present experiment, the result of water content for redfish varied from 68.16%-79.61% and the lipid content varied from 3.05-6.32%. These results were similar to the findings of Jonsdottir (2014) who studied the shelf life of chilled redfish fillets. The lipid content of the mackerel ranged from 25.5% to 28.68%, while the water content ranged from 40.89% to 54.11%.

### 5.2 Changes in colour

All the colour parameters of mackerel and redfish did not show significant changes during the storage time (figure 7, figure 8 and figure 9). These results were similar with Seokjiin *et.al* (2017) when American sirloin and mackerel (*Scomber japonicus*) were stored in -18°C. There was no significant correlation between frozen storage time, storage condition and colour parameters which means that frozen storage time within 6 weeks is not associated with quality of mackerel and redfish of colour parameter.

### 5.3 Changes in PV and TBARS

PV values of mackerel and redfish increased significantly during storage at fluctuating temperature. The increase in PV indicated formation of primary lipid oxidation products and the formation of hydroperoxide. Lipid hydroperoxides are formed by various pathways including the reaction of singlet oxygen with unsaturated lipids or the lipid oxygenise catalysed oxidation of PUFA. These results were agreement with the result observed by Ronam *et.al* (2002) who studied the effect of fluctuating versus constant frozen storage temperature in frozen salmon.

The TBARS values increased significantly stored at both temperatures in week 6 for two of species. These results were similar to the finding of Romotowska *et.al*, (2017) who studied the affected of temperature abuse during transportation in frozen Atlantic mackerel (*Scomber scombrus*). TBARS increased due to the decomposition of hydroperoxides into the secondary oxidation products, especially aldehydes.

### 5.4 Changes in Free fatty acid (FFA)

The FFA concentration increased in samples stored at fluctuating temperatures. These results were in agreement with the result observed by Namulema, Muyonga & Kaaya (1999) who studied quality deterioration in frozen Nile perch (*Lates niloticus*). The increase in FFA could have been due to depletion of substrate or oxidation of the FFA. Accumulation of FFA in fish muscle has no nutritional significance but it has undesirable secondary effects including muscle texture changes, acceleration of lipid oxidation and off-flavour (Manuel, J, Jose, Jorge, & Santiago, 2013). This study showed that lipid hydrolysis occurs both in fatty fish such as mackerel and semi fatty fish as redfish during frozen storage, but the accumulation is highly temperature depended.

## 6 CONCLUSIONS

In this study, the effect of fluctuating temperature on the quality of Atlantic mackerel and redfish was analysed on the physiochemical properties during storage time. The consistent storage temperature,  $-25^{\circ}\text{C}$  had more preservative effects than fluctuant storage temperature ( $-12^{\circ}\text{C}$  to  $-18^{\circ}\text{C}$ ). Fluctuating temperature had significant negative effects on increasing the lipid degradation including PV, TBARS and FFA. However, some parameters had no significant differences in this storage time. In the conclusion, lower and stable temperature can effectively reduce quality loss of Atlantic mackerel and redfish, compared with fluctuating temperature ( $-12^{\circ}\text{C}$  to  $-18^{\circ}\text{C}$ ).

Based on this study, it can be said that lower and stable temperature effects on fish quality are less significant provided. This study informs the fish processing industry in Myanmar about the importance of stable frozen storage temperature so the country can better access the high-quality frozen product markets. The information will be transferred to the fishery plants in Myanmar by training.

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## APPENDIX I

## Chemical compositions of the raw materials

The chemical compositions of the raw materials were measured for Atlantic mackerel and redfish. The determined results are shown in Table 2. Generally, the quality of raw material was rather good.

*Table 2 Chemical compositions of the raw materials of mackerel and redfish*

Raw material	Protein Content (%)	Lipid content (%)	Water content (%)	Cooking yield (%)
Mackerel	16.55±3	26.42 ±0.23	54.11 ±0.28	93.32 ±1.13
Redfish	16.45 ±3	3.05 ±0.01	79.61 ±0.71	75.61 ±1.5

\*: The results are expressed as Mean ± Standard Deviation.