High Quality Redfish Fillets for Export

Improving handling, processing and storage methods to increase shelf life

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DECLARATION

I hereby declare that this thesis is based on my own observations, is written by me and has not in any part or as a whole been submitted before to a higher academic degree.

YFIRLÝSING

Hér með lýsi ég því yfir að ritgerð þessi er byggð á mínum eigin athugunum, er samin af mér og hefur hvorki að hluta né í heild verið lögð fram áður til hærri prófgráðu.

Reykjavík, 1. maí 2014

Steinunn Áslaug Jónsdóttir

ABSTRACT

The main purpose of this project was to study how different processing and packaging methods affect the rate of lipid oxidation, microbiological spoilage, and therefore the shelf life of fresh redfish fillets. The project was divided into three experiments performed in May, June, and October 2013.

In experiment I, the fish was evaluated in terms of lipid oxidation. Two bleeding methods, including bleeding by gill cut and bleeding by throat cut, were compared to the traditional unbled fish. All fish was packed the same way into expanded polystyrene (EPS) boxes with ice on top and stored in a cooling chamber at -1 °C.

In experiment II, the fish was evaluated in terms of lipid oxidation, microbiological spoilage, and sensory acceptance. Three different packaging methods were compared, including the traditional method of air packaging using EPS boxes with the addition of ice (Air), modified atmosphere packaging (MAP, 40% CO_2: 60% N_2) using plastic boxes (M1), and modified atmosphere with the addition of CO_2 emitting pads using plastic boxes (M2). All fish was stored in a cooling chamber with the initial temperature of -1 °C. To imitate temperature fluctuations during a sea freight transport, temperature of the cooling chamber was increased to +1 °C on day 6, and to +2 °C on day 10.

In experiment III, the fish was evaluated in terms of lipid oxidation, microbiological spoilage, and sensory acceptance of the fish. Four different groups were compared, including bled fish by gill cut placed into a box with the skin side up (H1), the traditional unbled fish placed into a box with the skin side up (H2), unbled fish with sandwich placement into a box (H3), and unbled fish with sandwich placement into a box and the addition of CO_2 emitting pads (H4). All fish in this experiment was packed into EPS boxes and stored in a cooling chamber with the initial temperature of -1 °C. As in experiment II, the temperature of the cooling chamber was increased to +1 °C on day 6, and further to +2 °C on day 10.

Lipid oxidation measurements in experiment I showed some conflicting results as primary and tertiary oxidation measurements showed no benefit of bleeding whereas secondary measurements showed no secondary oxidation in either of the bled groups.

However, as heme iron content measured higher in both bled groups it can be assumed that the bleeding was not effective. The colour measurements showed that bleeding the fish did not significantly affect the color of the fillets. However, visual observation of the fillets showed a clear difference between the groups on day 0, as the bled fillets were lighter in colour than the unbled fillets. This colour difference evened out during storage.

MAP proved to be a useful packaging method in reducing lipid oxidation. In terms of microbes, MAP fish favored the growth of *P. phosphoreum* as *Pseudomonads* count was reduced. However, total viable count (TVC) was not reduced in the MAP fish. An increase in drip was observed in the M1 and M2 groups as MAP fish had a lower pH than the air packed fish. In fact the highest drip was observed in the M2 group, and the lowest in the Air group. The addition of CO_2 emitting pads did not appear to increase the benefits of a regular gas packaging any further. As colour measurements showed no significant difference between the three groups, it can be concluded that MAP did not affect the colour. Freshness period of the MAP fish was increased by 1.5 days, with no difference between the two MAP groups.

Results of experiment III indicated that the different treatments and packaging methods did not prove to be beneficial in increasing the quality of the fillets compared with traditional methods. The addition of the CO_2 pads did not prove to be effective in reducing the pH much, which was probably caused by the gas leaking out of the boxes. Therefore, EPS boxes without an airtight inside seal cannot be recommended when using CO_2 pads. Bleeding the fish by gill cut, did not prove effective in the reduction of lipid oxidation, or microbiological spoilage. Heme iron content proved to be just as high in the bled group as in the unbled group indicating that the bleeding was not efficient enough. Another factor, which may have affected the outcome of the bled group (H1), is that the fillets were much smaller than in the unbled groups (H2, H3, H4). The sandwich placement of the fish in the box (H3) did not affect the spoilage factors. The colour analysis showed no significant difference between the groups on any of the sampling days. However, bled fish had somewhat more lightness than the unbled groups and sandwich placed fish was somewhat lighter than fish with skin side up placement. Darker spots were more obvious in unbled groups than in the bled group in the beginning of storage, however, the colour difference evened out as storage prolonged.

ÁGRIP

Megin markmið verkefnisins var að rannsaka áhrif ólíkra vinnslu- og pökkunaraðferða á þránun fitu, skemmda af völdum örvera og líftíma á ferskum karfaflökum. Verkefninu var skipt upp í þrjár tilraunir sem framkvæmdar voru í maí, júní og október 2013.

Í tilraun I var fiskurinn metinn út frá þránunarmælingum á fitu. Tvær blóðgunaraðferðir voru framkvæmdar á karfanum, annars vegar blóðgun með skurði á lífodda og hins vegar blóðgun með skurði á tálkn. Þessar tvær blóðgunaraðferðir voru síðan bornar saman við hina hefðbundnu aðferð þar sem engin blóðgun fer fram. Öllum flökunum var pakkað í frauðplastkassa (EPS) og kassarnir geymdir í kælihermi við -1 °C.

Í tilraun II, var fiskurinn metinn út frá þránunarmælingum á fitu, örverumælingum og skynmatsmælingum. Þrjár ólíkar pökkunaraðferðir voru bornar saman. Í fyrsta hóp var notuð hefðbundin aðferð þar sem fisknum var pakkað í frauðplastkassa með ís (Air). Í öðrum hóp var fisknum raðað í plastbakka og settur í loftskiptar umbúðir (MAP, 40% CO_2: 60% N_2) (M1). Í þriðja hóp var fisknum pakkað eins og fisknum í öðrum hóp, og að auki notaðar 5 CO_2 mottur sem raðað var á botninn (M2). Allir kassarnir voru geymdir í kælihermi með upphafshitastigið -1 °C. Til að líkja eftir gámaflutningi á skipi til Evrópu, þá var hitinn hækkaður í +1 °C á degi 6, og í +2 °C á degi 10.

Í tilraun III, var fiskurinn metinn út frá þránunarmælingum á fitu, örverumælingum, og skynmatsmælingum. Fjórir ólíkir hópar voru bornir saman. Í fyrsta hóp (H1) var fiskurinn blóðgaður með skurði á tálkn og raðað á hefðbundinn hátt í kassa með roðhliðina upp. Í öðrum hóp (H2) var hefðbundin aðferð þar sem fiskurinn var ekki blóðgaður, raðað í kassa með roðhliðina upp. Í þriðja hóp (H3) var óblóðgaður fiskur, raðað með samlokuröðun í kassa. Í fjórða hóp (H4) var fiskurinn óblóðgaður, honum raðað með samlokuröðun í kassa og að auki var 8 CO_2 mottum komið fyrir á botni hvers kassa. EPS kassar voru notaðir í öllum hópum og allur fiskurinn var ísaður með ísflögum og þurrís. Öllum kössunum var komið fyrir í kælihermi við upphafshitastigið -1 °C. Eins og í tilraun II, þá var hitinn hækkaður í +1 °C á degi 6, og í +2 °C á degi 10 til að líkja eftir gámaflutningi á skipi til Evrópu.

Þránunarmælingar á fitu í tilraun I, leiddu í ljós nokkuð ósamræmdar niðurstöður. Fyrsta og þriðja stigs þránunarmælingar sýndu fram á að blóðgun hafði ekki áhrif á þránun fitunnar, en annars stig þránunarmælingar sýndu að engin þránun var í blóðguðu hópunum. Það er þó vert að taka það fram að mælingar á járni sem bundið er við hemoglobin sýndu að meira

magn mældist í blóðguðum fisk en í óblóðguðum. Af þessu má álykta að blóðgunin hafi ekki verið nægilega góð. Litamælingar leiddu í ljós að blóðgun hafði ekki marktæk áhrif á lit fisksins. Sjónrænt mat leiddi þó í ljós greinilegan mun á hópum þar sem blóðgaði fiskurinn var áberandi ljósari en óblóðgaði fiskurinn í byrjun. Þessi litamunur jafnaðist þó út er leið á geymslutímann.

Loftskiptar umbúðir reyndust vel í að takmarka þránun fitu í ferskum karfaflökum. Í sambandi við skemmdarferli af völdum örvera, þá drógu loftskiptu umbúðirnar ekki úr vexti örvera í heild. Sértækar skemmdarörverur af tegundinni *P. phosphoreum* náðu að dafna vel í flökum sem pakkað var í loftskiptar umbúðir og þola greinilega vel umhverfi þar sem ekkert súrefni er til staðar. Hins vegar var vöxtur *Pseudomonads* tegunda takmarkaður með loftskiptum umbúðum. Vatnstap (drip) var meira í báðum MAP hópum en í viðmiðunarhóp (Air), eða mest í M2 hóp og minnst í loft hóp. Lægra pH mældist í MAP hópum en í loft hóp, en CO_2 motturnar höfðu áhrif til enn meiri lækkunar. Borið saman við loftskiptar umbúðir með gasi (M1), þá hafði viðbót CO_2 motta (M2) ekki aukin áhrif á geymsluþol. Litamælingar leiddu í ljós að MAP hafði ekki marktæk áhrif á lit flakanna. Einn helsti ávinningur af loftskiptum umbúðum virðist þó vera aukning á ferskleika-tíma fisksins, en MAP fiskurinn (M1 og M2) hélt ferskleikanum um 1,5 degi lengur en viðmiðunarhópurinn (Air). Ekki var marktækur munur á MAP hópum.

Niðurstöður úr tilraun III leiddu í ljós að þær mismunandi vinnslu- og pökkunaraðferðir sem rannsakaðar voru, höfðu ekki áhrif á skemmdarferli fisksins. Samanborið við aðra hópa þá höfðu CO_2 motturnar (H4) ekki marktæk áhrif á geymsluþol, en pH gildi fisksins lækkaði einungis lítillega við notkun þeirra. Helstu skýringar á þessu er að mest af gasinu hafi lekið út um kassann, og er því ekki hægt að mæla með að EPS kassar séu notaðir án loftþéttrar innri filmu við gaspökkun. Blóðgun fisksins með skurði á tálkn reyndist ekki árangursrík til að draga úr þránun fitu, né til að draga úr örveruvexti. Mælingar á járni bundið við hemoglobin reyndist engu minna í blóðguðum fisk en í óblóðguðum sem gefur til kynna að blóðgun bar ekki nægan árangur. Annar þáttur sem ber að minnast á er að blóðgaði fiskurinn (H1) var töluvert minni en óblóðgaði fiskurinn (H2, H3 and H4). Slíkur stærðarmunur getur haft áhrif á útkomu mælinga og því erfitt að bera saman fisk af ólíkri stærð. Samlokuröðun í kassa (H3) hafði ekki áhrif á skemmdarferlið, en heldur meiri ljósleiki mældist í H3 í samanburði við H2. Litamælingar leiddu í ljós að ekki var marktækur munur á milli hópa, þó mátti sjá að blóðgaður fiskur var heldur ljósari en óblóðgaður. Dökkir blettir voru meira áberandi í óblóðguðum flökum en í blóðguðum, þó jafnaðist þessi munur út er leið á geymslutímann.

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ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
CBC	Combined blast and contact cooling
CFU	Colony forming units
CO_2	Carbon dioxide
CP	Corrugated plastic
DMA	Dimethylamine
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
EPS	Expanded polystyrene
EUR	Euro
FA	Fatty acids
FFA	Free fatty acids
H_2S	Hydrogen sulfide
HX	Hypoxantine
IA	Iron agar
IMP	Inosine monophosphate
LH	Long and Hammers (agar)
MA	Modified atmosphere
MAP	Modified atmosphere packaging
MRD	Maximum recovery diluent
MUFA	Monounsaturated fatty acids
N_2	Nitrogen gas
O_2	Oxygen
OFR	Fluorescence shift ratio
PV	Peroxide value
PUFA	Polyunsaturated fatty acids
QDA	Quantitative descriptive analysis
qPCR	Quantitative polymerase chain reaction
SFA	Saturated fatty acids
SSO	Specific spoilage organism
TBARS	Thiobarbituric acid reactive substances
TMA	Trimethylamine
TMAO	Trimethylamine oxide
TVB	Total volatile bases
TVB-N	Total volatile bases nitrogen
TVC	Total viable count

1 INTRODUCTION

During recent years, the export of fresh fish from Iceland to European markets has been on the rise while the export of fish frozen at sea has somewhat declined. This change has resulted in higher price for the Icelandic fishing industries.

Today, the export of fresh redfish fillets from Iceland to European markets is mainly by airfreight. Due to the short shelf life of fresh fish, exporting the fish by airfreight ensures a fast delivery to the consumer meaning that the fish is fresh upon arrival. However, temperature fluctuations are inevitable with airfreight as the fish awaits loading and transportation. Exporting the fresh redfish fillets by sea freight rather than airfreight is both environmentally and economically beneficial. The cost of airfreight export to European markets is about 1.5 EUR per Kg while the cost of sea freight export is about 0.2 EUR per Kg. This is a large cost difference, which greatly affects the profit margin of the export companies. However, even though temperature fluctuations are minimized by sea freight, the downside of sea freight is the relatively long shipping time of about five days. Therefore, sea freight has not been found feasible due to the short shelf life of fish. The shelf life of a fresh whole redfish is 16-19 days, while the shelf life of fresh redfish fillets is 12-13 days (Martinsdóttir, 1995; Martinsdóttir & Magnússon, 1993). Therefore, it is of great importance to the Icelandic fishing industry to find ways to improve the shelf life of fresh redfish fillets.

The quality deterioration of fish is caused by microbiological, chemical and enzymatic actions. The spoiling pattern is species dependent. Furthermore, different spoiling pattern has been noted within species depending on many factors including handling of the fish, environmental factors, age, time of year, and nutritional composition of the fish. It is in fact of great importance to find out the different spoiling pattern for each species to increase the freshness period, as well as the overall shelf life of the fish. Much study has been done on the different species of fish, especially cod. However, redfish has not been studied as extensively. As a medium fat fish species, redfish may be prone to lipid oxidation during storage. Bleeding of fish is practiced with most fish species, as it is known to improve overall quality of the fish including color. Hemoglobin and iron are both known as potent pro-oxidants in fish. Despite this

knowledge, bleeding of redfish has not been practiced. Therefore, discolorations of the flesh can be observed.

The present study aims at finding ways to improve the shelf life and colour of redfish by means of different processing, packaging and storage methods. In order to decrease microbial growth and reduce lipid oxidation, modified atmosphere packaging was used to eliminate oxygen.

2 LITERATURE REVIEW

2.1 Redfish

Redfish lives in the North-Atlantic Ocean. Three main species can be found all around Iceland but are most abundant out of the west- and south-west coast. The three species are golden redfish (*Sebastes marinus*), deep-sea redfish (*S. mentella*), and Norway redfish (*S. viviparus*). Depending on species, redfish lives in different depth strata. The Norway redfish generally lives in the most shallow sea, usually above 100 m. Golden redfish lives at about 100-400 m depth and deep sea redfish lives at even greater depth of about 500-700 m at temperature of 3-8 °C (Figure 2.1). It has been found at depths of 1000 m. The golden redfish can reach the size of 90 cm, and the weight of 12-15 kg, however the most common size at the time of catch is about 35-42 cm and 0.6-1.1 kg. The golden redfish migrates vertically, depending on the amount of light, and tends to stay deeper during daytime than during night. Redfish is a slow growing fish that reaches puberty at the age of 12-15 years of age. Unlike most other fish species that spawn unfertilized eggs, redfish has an internal fertilization. Life offspring, 37-350 thousand at a time, are born in April and May. Redfish feeds mainly off plankton, crustaceans, and fish fries. Older redfish feeds off herring, capelin, shrimp and some codfish as well (Magnússon, 2000).

Figure 2.1. *Golden redfish (Sebastes Marinus) (adopted from http://thisfish.info/fishery/species/redfish/)*

Initially, Icelanders regarded the redfish as a side product of cod fishing and often discarded it or used it for fishmeal and oil. However, the golden redfish has become one of the most commercially important fish species in Icelandic waters in recent times. In the 1950´s to the year 1978 foreign fleets, mainly West German, were coming to

Icelandic waters for redfish fishing and catching more redfish than the local fleet (Figure 2.2). As foreign fleets were expelled from Icelandic waters, markets opened up in Germany, and Icelandic boats increased their catch. As redfish catching has declined, due to fishing quotas, there has been an increase in deep-sea redfish catching. The main markets today are Western Europe and Eastern Asia, with Japan as the single largest buyer of golden redfish (Ministry of Fisheries and Agriculture, retrieved Jan. 20th 2014).

The main fishing grounds for the golden redfish around Iceland over the years have been the South West and West coast of Iceland (Björnsson & Sigurðsson, 2003). The catching of redfish is done by bottom trawl, by either freezer trawlers or wet fish trawlers. The whole processing, packaging and freezing of the fish is done on board freezer trawlers. Whereas on board wet fish trawlers the fish is pre-cooled, iced, and transported fresh for processing on land. Fresh fish is mainly exported by airfreight (HB Grandi, retrieved Jan. 20th 2014).

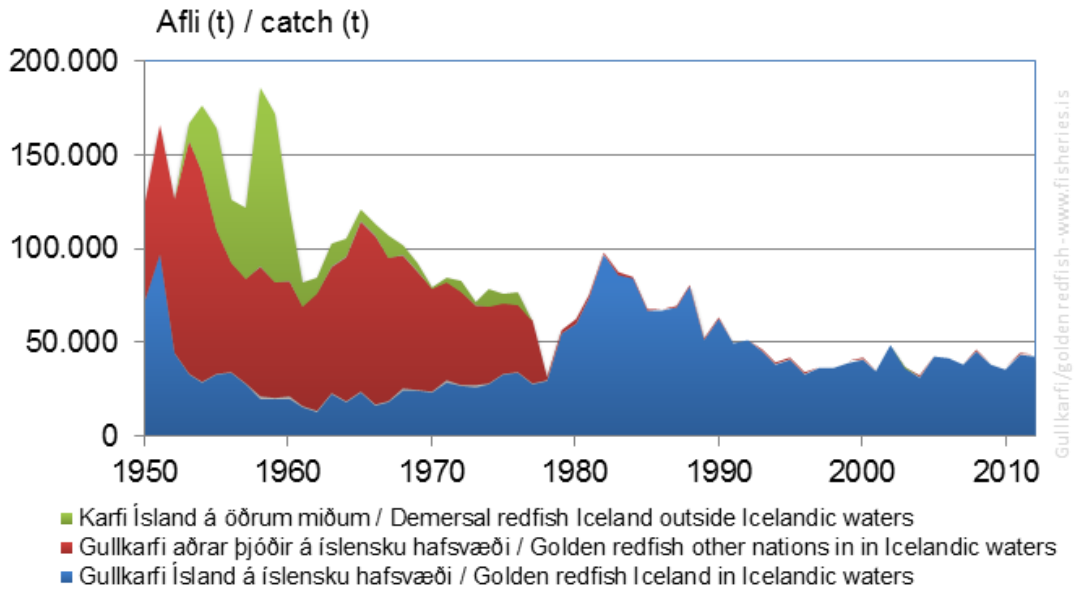

Figure 2.2. *Golden redfish catch in Icelandic waters (adopted from Ministry of Fisheries and Agriculture, 2014).*

2.2 Shelf Life of Fish

Sensory shelf life of fish is determined by the time from which the fish is processed until it is no longer fit for consumption. Total shelf life is the time from which the fish

is caught until processing plus the sensory shelf life of the fish (Lauzon *et al.*, 2010). As fish is perishable food, which spoils faster than any other muscle food, the shelf life of fish is very short compared to many other foods. This is mainly caused by the nutritional composition of fish, high pH and high water activity, which favors the growth of microbes (Huss, 1995). Many factors influence the shelf life of fish including initial microbial load, treatment of fish on board the ship and in the factory, as well as method of transportation. Therefore, shelf life varies from one species to another. Freshness period, determined by sensory evaluation, is the time from which a product is processed until the time when its freshness characteristics are lost (Torry score of 7). Sensory evaluation is the most efficient way of determining freshness of fish and quality deterioration (Connell, 1975; Ólafsdóttir *et al.*, 1997).

2.3 Postmortem Changes

Adenosine triphosphate (ATP) is the muscle cell energy carrier. After slaughter, anaerobic glycolysis becomes the metabolic pathway for ATP generation. Gradually the rate of ATP synthesis declines. ATP is no longer available for dissociating myosin from actin during contraction resulting in stiffening of the muscles, also known as rigor mortis. ATP hydrolysis generates and accumulates hydrogen ions which reduce the pH level of the muscle. Therefore, pH value is a good indicator of lactic acid accumulation (Damodaran *et al.*, 2008).

2.3.1 Enzymatic

Spoiling of fish begins with degradation of nucleotides by autolytic enzymes. Most of the ATP will be converted to ADP (adenosine diphosphate) and then to AMP (adenosine monophosphate) and from there to IMP (inosine monophosphate) and finally to HX (hypoxantine). IMP is very important in flavor masking of bitter flavors. Therefore, fresh fish flavour is lost as intermediate nucleotides, IMP, are lost. High levels of HX can make the fish unacceptable as it gives bitter flavor. The breakdown of nucleotides brought about by the autolytic changes, make catabolites available for bacterial growth (Church, 1998; Huss, 1995).

2.3.2 Microbial

Microorganisms are mainly found on the skin and gill surfaces of a live fish as well as in the gut. The number and type of microflora in fish flesh depends mainly on the environment in which the fish lives (Shewan, 1971). Generally, no microbes are found in the flesh of a living fish. Therefore, it is very important to minimize the cross contamination during handling of the fish both during storage and processing. Microbial flora of the cold water fish is dominated by psychrotrophic, gram negative bacteria like *Acinetobacter, Flvobacterium, Moraxella, Shewanella* and *Pseudomonas* (Huss 1995). Specific spoilage bacteria of iced, air stored fresh marine fish are mainly *Shewanella putrifaciens* and *Pseudomonas* species (Gram & Huss, 1996). *Pseudomonas* species are known to produce a number of volatile aldehydes, ketones, esters and sulphides (Edwards et al., 1987; Miller et al., 1973 a,b). Another bacteria of importance in the spoilage of fish, is *Photobacterium phosphoreum*. This bacteria lives in the gut of the fish and is common in deep sea fish. *P. phosphoreum* can grow in anaerobic conditions and is commonly seen in modified atmosphere packaging where the level of O_2 is excluded. In fact, *P. phosphoreum* has been shown to be the main spoilage bacteria in MAP fish, (Dalgaard *et al.*, 1997). The growth of *P. phosphoreum* becomes evident at temperature of 0-15 °C and it becomes a major spoiler of fresh air stored fish with increased temperature (Ólafsdóttir *et al*., 2003), however it is sensitive to freezing (Boknæs *et al.*, 2001, 2002).

2.3.3 Chemical

There are several chemical indicators of spoilage including trimethylamine (TMA), total volatile bases (TVB) and hypoxanthine contents of the flesh. The best known indicator of spoilage, TMA, is mainly derived from bacterial breakdown of trimethylamine oxide (TMAO) (Pedraso-Menabrito and Regenstein, 1990). TMAO is naturally found in significant numbers in marine fish, and serves as a part of the osmolyte system. TMA contributes to the stale aroma of fish (Damodaran *et al.* 2008). As TMA does not increase much in the early stages of spoiling, it should only be used as a discriminator in fish stored on ice and older than 6 days (Howgate, 1982). Shewanella *putrifacies* and *P. phosphoreum* both reduce TMAO to TMA but *P. phosporerum* is also CO_2 tolerant (Dalgaard, 1995). An alternative to measuring the TMA is total volatile basis (TVB-N), which includes ammonia, dimethylamine (DMA)

and TMA (Lauzon et al. 2010). Factors affecting the growth of microorganisms include temperature, duration of storage, chemical and nutritional composition of the fish as well as the availability of oxygen.

2.3.4 Lipid Hydrolysis and Oxidation

Free fatty acids (FFA) are known to cause problems in fish as they reduce the oxidative stability. Lipid hydrolysis involves the breakdown of lipids resulting in the formation of FFA. The mechanism involves the cleavage of triglyceride in the depot fat by triglyceride lipase from either the digestive tract or from microorganisms (Figure 2.3). Cellular phospholipases are believed to be involved in the production of FFA in lean fish species where phospholipids are cleaved (Figure 2.3) (Huss, 1995).

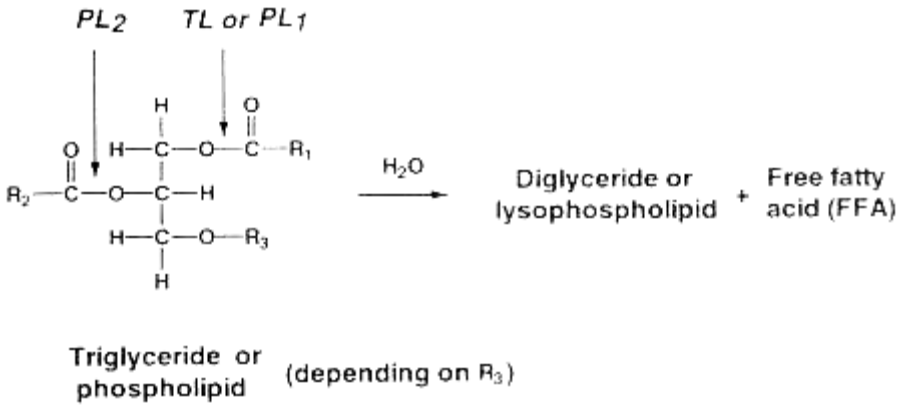

Figure 2.3. *Primary hydrolytic reactions of triglycerides and phospholipids. Enzymes: PL_1 & PL_2 phospholipases; TL, triglyceride lipase (Huss, 1995).*

Lipid oxidation is one of the major spoilage factors, resulting in quality deterioration of fish stored at refrigerated or frozen temperatures. Lipid oxidation refers to the interaction of lipids and oxygen. During this interaction, fatty acids esterified to triacylglycerols and phospholipids decompose to form small volatile molecules which give the off flavour and odour associated with lipid oxidation (Damodaran *et al.* 2008).

The rancid taste produced by lipid oxidation has been described as soapy, stale and linseed oil flavor. The taste of rancidity is an indicator of spoilage and is generally not accepted by the consumer. There are two types of rancidity: oxidative and hydrolytic. Hydrolytic rancidity is caused by microorganisms and moisture, and the off-flavor

comes from free amino acids. In oxidative rancidity, which is much more common, oxygen attacks unsaturated fatty acids giving hydro peroxides which then degrade into off-flavor compounds (Church, 1998). In addition to flavor and odor changes, lipid oxidation can cause color changes, reduce the nutritional value, and form free radicals, which can have harmful effects on one's health (Grey *et al*. 1996). As these compounds gradually accumulate, quantification of the compounds will give a measurement of quality deterioration (Connell, 1975).

Lipid oxidation has many mechanisms including enzymatic and non-enzymatic. Enzymatic oxidation of lipids is mainly caused by two enzymes including lipoxygenase and cylooxygenase. The non-enzymatic oxidation is mainly caused by autooxidation (free radical mechanism) and by photogenic oxidation (singlet oxygen mediated) (Ericson, 1999). Lipid autooxidation, the most common form of oxidation, starts as unsaturated fatty acid reacts with oxygen to form peroxides. There are three steps in the free radical mechanism including initiation, propagation and termination.

Initiation: $RH \rightarrow R\bullet + H^+$

$RH + O2 \rightarrow ROO\bullet + H^+$

Propagation: $R\bullet + O2 \rightarrow ROO\bullet$

$ROO\bullet + RH \rightarrow R\bullet + ROOH$

Termination: $2RO2\bullet \rightarrow O2 + RO2R$

$RO2\bullet + R\bullet \rightarrow RO2R$

In the initiation step, a hydrogen atom is removed from an unsaturated fatty acid (RH) resulting in a lipid radical (R•), which again reacts with oxygen (O_2) to form lipid peroxy radical (ROO•). Even though this reaction can be caused by irradiation, the most common is by interaction with oxygen. The next step, propagation, involves the interaction of lipid peroxy radical with an unsaturated fatty acid resulting in a lipid hydroperoxide (ROOH) and a new free radical. Lipid hydroperoxides (peroxides) are known as the primary product of lipid oxidation. This is a chain reaction which can go

on several times until the process is terminated either by two free radicals combining or by antioxidants (Gray, 1978). Peroxides are very unstable and break down easily into secondary products like aldehydes, ketones, alcohols and small alkanes (Figure 2.4). Peroxides cannot be detected by sensory evaluation as they don´t give off any distinctive odour or flavor. However, secondary oxidation products, like aldehydes, do have distinctive off flavors and are known to cause yellow colour of the fish muscle (Ericson, 1999).

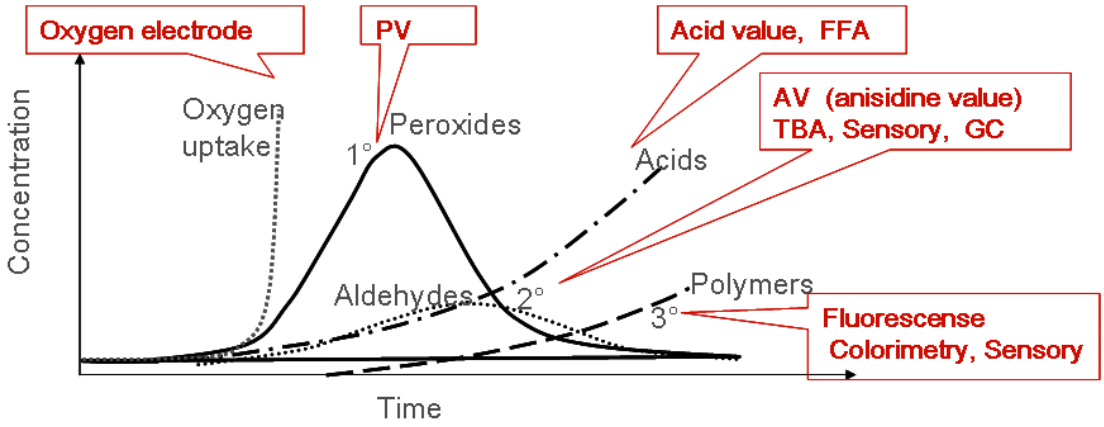

Figure 2.4. *Lipid oxidation progress, assessed by different methods (Jónsdóttir et al., 2008).*

Phospholipids, which are found within the cell membrane, are believed to be the key substrate in lipid oxidation as they contain high numbers of unsaturated fatty acids (Liang & Hultin, 2005). The unsaturated fatty acids are very unstable in the presence of oxygen and are easily oxidized (Shahidi & Dunajski 1994). The main catalysts of lipid oxidation are heme and non-heme iron (Hultin, 1994).

2.4 Bleeding

Spoilage bacteria can thrive in the post mortem flesh of unbled fish, as blood is a good nutrition for microbes. Due to increasing knowledge of how blood affects the quality of the fish, today, most fish is bled. Bleeding of fish is generally performed to remove as much blood from the flesh as possible for whiter flesh and less bacterial growth. In fact, bleeding has been shown to eliminate most of the hemoglobin in the flesh (Sohn *et al*., 2007). Hemoglobin, a protein found in the blood, has been found to be a major contributor of lipid oxidation in fish and fish products where blood has not been

removed prior to processing (Hultin & Richards, 2002). Proper bleeding has been shown to have adverse effect on oxidation of lipids in minced trout at 2 °C (Hultin & Richards, 2002). Furthermore, many other studies have shown that bleeding of fish is beneficial to reduce microbial growth and lipid oxidation (Benjakul & Maqsood, 2010; Hultin & Richards, 2002; Olsen *et al*. 2008; Sohn *et al*., 2005). Huss (1995) concluded that poorly bled fish will have shorter shelf life, discolored flesh and impaired taste.

There are mainly three different bleeding methods used today, throat cut, gill cut and pectoral cut. Throat cut, which is the most widely used method by the Icelandic fleet, involves cutting the blood vessel between the heart and the gill. Gill cut involves severing the gill arch or cutting up into the blood vessels (Figure 2.5). The pectoral cut involves a cut under the fin and is mainly used for tuna (Figure 2.5).

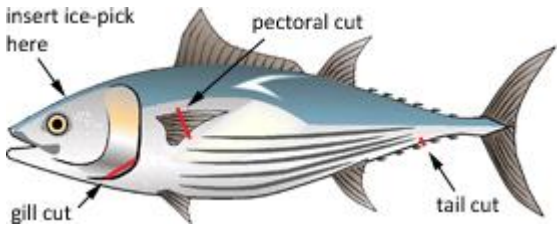

Figure 2.5. *Bleeding methods including gill cut and pectoral cut (adopted from http://www.go-saltwater-fishing.com/cleaning-fish.html)*

The time of bleeding as well as the pre mortem state of the fish is of great importance to the efficiency of the bleeding. Immediate bleeding after death, preferably before the heart stops beating, has been shown to be the most effective in removing the blood from the flesh. A study done by Roth *et al.*, (2005) showed that the time of bleeding is more important than the method of bleeding. Overall quality of wild cod was improved when bled before gutting. In this same study it was concluded that immediate bleeding of fish after catch was of great importance.

Handling all fish with care is very important during processing and storage. If the fish is knocked on hard surface, small blood vessels can rupture and blood leaks out into the flesh. The same can happen with the fish, which lies on the bottom of the trawl in a big catch. As this blood does not drain during bleeding, the flesh becomes discolored.

Even though bleeding has been shown to improve the overall quality and shelf life of fish, many fish species including redfish are still not bled. There are probably several reasons for this, including the small size of the redfish and the high number of individuals in each catch, as well as the fact that it has sharp spikes, which makes it difficult to handle.

2.5 Cooling

A number of studies have shown the importance of proper cooling in order to maintain freshness of fish during processing and storing. The main cooling methods used today include liquid cooling, slurry ice cooling, and combined blast and contact cooling (CBC). Liquid cooling involves the immersion of the fillets into tubs filled with lightly salted water. Slurry ice cooling involves immersion of the fillets into tubs filled with slurry ice. The two previously mentioned cooling methods have shown to increase the risk of contamination either from the salt or from the fish itself. According to Magnússon et al. (2009b), the brine can carry a large amount of microbes, including *P. phosphoreum*. Furthermore, temperature control can be difficult when applying liquid cooling. Valtýsdóttir *et al.*, (2010), measured a temperature fluctuation in a liquid cooler, from -0.4 °C to 4.0 °C with an average of 1-2 °C during a day of processing. CBC involves superchilling, which aims at lowering the temperature of the fish down to subzero temperatures, resulting in no higher than 10-20% of the water of the fish muscle to be frozen. The CBC method involves a process where the fillets, with the skin side down, are moved on a Teflon coated aluminum conveyor belt with a temperature of about -8 °C. Fans placed above the conveyor belt blow cold air on the fillets as they pass by. Before this process, the fillets go through slurry ice, which contains about 2.5 % salt, to prevent the flesh from freezing. Using this technique enables quick cooling of the fillets and packaging of fillets with a temperature of about -1 °C (Magnússon *et al*., 2009a). Studies have shown that maintaining subzero temperature throughout storage is effective in reducing the number of microbes and increasing the shelf life of the fish. A study done by Magnússon *et al.,* (2009a), showed that CBC cooling resulted in lower temperature during storage, longer freshness period and shelf life of cod fillets when compared to liquid ice cooling and plate ice cooling. Furthermore, CBC has been found to retard the growth of specific spoilage organisms like *P. phosphoreum* (Ólafsdóttir *et al.,* 2006 a, b).

Superchilling fish to a temperature of -1 °C has proved to be beneficial in reducing the number of microbes and extend shelf life. However, at such low temperature enzymatic reaction is enhanced, leading to different type of spoilage. This is due to partial freezing of the water phase, which enhances the substrate concentration. The higher the water content of the fish, the more is frozen at subzero temperature. Therefore, finding the proper temperature for each species is of great importance.

2.6 Packaging

2.6.1 MAP

Modified atmosphere packaging (MAP) is done to control the type of atmosphere that surrounds the product during storage. MAP generally results in longer shelf life when compared to traditional ice storage. The different factors that affect the shelf life include the composition of the gas mixture and the size of packaging, nutritional composition of the product, initial quality of the product, and storage temperature. The three types of gas generally used in MAP are carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2) at different levels. Lower levels of CO_2 are used to lower water loss and textural defects. However, when the concentration of CO_2 is high, the shelf life of fillets will increase (Molin *et al.,* 1983; Dalgaard *et al.,* 1993). Nitrogen is used as an oxygen replacer and does not affect the quality of the fish. As CO_2 is dissolved into the flesh of the fish, pH is lowered and water holding capacity of the protein decreases. Therefore, textural and taste defects can be expected (Davis, 1993; Lauzon *et al.,* 2009). The textural defects of too high CO_2 level have been described as tough and dry (Tiffney and Mills, 1982). Generally, however, fillets stored under high concentration of CO_2 have a longer shelf life (Molin *et al*., 1983; Dalgaard *et al*., 1993). Due to the high drip loss and weight loss and increased dryness of the fish, it is not preferable to use 100% CO_2. When packing lean fish, it has been found better to use O_2 instead of N_2 in combination with CO_2. Different ratios of the gases are needed depending on the fish species. Fish with higher fat content is, for example, vulnerable to lipid oxidation and therefore, tolerates less O_2 in the MAP. The use of oxygen in the gas mixture for MAP is generally not recommended for redfish which is a medium fat fish (Martinsdóttir *et al.,* 2003)

As with all storage methods, temperature plays a large role. Studies have shown that super chilling along with MAP considerably increases the shelf life of fresh fillets. *P. phosphoreum* is the main spoilage bacteria in MAP fish, (Dalgaard *et al.,* 1997) however it is sensitive to freezing (Boknæs *et al.,* 2001, 2002).

A study was done by Lauzon *et al.,* (2002), where a whole redfish was stored in bulk in modified atmosphere (MA) and subsequent redfish fillets stored in MAP, 60% CO_2, 40% N_2, were evaluated in terms of shelf life. Results showed that MA of whole redfish was not effective if stored for more than 5-10 days and negative effects were shown in texture. MAP fillets, processed form a 10 days old MA bulk stored whole redfish, showed a moderate increase in shelf life but negative effects in texture. The negative texture effects of fillets stored for 5 to 10 days in MAP were restored to some level when stored in air. Microbial levels were lower in MAP fish than in ice stored fish. However, TMA level was higher in the MAP fillets.

As mentioned before, temperature plays a large part in all storage. Partial freezing of samples has been shown to increase drip. Therefore, superchilling temperature closer to 0 °C has been shown to increase WHC in fillets when compared to frozen fillets in MAP (Gudjónsdóttir *et al.,* 2010).

2.6.2 Boxes

There are mainly two types of boxes, which have been used for storage and transport of fish: corrugated plastic (CP) boxes, and expanded polystyrene (EPS) boxes. EPS boxes are the traditional boxes used in the export of Icelandic fish as they are well insulated. However, these boxes are not very strong and can leak over time. CP boxes have now gained increased interest as they are environmentally and economically friendly. They are made of extruded corrugated plastic and can be folded to save space. Studies have indicated that CP boxes are poor insulators and weak (Anyadiegwu and Archer, 2002; Margeirsson *et al.*, 2009; Margeirsson *et al.,* 2011). A study done by Margeirsson *et al*., (2009), compared the effect of temperature abuse and the type of packaging on the shelf life of fish. Results showed that CP boxes have much lower insulation than EPS boxes. CP boxes can, however, be useful in export where temperature abuse is minimized (Lauzon *et al*. 2010).

2.7 Study Objectives

European buyers of fresh redfish fillets from HB Grandi ltd., have made some complaints about yellow spots appearing on the fillets as they age. This affects the price of the redfish fillets as the general consumer does not accept this as a high quality product. Therefore, the main purpose of this project was to investigate how different processing and packaging methods affect the shelf life of redfish with regard to lipid oxidation, colour, microbial growth and sensory evaluation. Three experiments were performed in an attempt to find out whether the yellow spots were caused by lipid oxidation and whether different processing and packaging methods could solve this problem.

The different processing methods included two different bleeding methods, compared with the conventional non-bleeding of the redfish. Different packaging methods included two types of MAP packaging, compared with a conventional air packaging. Two types of boxes were used, Færch plastic boxes and EPS boxes. In addition, different placement of the redfish fillets in the boxes was investigated, skin side up versus sandwich placement. The results indicate how these different parameters affect the overall freshness and shelf life of the redfish fillets. The main variables evaluated include temperature during storage, pH, drip loss, heme iron, non-heme iron, TMA, TVB-N, microbiological analysis (TVC, H_2S producing bacteria, *Photobacterum phosphoreum, and Pseudomonads),* free fatty acids, fatty acid analysis, lipid oxidation analysis (PV, TBARS, and fluorescence), sensory evaluation and colour analysis.

3 MATERIALS AND METHODS

3.1 Redfish Processing, Packaging, Storage and Sampling

3.1.1 Experiment I (May experiment)

The main purpose of this study was to measure lipid distribution, composition of fatty acids and lipid oxidation in different parts of the redfish fillet, as well as determining if different bleeding methods affect any of these parameters.

Redfish (*Sebastes marinus*) was caught by deep trawl on the 12th of May 2013 out of the west coast of Iceland. Three different methods of processing the fish on board were used, including no bleeding, and two different bleeding methods. The two bleeding methods involved a deep cut on the throat and a cut on the gill. The fish was iced and stored chilled until processed 3 days later, on May 15th, at HB Grandi (Reykjavík, Iceland). In this study, May 15th is referred to as day 0.

Once at the processing plant, the fish was beheaded, gutted, filleted, de-skinned and packed in EPS boxes, 5 kg in each, with a plastic film and ice on top. Packaging and treatment of the fish are listed in Table 3.1. Boxes were transported to Matís laboratory where they were randomly placed in a cooling chamber at -1° C +/- 0.5 °C for 15 days.

Sampling was performed on days 0, 5, 8, and 12 post-packaging. Five fillets were randomly selected each sampling day from each group (unbled, gill cut and throat cut) in duplicate. Each fillet was then cut into three sections, tail, middle and loin. Analyzes performed in experiment I are summarized in Table 3.4.

Table 3.1. *Experimental layout of experiment I, showing different processing and packaging methods.*

Groups	Section	Bleeding	MAP	Packaging
Unbled	Tail	No	No	-EPS box -Plastic film -Ice flakes -Absorbing pad
	Middle	No	No	
	Loin	No	No	
Throat cut	Tail	Yes	No	-EPS box -Plastic film -Ice flakes -Absorbing pad
	Middle	Yes	No	
	Loin	Yes	No	
Gill cut	Tail	Yes	No	-EPS box -Plastic film -Ice flakes -Absorbing pad
	Middle	Yes	No	
	Loin	Yes	No	

3.1.2 Experiment II (June experiment)

The purpose of this study was to observe how different packaging treatments affect the shelf life of the fresh fish with respect to chemical-, microbiological- and sensory evaluation.

The Redfish (*Sebastes marinus*) used in this study was caught on May 29th 2013 out of the west coast of Iceland by the fishing vessel Sturlaugur H. Böðvarsson, iced in tubs and stored chilled until processed (beheaded, filleted and skinned) 6 days later on June 4th at HB Grandi. In this study, June 4th is referred to as day 0.

Two packaging methods were used in this study, air packaging (A) and modified atmosphere packaging (MAP) (M1 and M2). The control fillets (A) were packed in expanded polystyrene (EPS) boxes (40402610, Promens Tempra), laid with an absorbing pad (capacity of 600 mL; McAirlaid´s Vliesstoffe GmbH & Co), and covered with a plastic sheet with ice placed on top (Figure 3.1). Treatments M1 (40% CO_2 and 60% N_2 gas packaging) and M2 (40% CO_2 and 60% N_2 gas packaging) with the addition of five CO_2 emitting pads (T-370, McAirlaid's Vliesstoffe GmbH & Co. KG,

Germany), were placed in Færch plastic boxes and covered with vacuum bags (cat. # 4031010114, 40x50 cm, PA/PE 85 µm; Oddi hf., Iceland) (Figure 3.1). Two boxes per group were analyzed each sampling day. The three different packaging and treatments are listed in Table 3.2. All boxes of fish were randomly placed on shelves in the cooling chamber.

Temperature inside containers maintain fairly constant during transport from Iceland to Europe. However, after 6 days, temperature has been shown to increase by 2 °C, from -1 °C to +1 °C upon arrival when the container is opened. Another increase in product temperature is expected on day 10 as the fish reaches retail stores. In order to imitate temperature change of a cooling chamber during transportation by sea freight from Iceland to European market temperature of the cooling chamber was set at -1 °C on day 0, then increased to +1 °C on day 6, and to +2 °C on day 10.

Figure 3.1. *Packaging box on the left side shows packaging of Air group in an EPS box covered with a plastic film and ice, then closed with EPS lid (not shown). Packaging box on the right side shows packaging of MAP groups in a plastic box, with no ice, covered with a plastic bag and sealed.*

To monitor temperature of the fish, loggers (iButton DS1922L, Maxim Integrated Products Inc, USA) were placed aseptically at two positions inside the boxes; one in the bottom corner on top of bottom layered fish, and one in the top corner underneath a top layer of the fish. The ambient temperature was recorded as well with 3 loggers altogether, one placed on top of a box furthest in the back, one in the middle, and one in the front of the cooling chamber. Temperature was recorded every 10 min. Loggers for treatment A were collected on day 14, while all other loggers were collected on day 16, at the end of storing time.

Sampling of fish was performed on day 0, 6, 10, and 14 post-packaging. Samples were taken from two boxes, A and B, for each group each sampling day. Analysis performed in experiment II are summarized in Table 3.4. For the sensory analysis, 7 fillets were

taken from the middle layer in each box analyzed and pooled together. For the microbiological analysis, one fillet from the top layer of each box, were pooled together into one sample (sample A) and one fillet from the bottom layer of each box were pooled together into one sample (sample B). Fillets were aseptically minced, assessing two pooled loins for each sample. Two replicate samples (upper and lower layers) were evaluated for each group. For the chemical analysis, two fillets from the top layer of each box were pooled together into one sample (sample A) and two fillets from the bottom layer of each box were pooled together into one sample (sample B). Only middle section was used for the chemical analysis.

Table 3.2. *Experimental layout of experiment II, showing different processing and packaging methods.*

Treatment	Bleeding	MAP	Packaging
A	No	No	-EPS box -Plastic film -Ice -Absorbing pad
M1	No	Yes (40% CO_2, 60% N_2)	-Plastic box -Plastic bag -Absorbing pad
M2	No	Yes (40% CO_2, 60% N_2) 5 CO_2 pads	-Plastic box -Plastic bag -5 CO_2 pads

3.1.3 Experiment III (October experiment)

The main purpose of this study was to evaluate how bleeding by gill cut versus no bleeding and different packaging methods affect the shelf life of fresh redfish fillets.

Redfish (*Sebastes marinus*) was caught on September 28th 2013 by the fishing vessel Sturlaugur H. Böðvarsson. Treatment on board included bleeding 25% of the fish (group H1) by gill cut and bled in air. All fish was then iced in tubs and stored chilled

until processed 3 days later, October 1st 2013, at HB Grandi. In this study October 1st is referred to as day 0.

At the processing plant, the following steps were undergone: beheading, filleting skinning, and thereafter, cooled in slurry ice before packaging. Packaging methods and treatment of the different groups can be seen in Table 3.3. Five kg of fish (all groups) was weighed into each box. All boxes were transported to Matís were they were randomly placed in a cooling chamber and stored for 16 days.

Temperature of the fish was recorded by loggers (iButton DS1922L, Maxim Integrated Products Inc, USA) inserted aseptically at two positions inside boxes; one in the bottom corner on top of bottom layered fish, and one in the top corner underneath top layer of the fish. The ambient temperature was recorded as well, with two loggers placed on top of boxes furthest in the back, two in the middle, and two in the front of the cooling chamber. Temperature was recorded every 10 min. All loggers were collected on day 16, at the end of storing time. All boxes of fish were randomly placed on shelves of the cooling chamber, and stored for 16 days (Figure 3.2). As in experiment II, temperature of the cooling chamber was set at -1 °C on day 0, then increased to +1 °C on day 6, and to +2 °C on day 10.

Figure 3.2. *Random placement of boxes in the cooling chamber.*

Sampling of fish was performed on days 0, 6, 10, 13 and 16 post-packaging. Analysis performed in experiment III are summarized in table 3.4. For sensory analysis, 10 filets were selected randomly from each box analyzed and pooled together. For microbiological analysis, three fillets were randomly selected from each box (box A

was sample A and box B was sample B). For chemical analysis, two fillets were randomly selected from each box (fish from box A was sample A and fish from box B was sample B).

Table 3.3. *Experimental layout of experiment III, showing different processing and packaging methods.*

Groups	**Bleeding**	**MAP**	**Placement of fish**	**Packaging materials**
H1	Yes – Gill cut	No	Skin side up	-Plastic film -750g ice flakes -80g dry ice -1 moisture absorbing pad -EPS box
H2	No	No	Skin side up	-Plastic film -750g ice flakes -80g dry ice -1 moisture absorbing pad -EPS box
H3	No	No	Skin side together	-Plastic film -750g ice flakes -80g dry ice -1 moisture absorbing pad -EPS box
H4	No	No	Skin side together	-Plastic film -750g ice flakes -80g dry ice -8 CO_2 pads -EPS box

3.2 Methods

The redfish fillets from the three experiments were analyzed with several analytical methods. The methods used for each experiment are summarized in Table 3.4.

Table 3.4. *Parameters evaluated, x marking indicates that the parameter was evaluated in the experiment.*

Parameters evaluated	**Experiment I**	**Experiment II**	**Experiment III**
Temperature		X	X
Gas		X	X
Drip		X	X
pH		X	X
TVB-N		X	X
TMA		X	X
TVC		X	X
PCR-Pp		X	
H2S		X	X
% Water	X		X
% Lipid	X	X	X
FFA	X	X	
FA analysis	X		X
Heme iron	X	X	X
Non-heme iron	X	X	
Colour - Lens eye	X	X	X
Colour - Minolta			X
PV	X	X	
TBARS	X	X	X
Fluorescence	X	X	
Sensory analysis		X	X

3.3 Headspace Gas Analysis

Headspace gas composition in the MAP packaging (experiment II) was evaluated using a PBI Dansensor (CheckMate 9900, Denmark) gas measuring device. A septum was put on the bag to enable measurements, a gas sample collected twice via the sampling needle and the latter measurement recorded for each pack.

3.4 Drip Analysis

The drip was calculated as the ratio of the water lost during storage to the original weight of the fish.

$$\text{Drip loss (\%)} = \frac{\text{g fillets before storing} - \text{g fillets after storing}}{\text{g fillets before storing}} \times 100$$

3.5 pH Measurement

For each treatment, the fish pH was measured directly in two whole fillets (loin side) as well as in the mince of two pooled fillets (5 g of mince mixed with 5 ml of deionised water) using pH electrode (SE 104 Mettler Toledo GmbH, Greifensee, die Schweiz) connected to a Knick pH meter (Portmes 913 pH, Knick, Berlin, Germany). Measurements were performed in top and bottom fish layers of each pack sampled, and average pH values were calculated with their standard deviation. Measurements were done within 30 min from sampling for MAP fish. The pH meter was calibrated using the buffer solutions of pH 7.00 +/- 0.01 and 4.01 +/- 0.01 (25°C).

For experiment III, the pH was measured in 5 g of minced loins mixed with 5 ml of deionised water using the Radiometer PHM 80, while pH measurement of whole loins (loin and tail sides) was performed with a pH electrode (SE 104 Mettler Toledo GmbH, Switzerland) connected to a Knick pH meter (Portames 913 pH, Knick, Germany). Measurements were performed within 30 min from sampling for MAP fish. The pH meters were calibrated using buffer solutions of pH 7.00 ± 0.01 and 4.01 ± 0.01 (25 °C).

3.6 Total Volatile Bases Nitrogen (TVB-N) and Trimethylamine (TMA)

The method of Malle and Tao (1987) was used to evaluate TVB-N and TMA content in the previously prepared mince. TVB-N was measured by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen) and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic acid (TCA) solution. The distilled TVB-N was collected in boric acid solution and titrated with sulphuric acid solution. TMA was measured in the TCA extract by adding 20 ml of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine. All

chemical analyses were performed in duplicate.

3.7 Microbiological Analysis

Fillets were aseptically minced, assessing two pooled loins for each sample. Minced flesh (20 g) was mixed with 180 g of chilled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 minute. Successive 10-fold dilutions were done as required.

Total viable psychrotrophic counts (TVC) were performed on iron agar (IA) as described by Gram et al. (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. In addition, TVC were performed on Long and Hammers (LH) agar, modified from: Van Spreekens K. J.A (1974). Counts of H_2S producing bacteria were evaluated on IA. Plates were spread-plated and incubated at 17 °C for 5 days. Counts of all colonies (both white and black) on IA gave the number of total count and counts of black colonies gave the number of H_2S producing bacteria.

Enumeration of presumptive *pseudomonads* was performed, using modified Cephaloridine Fucidin Cetrimide (mCFC) agar as described by Stanbridge and Board (1994). Pseudomonas Agar Base (Oxoid, UK) with CFC selective Agar Supplement (Oxoid) was used and the Plates were spreadplated and incubated at 22 °C for 3 days. Mean bacterial numbers are presented as log10 numbers of colony-forming units (CFU) per gram fish.

A quantitative Polymerase Chain Reaction (qPCR) method for estimation of *Photobacterium phosphoreum* (Pp) counts, developed at Matís ohf. (Reynisson *et al*., 2008), was performed on fish samples collected. Briefly, 1 of ml of the 10-fold diluted fish sample in MRD buffer was frozen at -20 °C for later DNA extraction. For the DNA extraction, the diluted samples were centrifuged at 11.000 x g for 5 min to form a pellet. The supernatant was discarded and DNA was recovered from the pellet using the promeganesil KF, Genomic system (MD1460) DNA isolation kit (Promega Corporation, Madison, USA) in combination with King Fisher magnetic beads automatic DNA isolation instrument (Thermo Lab systems, Waltham, USA) according to the manufacturers' recommendations. All PCR reactions were done using the MX 3005p instrument. The PCR was done using Universal Mastermix (Applied Bio-systems). Primers were synthesized and purified with HPLC (MWG, Ebersberg,

Germany). The DNA standard used for quantification of *P. phosphoreum* was previously calibrated against the PPDM-Malthus conductance method (Dalgaard et al. 1996; Lauzon, 2003) using fish samples from storage trials.

3.8 Water Content

The water content of the fillets was measured with two replicates per group. The fillet was minced in a grinder for 10-15 seconds at low speed. Approximately 5 g of the minced sample was weighed and spread in a thin layer on a porcelain dish and then left to dry for 4 hours in an oven at 103±2 °C. The dishes were then removed from the oven and cooled down in a desiccator at ambient temperature for 30 minutes before weighed again. The water content was calculated according to the ISO 6496:1999 standard, as follows:

$$W = \frac{m1 - m2}{m1} \times 100(\%)$$

Where:

W is water content of sample (%).

m1 is the mass of the sample before drying (g).

m2 is the mass of the sample after drying (g).

3.9 Lipid Extraction

The extraction method according to Bligh and Dyer (1959) was used to measure the lipid content of the redfish muscle samples. The samples were minced for several seconds at medium speed. Approximately 25 g (80±1% water) minced sample was weighed and homogenized by homogenizer (Ultra-turrax T25, IKA-Labor technik) with 25 mL of chloroform and 50 mL of methanol on cooling condition for 2 min to obtain a monophasic system. Then, 25 mL of chloroform was added and mixed for 1 min. Subsequently, 25 mL of 0.88% (w/v) KCl solution was added and homogenized again for 1 min. The next step was centrifugation at 2500 rmp (1017 x g) for 20 min at 0-5 °C (Sorwall, RC-5B, GSA rotor) to separate the mixture solution. The chloroform layer was separated, collected and filtered through glass filter paper (Watman GH/D) under

suction. The filtrate was collected and poured into a 50 mL volumetric flask (which was made up to mark). To determine the lipid content, 3 mL of the chloroform phase was transferred into pre-weighed test tubes and placed under nitrogen stream at 50-60 °C until all chloroform was evaporated. Then, test tubes were cooled down at room temperature and weighed again.

3.10 Fatty Acid Analysis

The methylation method was based on AOCS official Method Ce 1b-89 with minor adjustments. Extracted fat was weighed (70 mg) in a test tube with a stopper. Then, 1.5 mL of 0.5N NaOH (in methanol) was added, then mixed and heated for 7 min. at 100 °C. Thereafter, test tubes were cooled down to room temperature, then 1 mL of standard solution and 5 mL of concentrated NaCl solution were added to the test tubes. Then the solution was mixed in the test tubes by placing them on a vortex for half a minute. When the isooctane layer separated from the aqueous layer, it was transferred to clean test tubes with a small amount of natriumsulfate. Then, repeated with 1 mL of clean isooctan and mixed again with a vortex. Isooctan layer (1.5 mL) moved to a small glass tube for gas chromatography for fatty acid analysis.

3.11 Lipid Hydrolysis

Lipid hydrolysis was evaluated by following formation of free fatty acids (FFA). FFA content was determined on the total lipid extracts according to Lowry & Tinsley (1976), with modifications from Bernardez *et al.*, (2005). The FFA concentration was calculated as micromolar quantities of oleic acid based on a standard curve spanning a 2-22 µmol range. Results were expressed as grams FFA / 100 g of total lipids.

3.12 Lipid Oxidation

Lipid oxidation was evaluated by the mean of lipid hydroperoxide (PV), thiobarbituric reactive substances (TBARS) and fluorescent properties in the organic phase (or; tertiary oxidation product) resulting from the lipid extraction.

3.12.1 Peroxide Value - (PV; primary oxidation product)

Peroxide value (PV) was determined with a modified version of 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) in to the mixture and homogenised for 30 sec before centrifuging at 5100 rpm for 5 min (TJ-25 Centrifuge, Beckmann Coulter, USA). The chloroform layer was collected (500 µL) and completed with 500 µL chloroform:methanol solution. A total amount of 5 µL 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 min and read at 500 nm (Tecan Sunrise, Austria). A standard curve was prepared using cumene hydroperoxides. The results were expressed as mmol lipid hydroperoxides per kg of wet muscle.

3.12.2 Thiobarbituric Acid Reactive Substance - (TBARS; secondary oxidation product)

A modified method of Lemon (1975) was used for measuring thiobarbituric acid reactive substance (TBARS). A sample (5.0 g) was homogenized with 10.0 mL of trichloroacetic acid (TCA) extraction solution (7.5% TCA, 0.1% propyl gallate and 0.1% EDTA mixture prepared in ultra-pure water) using a homogenizer at maximum speed for 10 seconds (Ultra-Turrax T-25 basic, IKA, Germany). The homogenized samples were then centrifuged at 5100 rpm for 20 min (TJ-25 Centrifuge, Beckmann Coulter, USA). Supernatant (0.5 mL) was collected and mixed with the same volume of thiobarbituric acid (0.02 M) and heated in a water bath at 95 °C for 40 min. 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 µmol of malomaldehyde diethylacetal per kg of wet muscle.

3.12.3 Tertiary Oxidation Products (or)

Formation of fluorescent compounds was determined with a Perkin Elmer LS 50B fluorescent spectrometer by measurements at 393/463 and 327/415 nm excitation/emission maxima, according to other researchers (Aubourg & Medina 1997;

Aubourg & Medina 1999). The relative fluorescence (RF) was calculated as $RF=F/F_{st}$, where F is the sample fluorescence intensity at each excitation/emission maximum and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/mL in 0.05M H2SO4) at the corresponding wavelength. The fluorescence shift (or) was calculated as the ratio between the two RF values, i.e. or=RF393/463nm / RF327/415nm, and was analysed on the organic phase resulting from the lipid extraction (Bligh & Dyer 1959).

3.13 Heme Iron and Non-Heme Iron

Heme iron content was determined according to the method of Gomez-Basauri & Regenstein (1992) with a slight modification. First, samples were minced very well. Then, 20 mL of cold 40mM phosphate buffer (pH 6.8) was added to 2 g of the minced sample. The sample was then homogenized at 13,500 rpm for 10 seconds and centrifuged at 3000 rpm for 30 minutes at 4 °C. The supernatant was filtrated with Whatman No.1 paper (Whatman International Ltd., Maidstone, UK) and the absorbance read at 525 nm using spectrophotometer. Myoglobin content was calculated from millimolar extinction coefficient 7.6 and a molecular weight of 16,110. Heme iron content was expressed as mg/100g sample.

Non-heme iron content was determined according to the method of Schricker, Miller, and Stouffer (1982). The ground sample (1.0 g) was transferred into a screw cap test tube and 50 µl of 0.39% (w/v) sodium nitrite were added. Four mL of 40% trichloroacetic acid and 6 M HCl (ratio of 1:1 (v/v), prepared freshly) were added. The tightly capped tubes were placed in an incubator shaker (W350, Memmert, Schwabach, Germany) at 65 °C for 22 h and then cooled at room temperature for 2 h. The supernatant (400 µl) was mixed with 2 mL of the non-heme iron colour reagent (prepared freshly). After vortexing, using a Vortex-Genie2 mixer (Scientific Industries, Bohemia, NY, USA) and standing for 10 min, the absorbance was measured at 540 nm. The colour reagent was prepared by mixing a 1:20:20 ratio (w/v/v) of: (1) bathophenantroline (0.162 g dissolved in 100 mL of double deionised water with 2 mL thioglycolic acid [96–99%]); (2) double deionised water; (3) saturated sodium acetate solution.

The non-haem iron content was calculated from an iron standard curve. The iron standard solutions (Fe(NO3)2 in HNO3) with concentrations ranging from 0 to 5 ppm

were used. The concentration of non-heme iron was expressed as mg/100 g sample.

3.14 Colour Determination

Colour was measured by two methods: Lens Eye (computerized program) and by Minolta CR-400 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan). The colour was analyzed using the CIE Lab scale, with L* (black 0 to light 100), a* (red 60 to green -60) and b* (yellow 60 to blue -60) to measure lightness, redness and yellowness respectively (Figure 3.4).

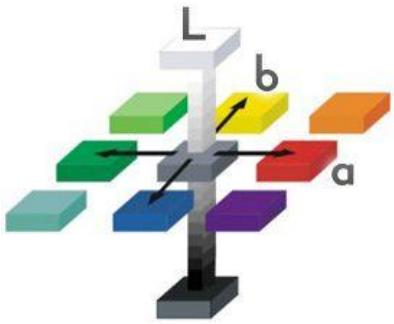

Figure 3.4. *Three-dimensional model of Lab colours, the L stands for the lightness of the color, with 0 producing black and 100 producing a diffuse white. The "a" is the redness vs. greenness, while the "b" is the yellowness vs. blueness (adopted from http://learn.colorotate.org/color-models/#.UxdbhCjkpS8).*

3.15 Sensory Evaluation

Samples of redfish fillets from experiment II and experiment III were evaluated by sensory methods. The main purpose was to evaluate differences in quality deterioration and shelf life according to sensory evaluation by a trained panel. Quantitative Descriptive Analysis (QDA), as introduced by Stone and Sidel (2004), and Torry freshness score sheet (Shewan et al., 1953; Table A.2 in appendix A) were used to assess cooked samples. Eleven panelists participated in the sensory evaluation of experiment II. Eight panellists participated in the sensory evaluation but five to six panellists took part in each session in experiment III. They had all been trained according to international standards (ISO 8586, 1993); including detection and recognition of tastes and odours, use of scales and in the development and use of descriptors. The members of the panel were experienced in using the QDA method and Torry freshness score sheet for medium fat fish. The intensity of each attribute for a given sample was evaluated using a 15 cm unstructured scale which in statistical

analysis was transformed to numbers from 0 to 100. Most of the attributes were defined and described by the sensory panel during other projects. The sensory attributes were 29 and are described in Table 3.4.

Each sample consisted of half a loin from a redfish fillet. The samples were placed in aluminum boxes coded with three-digit random numbers and cooked for 5 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95-100 °C with air circulation and steam and served warm to the panel (Figure 3.2). Each panelist evaluated duplicates of each test group in a random order in six sessions (maximum four samples per session).

Figure 3.5. *Half a loin of redfish fillets in aluminum box ready for cooking, and then tasting by the sensory panel.*

***Table 3.5**. Sensory attributes for cooked redfish and their descriptions.*

SENSORY ATTRIBUTE	SHORT NAME	SCALE	DEFINITION
ODOUR			
SWEET ODOUR	O-sweet	none || much	Sweet odour of fresh redfish
COD LIVER	O-liver	none || much	Boiled cod liver
SHELLFISH, ALGAE	O-shellfish	none || much	Characteristic, fresh odour
VANILLA/WARM MILK	O-vanilla	none || much	Vanilla, sweet warm milk
BOILED POTATOES	O-potatoes	none || much	Whole, hot, boiled potatoes in a saucepan
RANCID	O-rancid	none || much	Rancid odour
DISH CLOTH	O-cloth	none || much	Dirty damp dish cloth from the kitchen (left for 36 hrs.)
TMA	O-TMA	none || much	TMA odour, reminds of dried salted fish, amine
QUEASY SWEET	O-queasy	none || much	Spoilage odour, queasy sweet, overripe fruits
SPOILAGE SOUR	O-sour	none || much	Spoilage sour, sour odour, sour milk, acetic acid
SULPHUR	O-sulphur	none || much	Sulphur, matchstick, boiled cabbage
APPEARANCE			
COLOUR	A-colour	light || dark	light: white colour. Dark: yellowish, brownish, grey
HETEROGENEOUS	A-heterog.	homogeneous || heterogeneous	homogeneous: even colour. Heterogeneous: stains, uneven colour
WHITE PRECIPITATION	A-precipit.	none || much	White precipitation on the sample surface
FLAKINESS	A-flakes	none || much	The fish sample slides into flakes when pressed with a fork
FLAVOUR			
COD LIVER	F-liver	none || much	Boiled cod liver
METALLIC	F-metallic	none || much	Characteristic metallic flavour of fresh redfish
SWEET	F-sweet	none || much	Characteristic sweet flavour of fresh redfish
RANCID	F-rancid	none || much	Rancid flavour
PUNGENT	F-pungent	none || much	Pungent flavour
QUEASY SWEET	F-queasy	none || much	Spoilage flavour queasy sweet, overripe fruits
SOUR	F-sour	none || much	Spoilage sour, sour taste,
TMA	F-TMA	none || much	TMA flavour, reminds of dried salted fish, amine
OFF-FLAVOUR	F-off	none || much	Intensity of off-flavour (spoilage flavour)
TEXTURE			
SOFT	T-soft	firm || soft	Softness in first bite
JUICY	T-juicy	dry || juicy	Dry: draws liquid from mouth. Juicy: releases liquid when chewing
TENDER	T-tender	tough || tender	Tenderness when chewing
MUSHY	T-mushy	none || much	Mushy, porridge like texture
STICKY	T-sticky	none || much	Glues together teeth when biting the fish.

3.16 Data Analysis

Microsoft Excel 2004 for Mac version 11.6,6 (111101) was used to sort the data and calculate mean values and statistical difference between groups. One way ANOVA (analysis of variance) was performed on mean values and carried out in the statistical program SigmaStat 3.5 (Dundas Software Ltd., GmbH, Germany). The significance level was set at 5%.

For the sensory analysis, a computerised system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording. Panelcheck V1.3.2. (Nofima, Tromsø, Norway) was used to analyse panel performance. Analysis of variance (ANOVA) on QDA data was carried out in the statistical program NCSS 2000 (NCSS, Utah, USA). Comparison of data with respect to treatments was performed using the Duncan's multiple comparison test. The significance level was set at 5%.

4 RESULTS

The quality of redfish fillets was evaluated in terms of lipid oxidation, microbial evaluation, and sensory evaluation. Three experiments were performed in order to determine how different processing and packaging methods affect the quality of fresh redfish fillets.

4.1 Experiment I

The main purpose of this study was to measure lipid oxidation in fresh redfish fillets, comparing different parts of the fish (tail, middle, and loin) as well as top and lower layers in the box. The different processing methods involved unbled fish, gill cut fish, and throat cut fish.

4.1.1 Water and Lipid Content

The results of water content measurements showed that the redfish contained an average of 81.1% (+/- 0.6%) water. Results of lipid measurements are listed in Table 4.1 No significant change in lipid content was measured between sampling days in throat cut and gill cut fish. Lipid content was significantly higher in the middle section of throat cut fish on day 8, than that of unbled fish ($p<0.05$). The middle section of unbled fish had a significantly higher value than the tail section on day 8. The lowest value, 0.9%, in throat cut fish was measured in the loin section, day 0 and highest, 2.9%, in the middle section, day 12 ($p<0.05$). The lowest value in gill cut fish, 1.7%, was measured in the tail section on day 0. The highest value in gill cut fish was 3.8% in middle section on day 8. In unbled fish, the lowest value 0.8% was measured in loin section, day 0, whereas the highest value was measured in the middle section on day 12.

***Table 4.1**. Lipid content (g lipids/100g fish) for three different sections (tail, middle, and loin) of throat cut, gill cut, and unbled redfish measured on days 0, 5, 8, and 12, (mean +/- SD, n=4).*

Section	Days	Throat cut	Gill cut	Unbled
Tail	0	1.2 ± 0.1	1.7 ± 0.1	1.0 ± 0.2
	5	2.3 ± 1.1	2.0 ± 0.9	1.5 ± 0.3
	8	1.4 ± 0.6	2.4 ± 0.8	1.8 ± 0.2
	12	1.8 ± 0.5	1.9 ± 0.2	1.1 ± 0.0
Middle	0	1.3 ± 0.1	2.5 ± 0.1	1.2 ± 0.4
	5	2.1 ± 1.1	2.5 ± 1.7	2.4 ± 0.2
	8	2.1 ± 0.6	3.8 ± 1.9	3.9 ± 0.7
	12	2.9 ± 1.2	2.4 ± 0.3	1.4 ± 0.4
Loin	0	0.9 ± 0.0	1.8 ± 0.1	0.8 ± 0.1
	5	1.5 ± 1.2	1.9 ± 1.2	1.7 ± 0.1
	8	1.4 ± 0.6	2.4 ± 1.2	2.4 ± 0.2
	12	1.8 ± 0.2	1.6 ± 0.8	1.0 ± 0.5

4.1.2 Fatty Acid (FA) Analysis

Results of SFA, MUFA, PUFA, EPA and DHA are listed in Table 4.2. On day 0, SFA in tail, middle and loin sections of unbled fish measured 23.8% ± 0.2%, 24.2 % ± 0.3%, and 24.5% ± 0.25% respectively. MUFA in tail, middle and loin sections of unbled fish measured 32.4% ± 3.5%, 33.6% ± 3.5% and 30.1% ± 3.7% respectively. PUFA in tail, middle and loin sections of unbled fish measured 35.2% ± 3.8%, 34.1% ± 4.0% and 37.3% ± 4.2% respectively. On day 0, DHA in tail, middle and loin sections of unbled fish measured 20.8% ± 3.5%, 20.2% ± 4.1% and 23.4% ± 4.6% respectively. EPA in tail, middle and loin sections of unbled fish measured 8.4% ± 0.3%, 8.1% ± 0.0%, and 8.4% ± 0.1% respectively.

A significant decrease in SFA was observed in the middle section of unbled fish between days 0 and 12 ($p<0.05$). Otherwise, there was no significant difference between treatments or sections. A decrease in SFA was observed in all groups between day 0 and 12, however, not significant. Results showed that levels of MUFA increased slightly with storage time ($p>0.05$). Even though there was no significant difference in levels of MUFA between groups or sections of each treatment, there was a trend with

the highest value of MUFA noted in the middle section. No significant difference was observed in levels of PUFA between sampling groups, sections or days. However, with storage time some decrease in PUFA was observed within all treatment groups. Lower values of PUFA were generally seen in the middle part of the fillet. No significant difference was observed in EPA between sampling days within each group and no significant difference between the groups. A small decrease ($p>0.05$) in DHA levels was observed with storage time. However, no significant difference was observed between groups or sections. A trend of lower DHA values was observed in the middle part of the fillet compared to loin and tail sections.

Table 4.2. *Fatty acid analysis (g fatty acids/100 g lipids) for tail, middle and loin sections of unbled, gill cut and throat cut fish on days 0 and 12 (Mean +/- SD, n=4).*

Groups	Days	Section	SFA	MUFA	PUFA	DHA	EPA
Unbled	0	Tail	23.8 ± 0.2	32.4 ± 3.5	35.2 ± 3.8	20.8 ± 3.5	8.4 ± 0.3
		Middle	24.2 ± 0.3	33.6 ± 3.5	34.1 ± 4.0	20.2 ± 4.1	8.1 ± 0.0
		Loin	24.5 ± 0.2	30.1 ± 3.7	37.3 ± 4.2	23.4 ± 4.6	8.4 ± 0.1
	12	Tail	19.8 ± 0.8	39.5 ± 1.3	32.8 ± 3.5	19.9 ± 0.6	6.1 ± 1.2
		Middle	19.1 ± 0.8	44.6 ± 3.4	28.9 ± 4.4	16.9 ± 3.3	5.1 ± 0.4
		Loin	20.7 ± 1.2	37.5 ± 7.4	33.8 ± 5.4	21.3 ± 6.1	6.1 ± 0.1
Gill cut	0	Tail	22.8 ± 0.3	34.5 ± 2.3	33.9 ± 4.0	20.7 ± 2.2	6.3 ± 0.6
		Middle	22.7 ± 0.2	40.3 ± 3.8	28.6 ± 4.0	16.0 ± 2.6	5.4 ± 0.5
		Loin	22.8 ± 0.3	37.4 ± 4.3	31.5 ± 4.0	19.0 ± 2.7	5.7 ± 0.6
	12	Tail	21.5 ± 1.1	40.3 ± 2.8	30.6 ± 4.2	17.2 ± 1.5	6.4 ± 1.0
		Middle	21.1 ± 1.5	45.6 ± 5.2	25.9 ± 4.4	13.2 ± 2.9	5.7 ± 1.4
		Loin	21.5 ± 0.5	40.2 ± 1.1	30.2 ± 3.4	17.2 ± 1.5	6.3 ±0.6
Throat cut	0	Tail	22.9 ± 1.3	35.2 ± 3.1	33.7 ± 4.0	20.1 ± 4.0	7.4 ± 0.6
		Middle	22.7 ± 1.2	37.2 ± 0.3	31.7 ± 4.0	19.5 ± 1.2	6.0 ± 0.9
		Loin	23.2 ± 1.5	31.5 ± 0.3	36.7 ± 4.1	23.8 ± 1.0	6.9 ± 0.8
	12	Tail	20.5 ± 0.0	42.4 ± 1.5	29.5 ± 4.0	16.3 ± 1.4	6.5 ± 0.0
		Middle	20.9 ± 1.8	46.6 ± 2.4	25.0 ± 4.1	12.1 ± 0.1	6.0 ± 0.5
		Loin	21.6 ± 1.2	41.1 ± 3.6	29.8 ± 3.9	16.1 ± 1.7	7.0 ± 1.1

4.1.3 Free Fatty Acids (FFA)

Free fatty acids (FFA) are formed as a result of lipid hydrolysis. The results of FFA measurements are shown in Figures 4.1, 4.2, and 4.3. Generally, the same trend was observed in the amount of FFA measured in the redfish of both unbled and bled groups. The amount of FFA increased with time but some difference between sections of fish was observed. The highest value 5.19 g FFA was measured in the tail section of unbled fish on day 12, and the lowest value 1.01 g FFA was measured in the middle section of gill cut fish on day 0. Significant change in FFA between storage days was observed in loin, middle and tail sections of unbled fish, in the tail section of throat cut fish, and in tail and loin section of gill cut fish ($p<0.05$). On days 8 and 12, tail section of throat cut fish showed a significantly higher value of FFA than middle and loin sections ($p<0.05$). Tail section of unbled fish showed a significantly higher value on day 8, than middle and loin sections ($p<0.05$). On day 8, tail section of throat cut fish had a significantly higher value than tail section of gill cut fish ($p<0.05$). Otherwise, no significant difference was between groups.

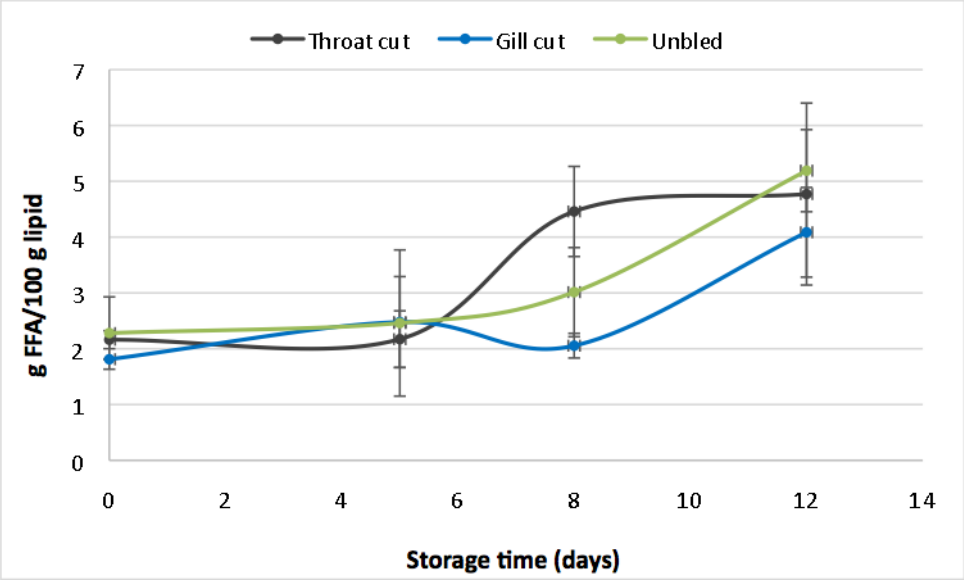

Figure 4.1. *The amount (g) of free fatty acids in 100 g of lipid for the tail section of redfish. Vertical bars show standard deviation from the mean value (n=4).*

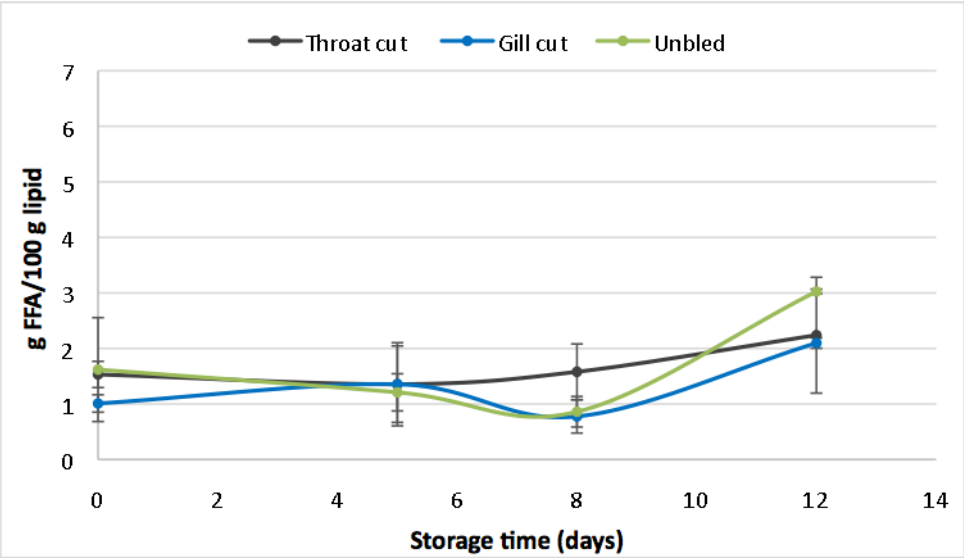

Figure 4.2. *The amount (g) of free fatty acids in 100 g of lipid for the middle section of redfish. Vertical bars show standard deviation from the mean value (n=4).*

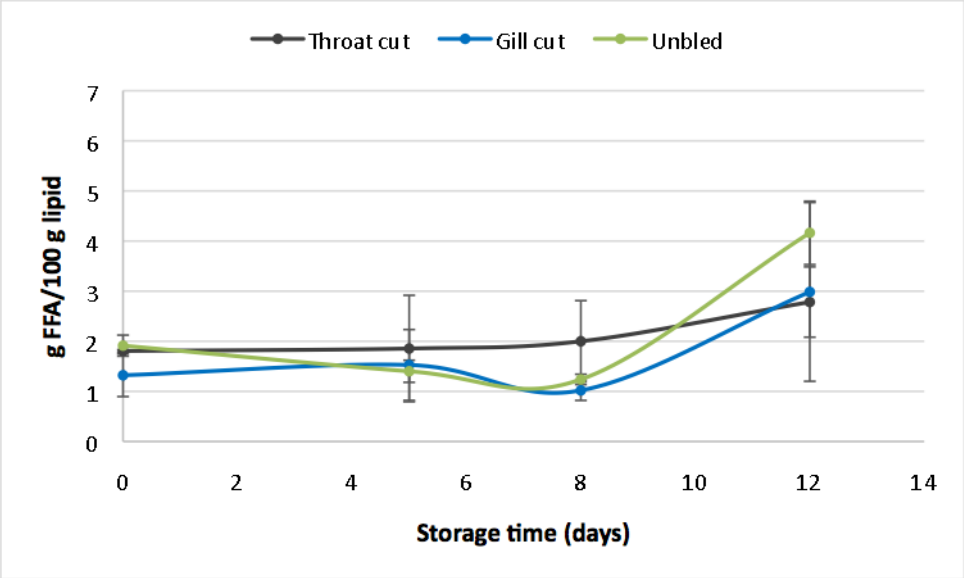

Figure 4.3. *The amount (g) of free fatty acids in 100 g of lipid for the loin section of redfish. Vertical bars show standard deviation from the mean value (n=4).*

4.1.4 Lipid Oxidation Analysis

Peroxide Value (PV)

Results of peroxide evaluation performed on tail section of the redfish showed that throat cut and unbled fish followed the same pattern with the highest value seen on day 8 post-packaging (Figure 4.4). However, from day 6 and onward, unbled fish had higher levels of peroxide than throat cut fish. Gill cut fish had a different pattern from

the other two groups, with the highest peroxide value on day 5 post-packaging. Gill cut and unbled fish both reached the highest value of about 50 µmol/g on days 5 and 8 respectively. Throat cut redfish reached its high point of about 45 µmol/g in the middle section on day 8.

Peroxide evaluation performed on middle and loin sections of the redfish showed same pattern within the groups as evaluation of the tail section (Figures 4.4, 4.5 and 4.6). Peroxide value changed significantly between days in all sections of both gill cut fish and unbled fish, and in tail and middle sections of throat cut fish ($p<0.05$). No significant change in peroxide value was observed in the loin section of throat cut fish indicating little or no oxidation. No significant difference was observed between unbled and throat cut fish.

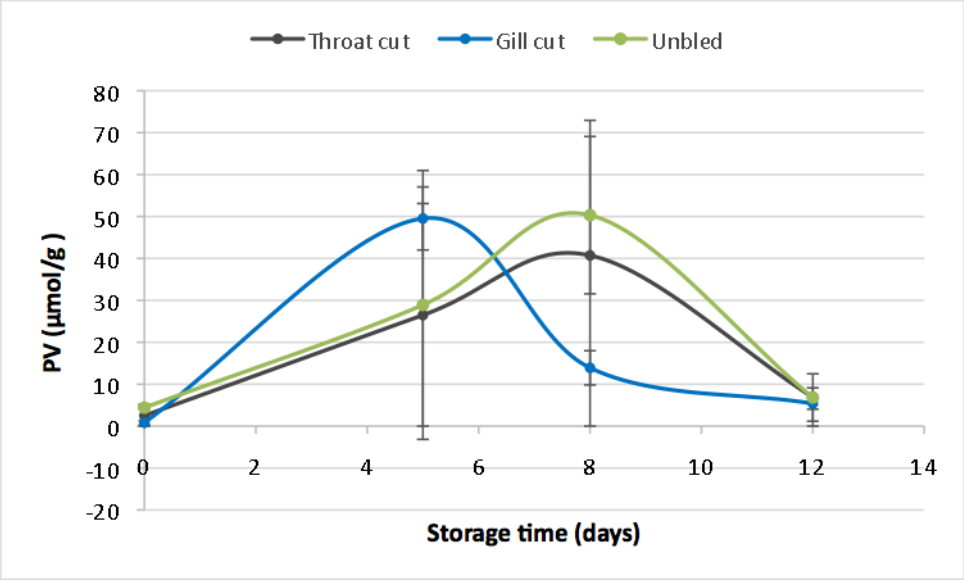

Figure 4.4. *Peroxide value (PV) µmol/g redfish for the tail section of throat cut, gill cut, and unbled fish. Vertical bars show standard deviation from the mean value (n=8).*

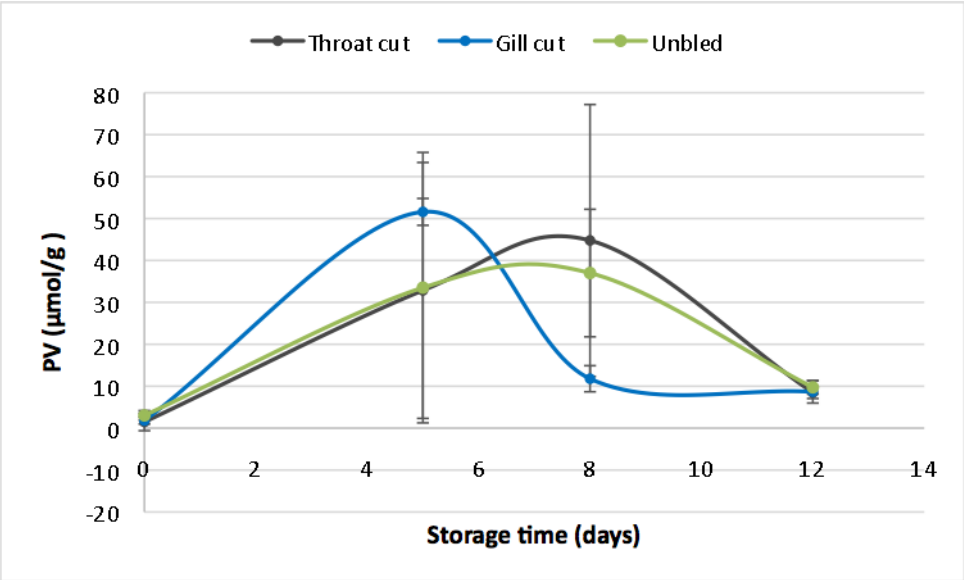

Figure 4.5. *Peroxide value (PV) µmol/g redfish for the middle section of throat cut, gill cut, and unbled fish. Vertical bars show standard deviation from the mean value (n=8).*

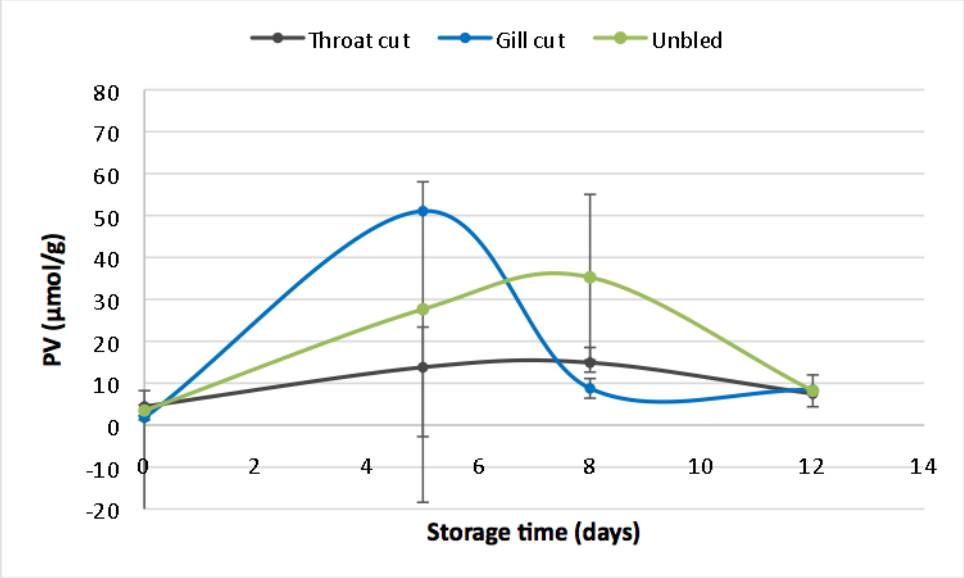

Figure 4.6. *Peroxide value (PV) µmol/g redfish for the loin section of throat cut, gill cut, and unbled fish. Vertical bars show standard deviation from the mean value (n=8).*

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS analysis results showed the same pattern for all groups with the highest point on day 8, except for unbled and gill cut middle section, which was still increasing on day 12 post-packaging (Figures 4.7, 4.8 and 4.9). On day 8, unbled fish had higher value than bled fish with the highest value, 22.3 µmol/kg, measured in the tail section. The highest value, 3.5 µmol/kg, for gill cut, loin section was observed on day 8.

Significant increase in TBARS was observed in unbled fish, in the loin section, between days 5 and 8, and in the middle section between days 5 and 12 ($p<0.05$). Tail section of the unbled fish showed significantly higher value than both middle section and loin section on day 8 ($p<0.05$). No significant increase in TBARS value for the loin section was seen between days for the bled fish (gill cut and throat cut), indicating little or no secondary oxidation.

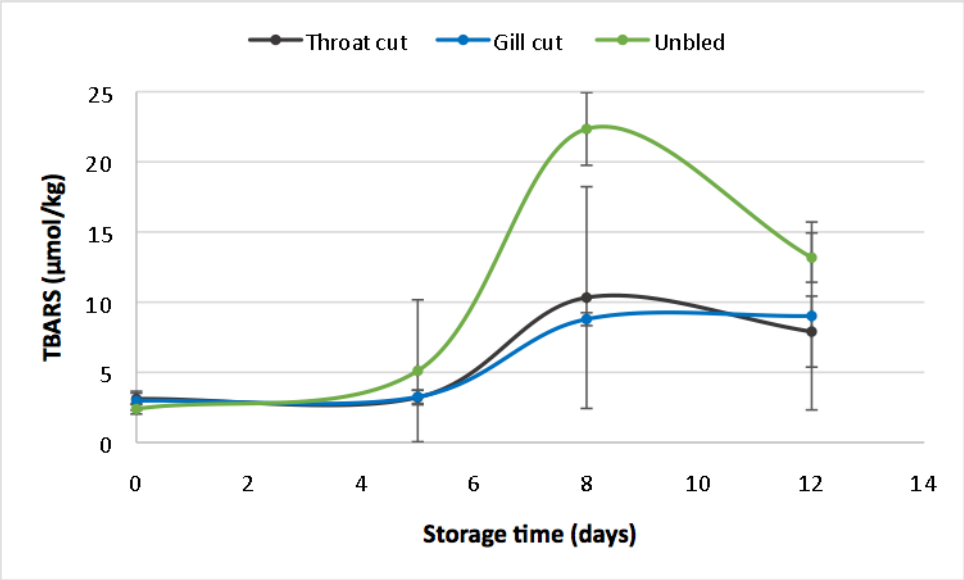

Figure 4.7. *TBARS µmol/kg redfish for the tail section of throat cut, gill cut, and unbled fish. Vertical bars show standard deviation from the mean value (n=4).*

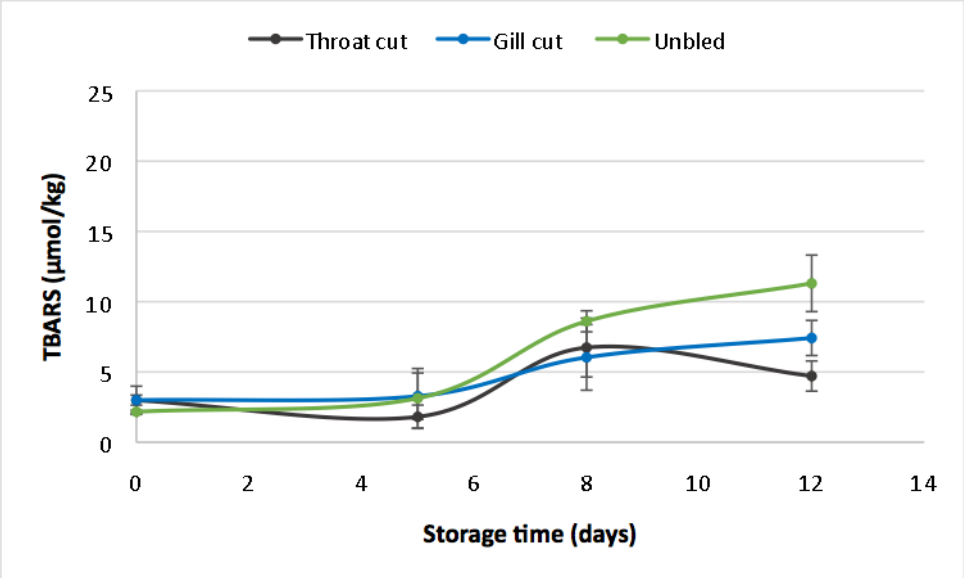

Figure 4.8. *TBARS µmol/kg redfish for the middle section of throat cut, gill cut, and unbled fish. Vertical bars show standard deviation from the mean value (n=4).*

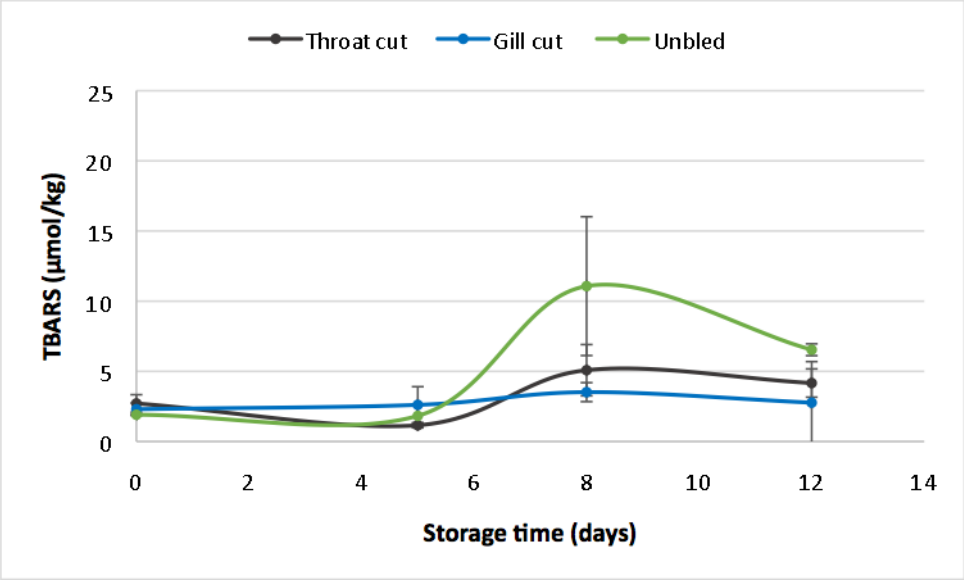

Figure 4.9. *TBARS µmol/kg redfish in loin section for throat cut, gill cut, and unbled fish. Vertical bars show standard deviation from the mean value (n=4).*

Fluorescence Shift (or) Analysis

Generally, the highest value in all sections of throat cut fish was observed on day 8, whereas the values were still increasing on day 12 in both gill cut and unbled (Figures 4.10, 4.11 and 4.12). A significant increase was observed between days 0 and 5 and between days 5 and 12 for the loin section of gill cut fish, with the highest value on day 12 ($p<0.05$). A slow but significant increase was observed for the middle section of gill cut fish from day 0 to 12 ($p<0.05$). No significant increase was observed in the tail section of gill cut fish. A steady but significant increase was observed for the loin section of throat cut fish from day 0 to 5 and 0 to 8, with the highest value on day 8 ($p<0.05$). For the middle section of the throat cut fish, a sharp and significant increase was observed from day 5 to 8 ($p<0.05$). For the tail section of throat cut fish, a significant increase was noted from day 5 to 8 ($p<0.05$). For the unbled group, a significant increase was only observed for the loin section from day 0 to 12 ($p<0.05$). Unbled fish showed significantly lower value than throat cut fish, for both tail and loin sections, on day 8 of storage ($p<0.05$). Loin of gill cut fish had a significantly higher value than the tail on day 12 ($p<0.05$).

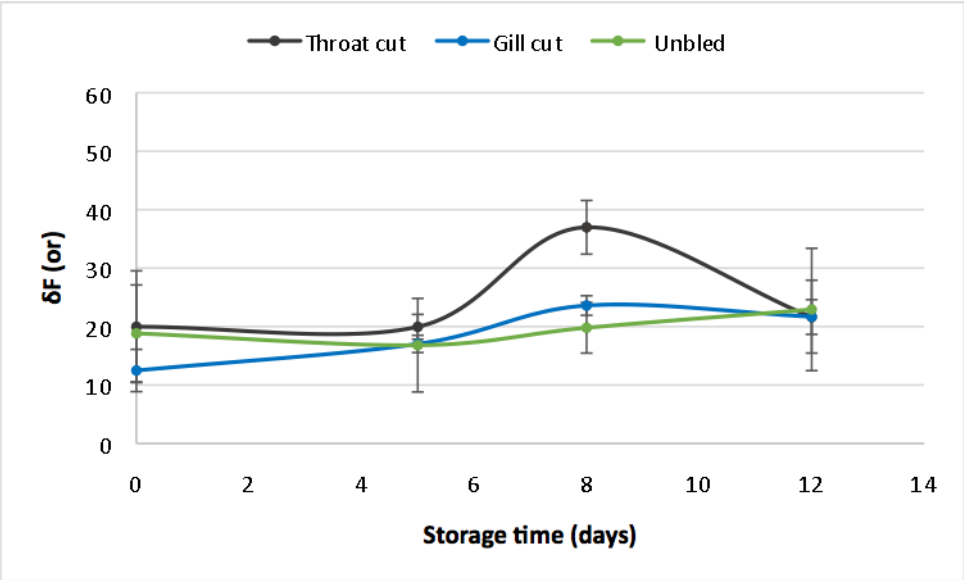

Figure 4.10. *Fluorescence value for the tail section of throat cut, gill cut, and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

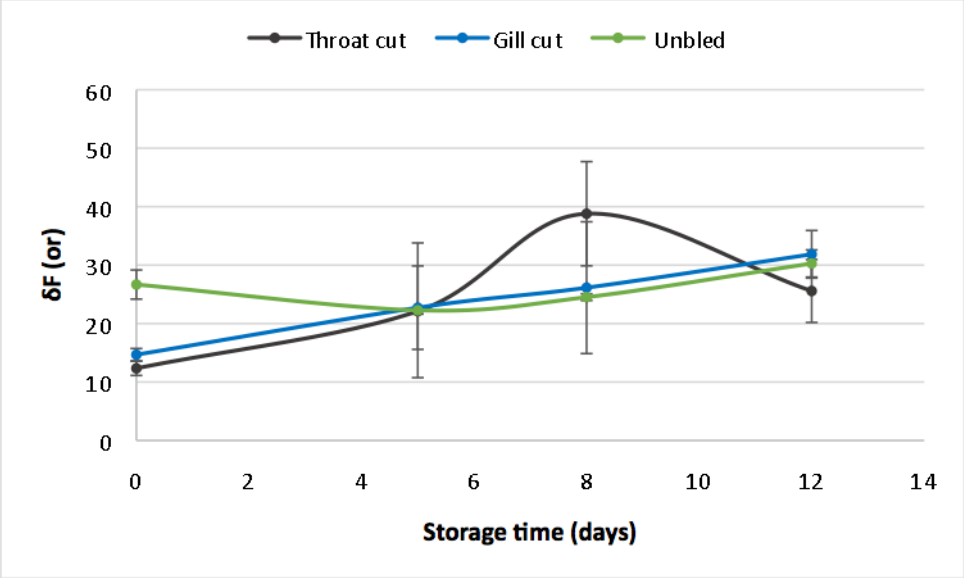

Figure 4.11. *Fluorescence value for the middle section of throat cut, gill cut, and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

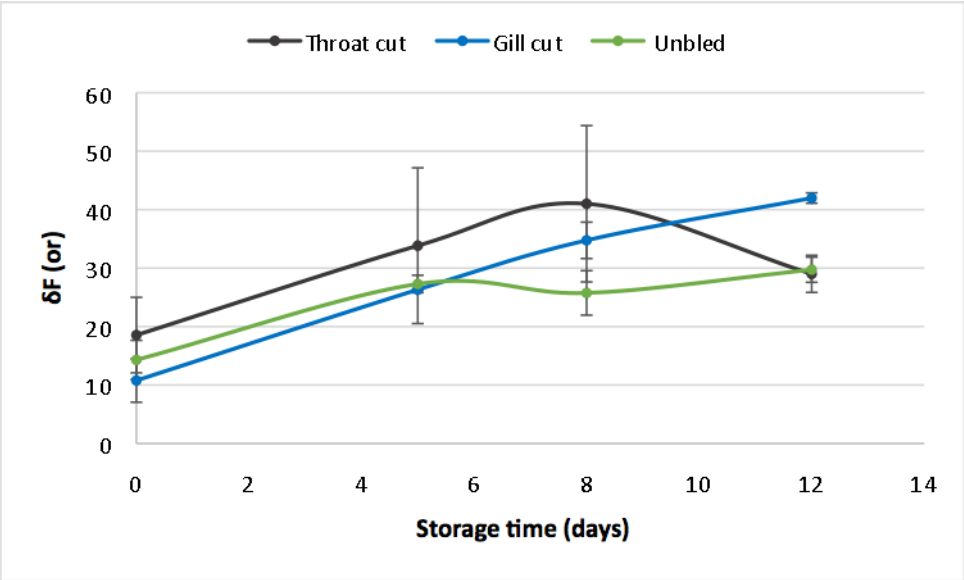

Figure 4.12. *Fluorescence value for the loin section of throat cut, gill cut, and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

4.1.5 Heme Iron

On day 0, unbled redfish had significantly lower levels of heme iron in all sections than throat cut fish ($p<0.05$) (Figures 4.13, 4.14, and 4.15). When compared to gill cut fish, unbled fish had significantly lower levels of heme in loin and middle sections. No significance was noted between the sections (loin, middle, and tail) of the fish except for the gill cut fish, where the middle section had significantly higher heme iron content than tail section on day 0. A significant change in heme level was observed in all sections of the different treatment groups ($p<0.05$).

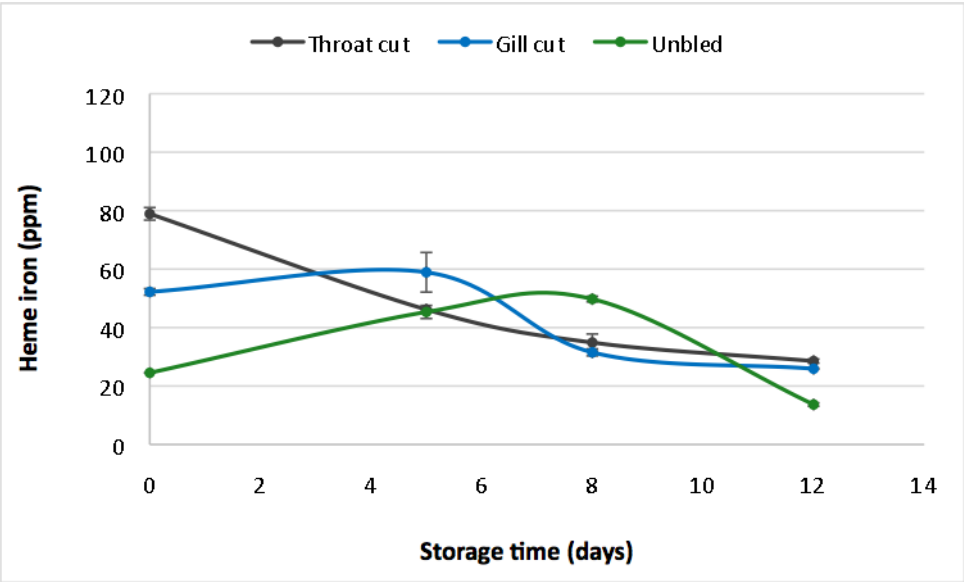

Figure 4.13. *Heme iron (ppm) for the tail section of throat cut, gill cut and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

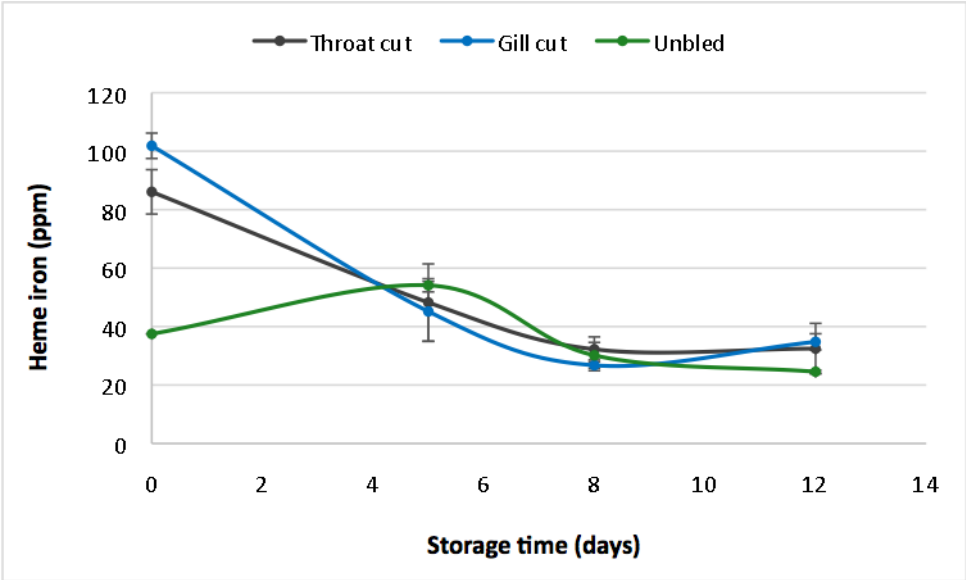

Figure 4.14. *Heme iron (ppm) for the middle section of throat cut, gill cut and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

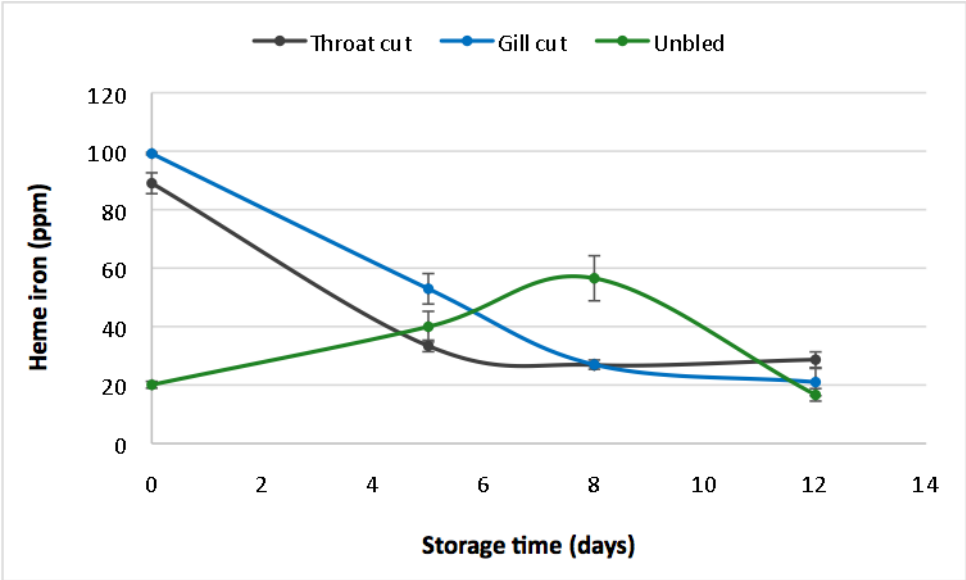

Figure 4.15. *Heme iron (ppm) for the loin section of throat cut, gill cut and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

4.1.6 Non-Heme Iron

Generally, it was noted that the groups followed the same pattern with little change over storage time (Figures 4.16, 4.17 and 4.18). No significant difference was observed between groups or sections of the fish ($p>0.05$).

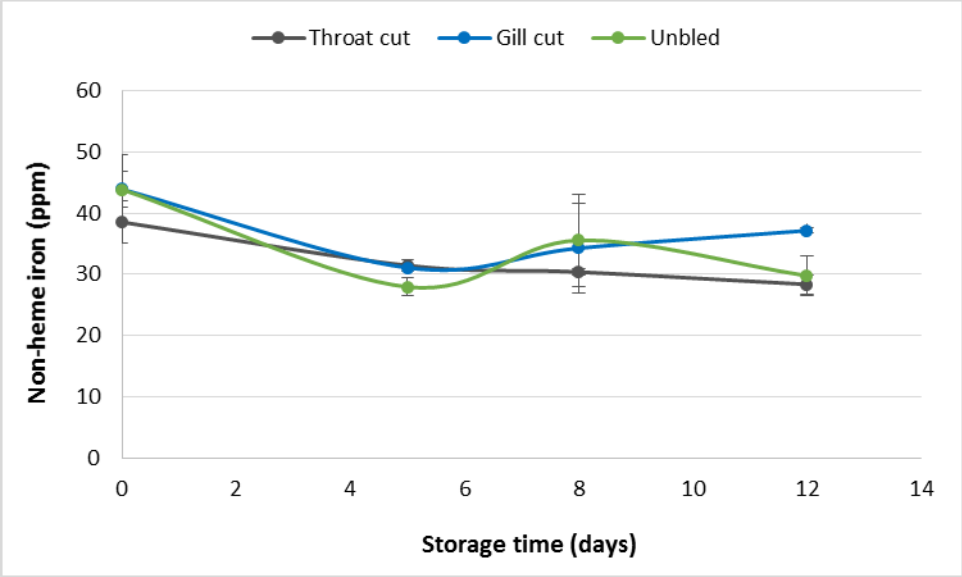

Figure 4.16. *Non-heme iron (ppm FE) for the tail section of throat cut, gill cut and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

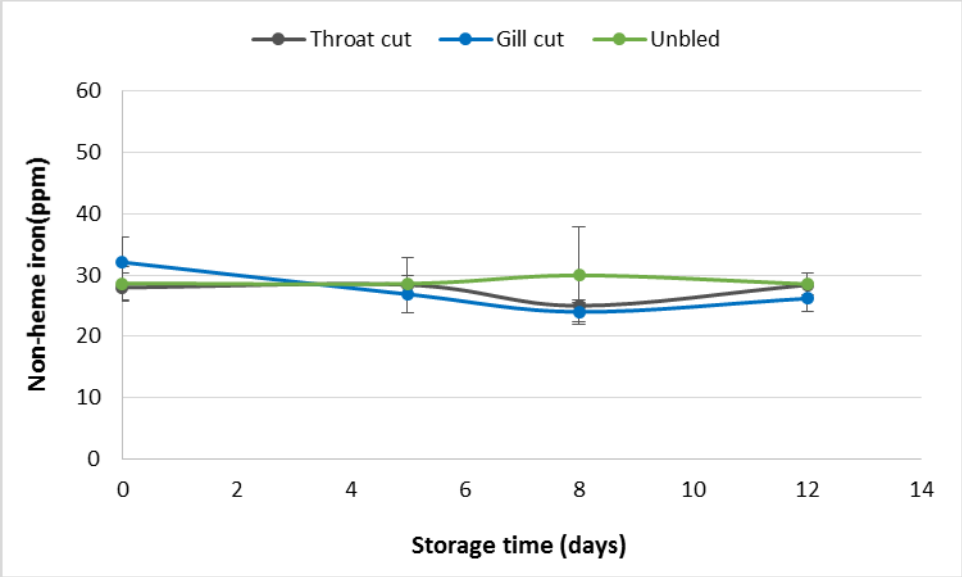

Figure 4.17. *Non-heme iron (ppm FE) for the middle section of throat cut, gill cut and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

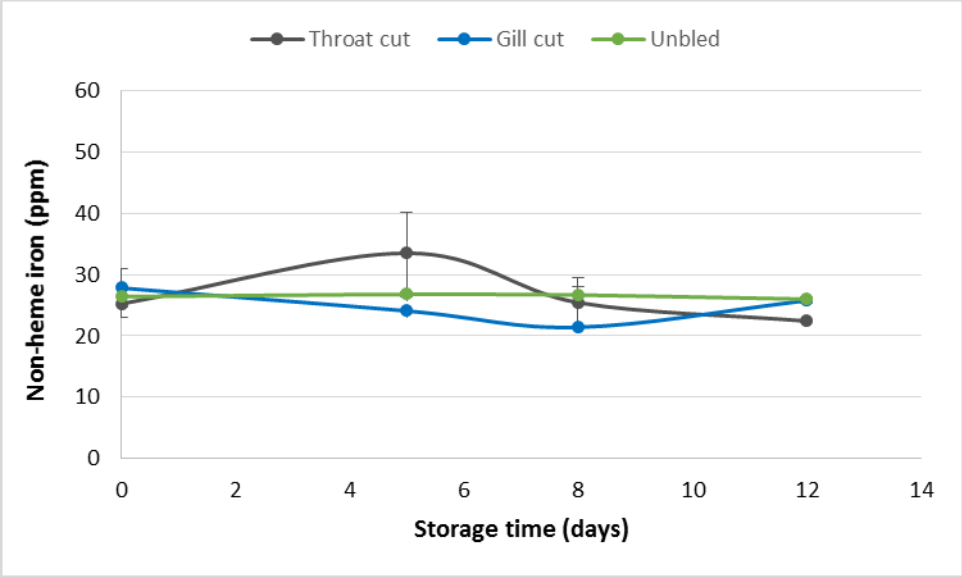

Figure 4.18. *Non-heme iron (ppm FE) for the loin section of throat cut, gill cut and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

4.1.7 Colour Analysis

Colour analysis revealed little or no change in lightness, redness or yellowness values for the three groups. Results showed no significant difference in lightness and redness for any of the groups (Figure 4.19 and Figure 4.20, respectively). However, a trend was observed in all groups as lightness decreased somewhat between days. A significant difference was observed in yellowness (b* value) between sampling days of gill cut fish and throat cut fish as yellowness increased from day 0. No significant difference was observed between the groups (Figure 4.21).

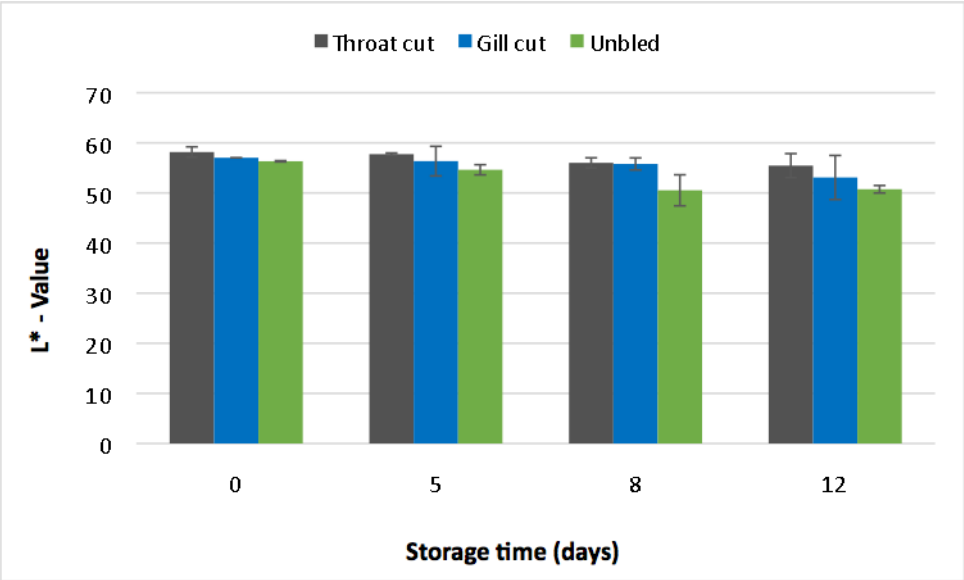

Figure 4.19. *Lightness (L* value) for throat cut, gill cut, and unbled groups. Vertical bars show standard deviation from the mean value (n=6).*

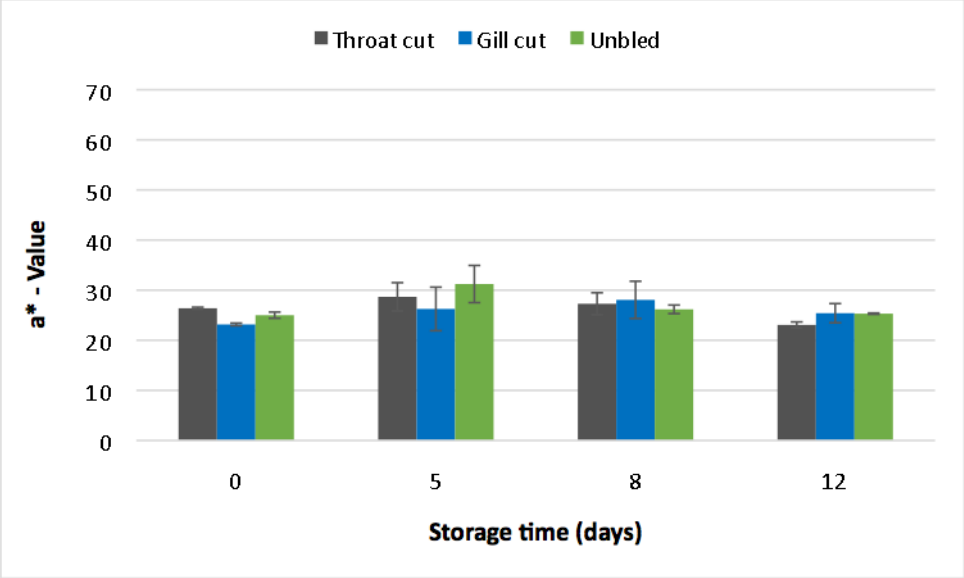

Figure 4.20. *Redness (a* value) for throat cut, gill cut, and unbled groups. Vertical bars show standard deviation from the mean value (n=6).*

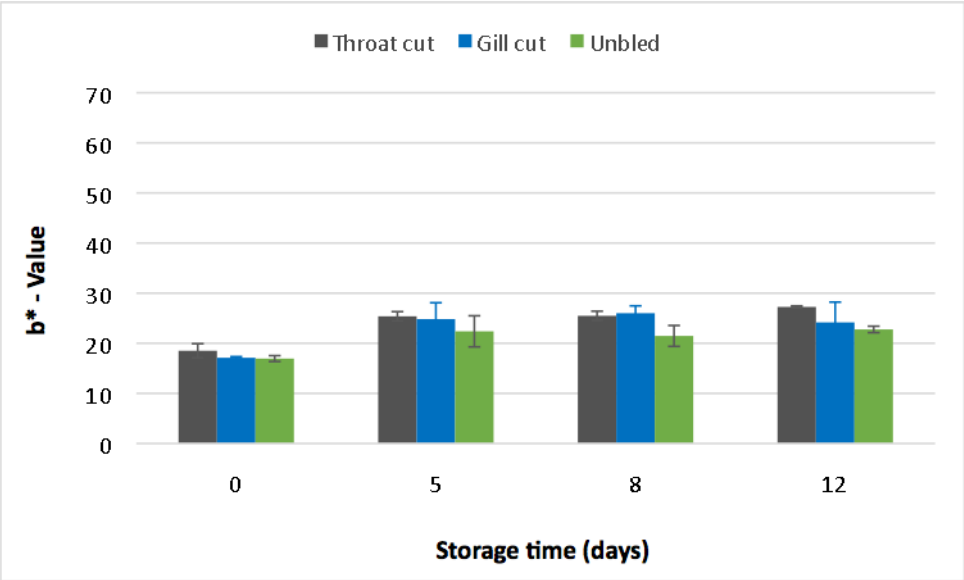

Figure 4.21. Yellowness (b value) for throat cut, gill cut, and unbled groups. Vertical bars show standard deviation from the mean value (n=6).*

4.2 Experiment II

The purpose of this study was to observe how different packaging treatments affect the shelf life of the fresh fish with respect to chemical-, microbiological- and sensory evaluation. Temperature of the three groups of fish (A, M1 and M2) was followed during the storage trial. Due to the fact that the air group looked spoiled on day 14, sensory analysis, as well as TVB-N and TMA measurements were not performed after day 10.

4.2.1 Temperature Monitoring

The temperature of the three groups in the beginning of storage (initial temperature) was 1.0 ± 0.9 ºC for the Air group, 4.4 ± 0.9 for the M1 group and 4.3 ± 0.4 for the M2 group (Table 4.3.). Higher initial mean temperature of M1 and M2 groups may be due to the fact that these groups had to be repackaged.

The ambient temperature over the 14 days period was 0.2 ± 1.1 °C (Table 4.3). Generally, product temperature (Table 4.3) of the Air group (A) stayed fairly constant throughout the storage time with very little increase in temperature on days 6 and 10 following temperature abuse (Figure 4.22). Temperature of the Air group (A) never reached much above 0 °C. Temperature of groups M1 and M2 showed greater increase on days 6 and 10 compared to group A. The lowest temperature (-0.4 ± 0.3 °C) was observed in the Air group (A) and the highest (0.5 ± 0.9 °C) in the M2 group.

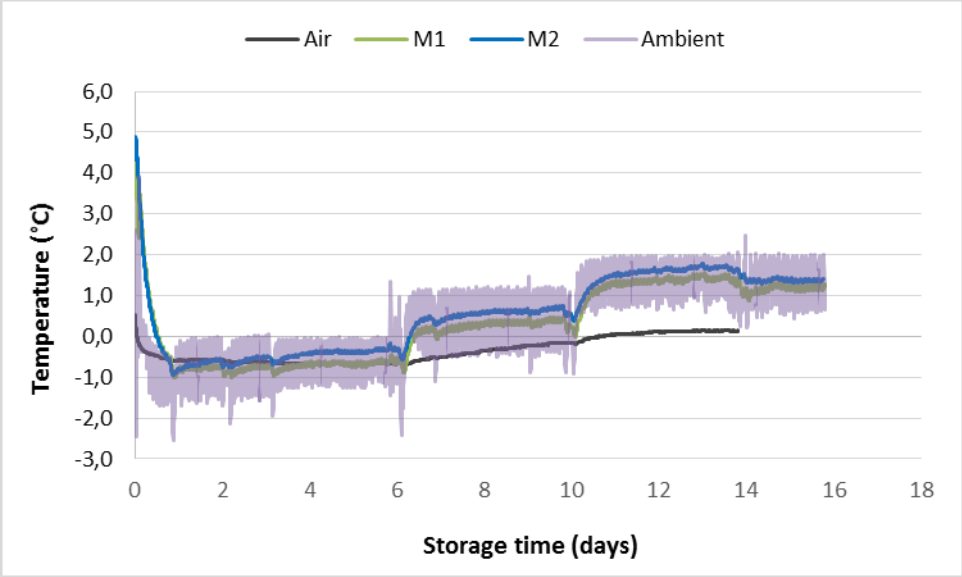

Figure 4.22. *Mean temperature (°C) of 4 loggers per group and 3 loggers in storage room, recorded every 10 minutes throughout storage time of 14 days. Temperature in cooling chamber was set at -1 (°C) on the day 0, increased to +1 (°C) on day 6, and to +2 (°C) on day 10.*

Table 4.3. *Mean initial product temperature (°C) recorded in the beginning of storage, mean temperature during storage (including the initial temperature) and time to reach 0 °C during storage.*

Treatment	Mean initial temperature (°C)	Mean temperature (°C) throughout storage	Days to reach 0 °C
A	1.0 ± 0.9	-0.4 ± 0.3	10
M1	4.4 ± 0.9	0.3 ± 0.9	6.5
M2	4.3 ± 0.4	0.5 ± 0.9	6.5

4.2.2 Headspace Gas Composition

Headspace CO_2 in the M1 group decreased from 39.3% on day 0 to 25.6% on day 6 and kept around 25 % throughout storage time (Figure 4.23). Headspace CO_2 in the M2 group increased from day 0 (39.3%) to day 6 (45.7%). The CO_2 was significantly higher in the M2 group than in the M1 group throughout storage time. This increase in CO_2 is due to the fact that in addition to the initial level 39.3% of CO_2, M2 group had 5 CO_2 emitting-pads placed on the bottom of the plastic boxes. The M2 group showed a slow decrease in CO_2 from 45.7 on day 6 to 44.0% on day 10, and to 40.9% on day 14. The N_2 level was significantly higher in M1 than in M2 throughout storage time. As CO_2 decreased with storage, significant increase in N_2 was noted in M2 group from day 6 to day 14. No significant difference in O_2 was observed between the two MAP groups.

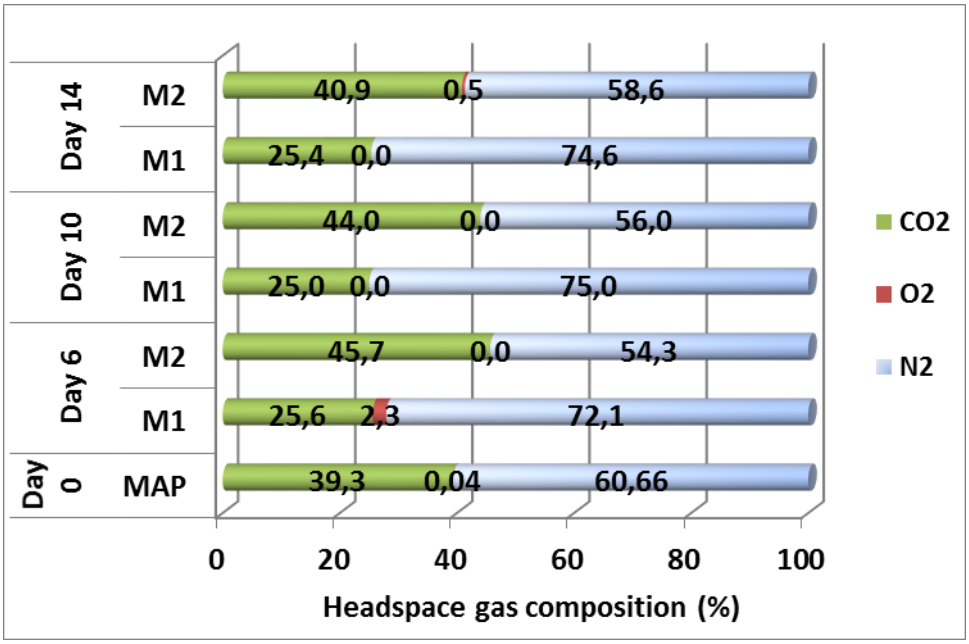

Figure 4.23. *% Headspace gas composition of MAP treated groups M1 and M2. In addition to regular gas filling M2 had 5 CO_2 emitting pads placed on the bottom of each box.*

4.2.3 Drip Analysis

Drip was estimated on days 6, 10, 14 and 16, post packaging for M1 and M2 groups (Figure 4.24). Water loss was estimated only on day 6 for the Air (A) group. On day 6, drip loss was significantly lower in Air group (2.3% +/- 0.0%) than in M1 (4.4% +/- 0.1%) and M2 (6.7% +/- 0.2%) ($p<0.05$). Furthermore, drip loss was significantly higher in the M2 group than in the M1 group on days 6, 10, and 14 ($p<0.05$).

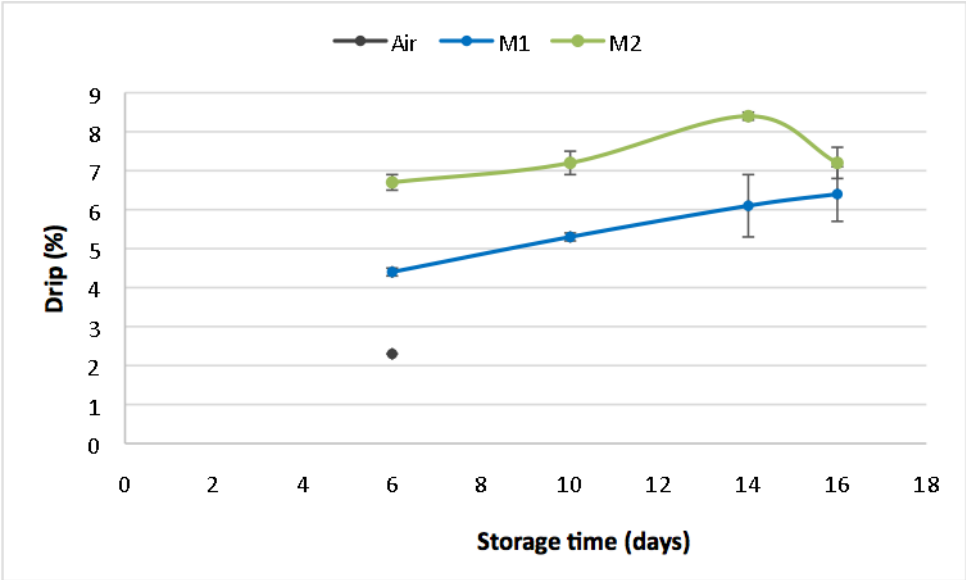

Figure 4.24. *Drip loss (%) for the Air (A) M1 and M2 groups on days 6, 10, 14, and 16. Vertical bars show standard deviation from the mean value (n=2).*

4.2.4 pH

Measurements of pH were performed on all sampling days (6, 10, and 14) both on a whole fillet and on a fish mince for all groups (Figure 4.25). In addition, pH was measured in a mince of the Air group on day 0 and on a whole fillet of the M1 and M2 groups on day 16. The results showed that pH was generally highest for the Air (A) group, and lowest for the M2 group when measured inside the fillet within 30 min from sampling. The top layer of Air group had a significantly higher pH than the top layer of M2 group on days 6 and 10 ($p<0.05$). A significant increase in pH was noted in both top and lower layers of M1 group from day 10 to 16, and in lower layer only, from day 6 to days 14 and 16, and from day 10 to 14 ($p<0.05$). Furthermore, a significant increase in pH was observed in the top layer of M2 group from day 6 to 16, and from day 10 to 16 ($p<0.05$). No significant increase was noted in the lower layer of M2 group. Generally, fish mince had a higher pH.

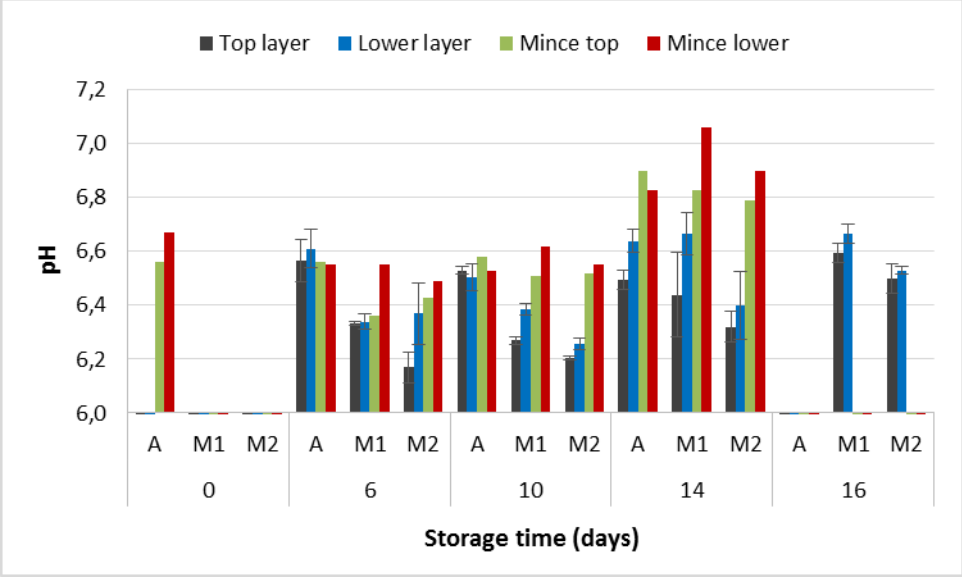

Figure 4.25. *Measurements of pH for the Air (A), M1 and M2 groups during storage (Experiment II). pH was measured both by inserting the probe into the fillet as well as into redfish mince. Samples were collected both from the top and the lower layer of the box. Vertical bars show standard deviation from the mean value (n=2).*

4.2.5 Total Volatile Basic Nitrogen (TVB-N) and Trimethylamine (TMA)

TVB-N and TMA measurements were performed on days 0 and 10 post packaging for the Air (A) group, and on days 10 and 14 post packaging for M1 and M2 treatment groups (Figure 4.26 and Figure 4.27, respectively). TVB-N results showed that on the

packaging day (day 0) the fish had a 12.36 mg N/100 g. All groups can be compared on day 10. Results for day 10 show that the Air (A) group had the lowest value of 15.6 mg N/100 g when compared to the M1 and M2 groups, which had a TVB-N value of 18.94 mg N/100 g and 16.04 mg N/100 g respectively. On day 14, both MAP groups had passed the TVB-N EU limit for consumption (35 mg N/100 g), but the M2 group had a lower value, 55.13 mg N/100 g, than M1, 56.58 mg N/100 g ($p > 0.05$).

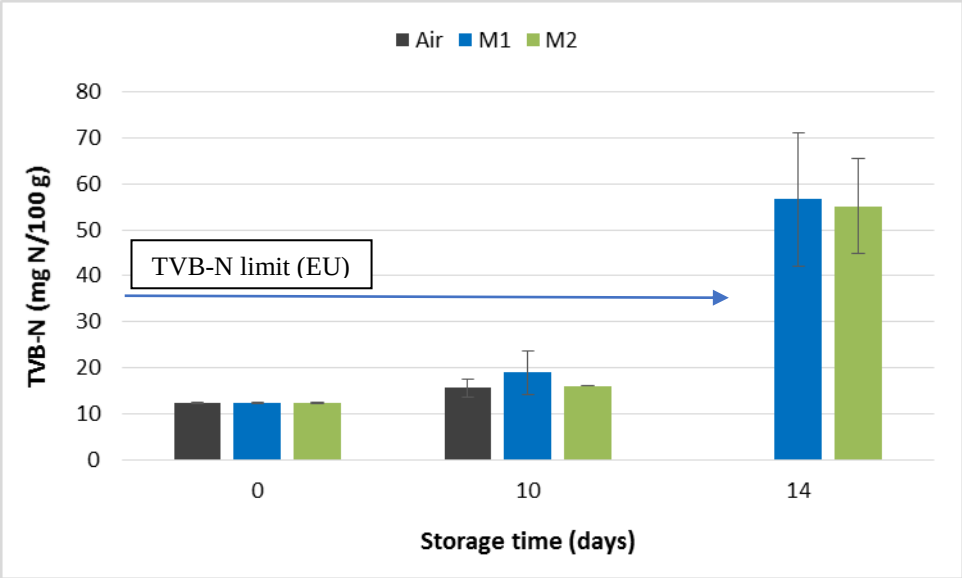

Figure 4.26. *Total Volatile Basic Nitrogen (TVB-N) mgN/100 g fish for the Air (A), M1, and M2 groups. Vertical bars show standard deviation from the mean value (n=2). Blue line shows TVB-N limit (EU).*

On the packaging day, the redfish had a very low TMA value of 0.67 mg N/100 g. On day 10, TMA value was lowest for the A (Air) group, 2.46 mg N/100 g, when compared to the M1 and M2 groups, which showed a TMA value of 7.47 mg N/100 g and 5.02 mg N/100 g respectively. A significant increase in TVB-N and TMA was noted in both M1 and M2 groups from day 10 to 14 ($p<0.05$). On day 14 both MAP groups reached or surpassed the proposed TMA limit of (15 mg N/100 g) (Connell 1995). No significant difference was observed within any of the groups or between the different groups (Air, M1 and M2) during the first 10 days of storage.

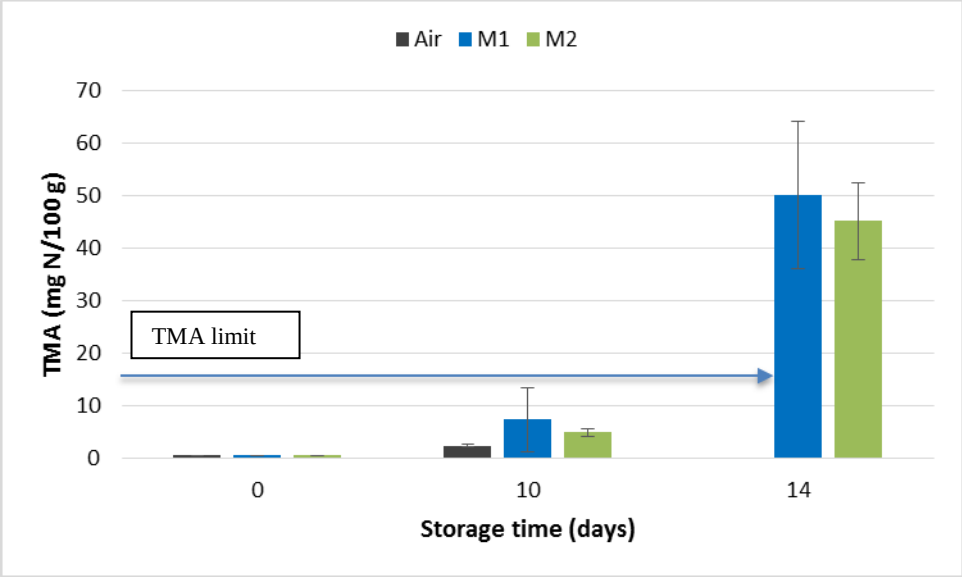

Figure 4.27. *Trimethylamine (TMA) mgN/100 g fish for the Air (A), M1, and M2 groups. Vertical bars show standard deviation from the mean value (n=2). Blue line shows TMA proposed limit.*

4.2.6 Microbiological Analysis

Total Viable Counts (TVC)

TVC was performed on both IA (iron agar) and LH (Long and Hammers) agar on days 0, 6, 10, and 14 for all groups (Figure 4.28). For the IA analysis, all three groups followed the same pattern with a steady, but significant, increase from day 0 to 10, and day 10 to 14. No significant difference was found between the different groups. Same pattern, but somewhat higher count was seen for the LH agar analysis (Figure D.1 in appendix D).

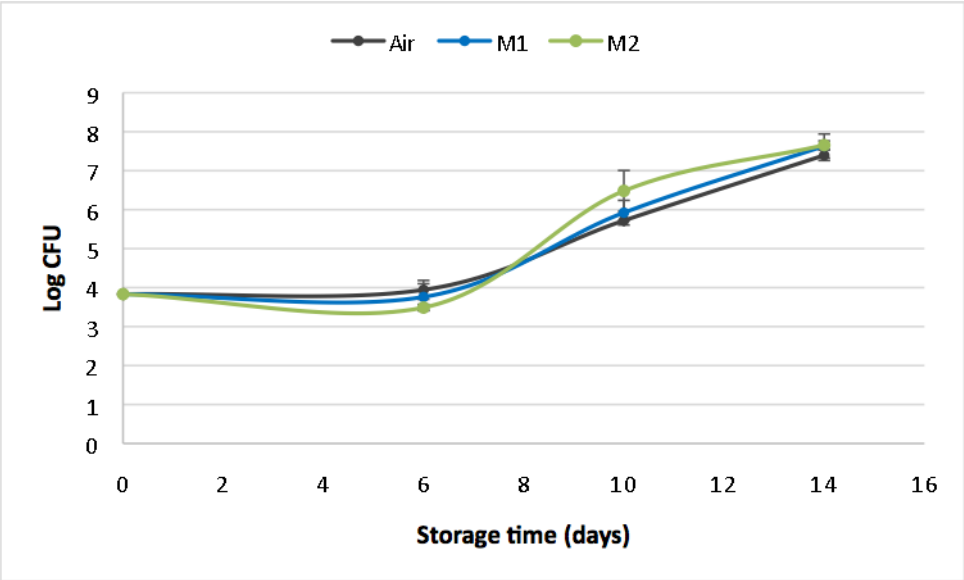

Figure 4.28. *Total viable count (TVC) on iron agar (IA) in log numbers for the Air (A), M1 and M2 groups. Vertical bars show standard deviation from the mean value (n=2).*

PCR-*Pp*

Results of PCR analysis of *Photobacterium phosphoreum* (*Pp*) (Figure 4.29), measured in log numbers, revealed that the Air group had the highest count of log 6.4 on day 14 when compared to other groups. The M1 and M2 groups had the highest count on day 10 with a log value of 5.7 and 6.1 respectively. On day 6, the lowest value 3.9 was observed in the M2 group. A significant increase in Pp was observed within all groups ($p<0.05$), however no significant difference was observed between the groups.

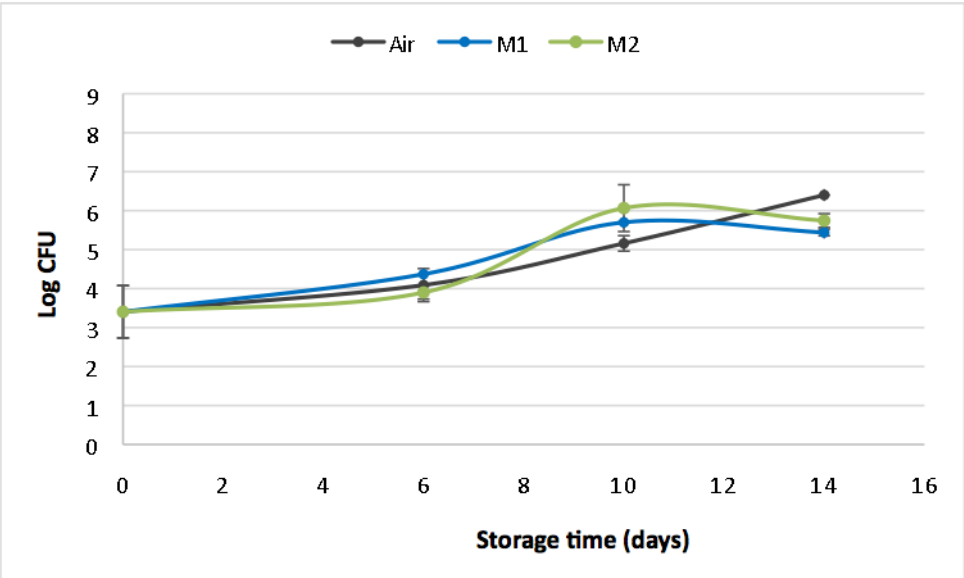

Figure 4.29. *Photobacterium phosphoreum (Pp) for the Air, M1 and M2 groups. Vertical bars show standard deviation from the mean value (n=2).*

H_2S Producing Bacteria

Analysis of H_2S producing bacteria, showed that at the time of packaging, the redfish had a 1.6 log CFU (Figure 4.30). The highest value was observed for M1 group throughout storage time. A significant increase of H_2S producing bacteria was observed in all groups ($p<0.05$), however there was no significant difference between the different groups, except on day 14 when M2 was significantly lower than M1.

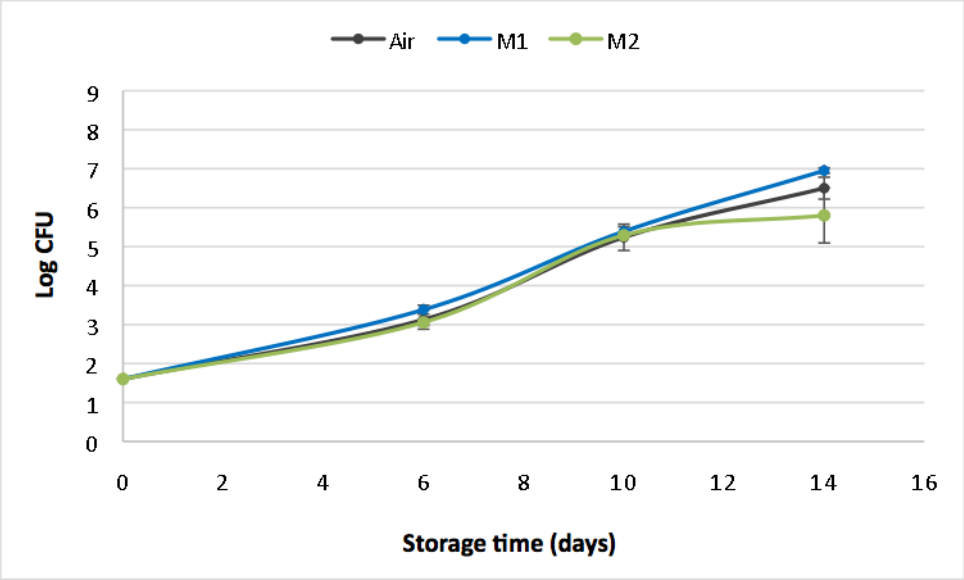

Figure 4.30. *H_2S producing bacteria for the Air (A), M1 and M2 groups. Vertical bars show standard deviation from the mean value (n=2).*

Pseudomonads

Results of *Pseudomonads* count showed a significant increase in the Air group from day 6 to 14 and 10 to 14 (Figure 4.31). Comparing the Air group and the two MAP groups showed that the Air group had a significantly higher *Pseudomonads* count than M1 on days 6 and 14, and a significantly higher count than M2 on days 10 and 14.

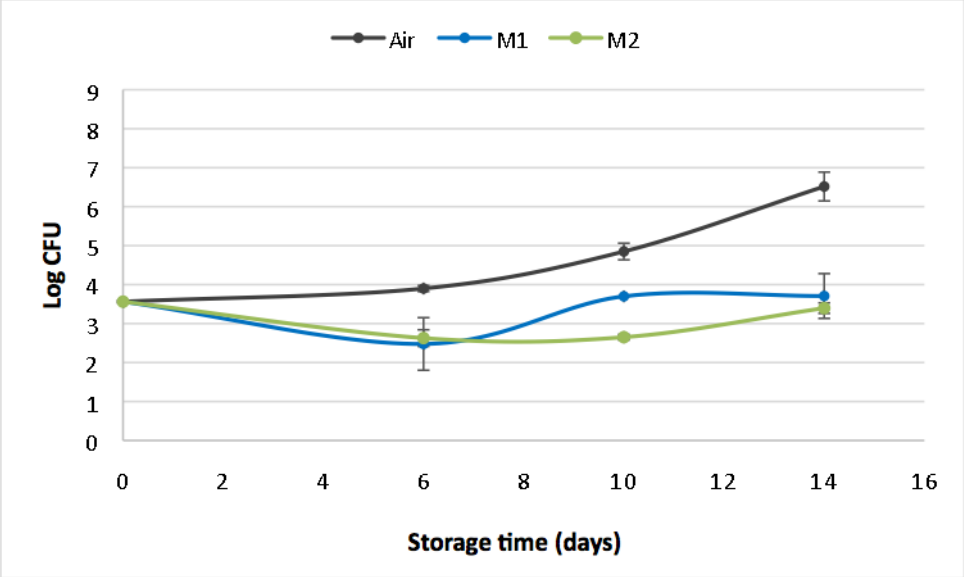

Figure 4.31. *Pseudomonads colony forming units (CFU) for the Air (A), M1 and M2 groups. Vertical bars show standard deviation from the mean value (n=2).*

4.2.7 Lipid Content

Some changes were observed in the lipid content of the fillets during the storage (Table 4.4). On day 0, lipid content of the redfish was 1.69%. The highest value 7,32% ± 1,25% was measured in the lower layer of the air group on day 10. The lower layer of the Air group and top layer of M1 group showed a significant change in lipid content with an increase from day 6 to 10 and a decrease from day 10 to 14 ($p<0.05$). Little change was however noted in the M2 group ($p>0.05$). Comparison between the top and lower layer generally showed no difference with the exception of M1 group, where the top layer had a significantly higher lipid content than the lower layer of the same group on day 10 ($p<0.05$). No significant difference was observed between the groups except on day 10, when the lower layer of Air group was significantly higher than the lower layers of M1 and M2 groups ($p<0.05$). In addition, the top layer of M1 group had a significantly higher lipid content than the top layer of Air group on day 10 ($p<0.05$).

Table 4.4. *Lipid content (g lipids/100g fish) for Air, M1 and M2 groups, samples taken from top and lower layers in the box (mean +/- SD, n=2).*

	Days	AIR	M1	M2
Top layer	0	1,69 ± 0,00	1,69 ± 0,00	1,69 ± 0,00
	6	3,15 ± 0,43	2,85 ± 0,23	3,36 ± 0,20
	10	2,33 ± 0,04	5,27 ± 1,09	3,52 ± 0,16
	14	2,47 ± 0,40	1,05 ± 0,30	2,75 ± 0,46
Lower layer	0	1,69 ± 0,00	1,69 ± 0,00	1,69 ± 0,00
	6	2,80 ± 0,41	3,22 ± 0,30	3,43 ± 0,01
	10	7,32 ± 1,25	1,43 ± 0,30	1,98 ± 0,15
	14	3,33 ± 0,85	2,85 ± 0,90	1,85 ± 0,12

4.2.8 Free Fatty Acids (FFA)

Similar pattern of FFA was noted between the groups (Figures 4.32 and 4.33). The FFA value on day 0 and 6 was similar for all groups, 1 g FFA/100 g lipids. Air group and M1 group showed no significant change in FFA throughout storage time. A significant increase was observed in both top layer and lower layer of the M2 group from day 6 to 14 ($p<0.05$). On day 14, the lower layer of the M2 group had a significantly higher FFA value than lower layer of the Air group ($p<0.05$).

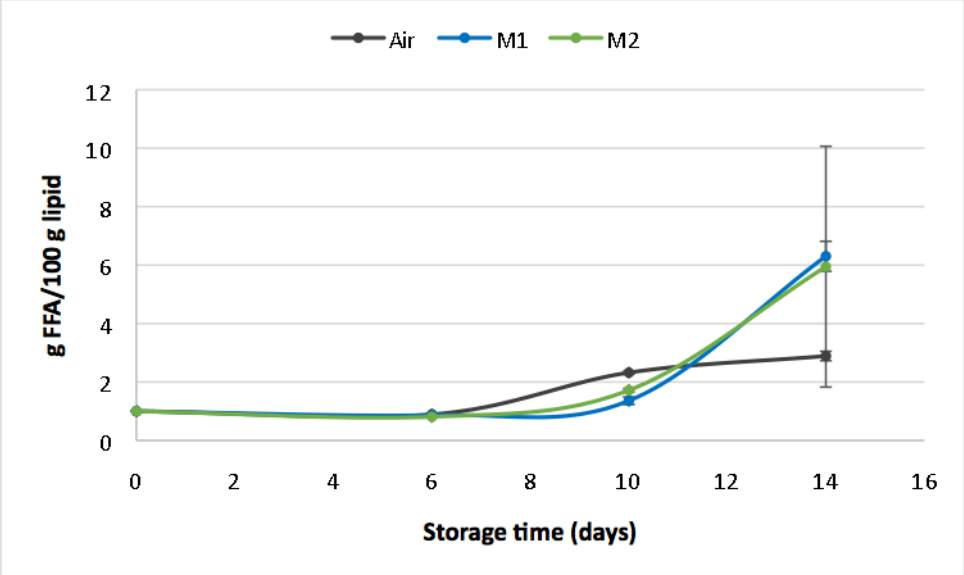

Figure 4.32. *Free fatty acids (FFA) g per 100 g of lipid for the Air, M1, and M2 groups. Samples taken from the top layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

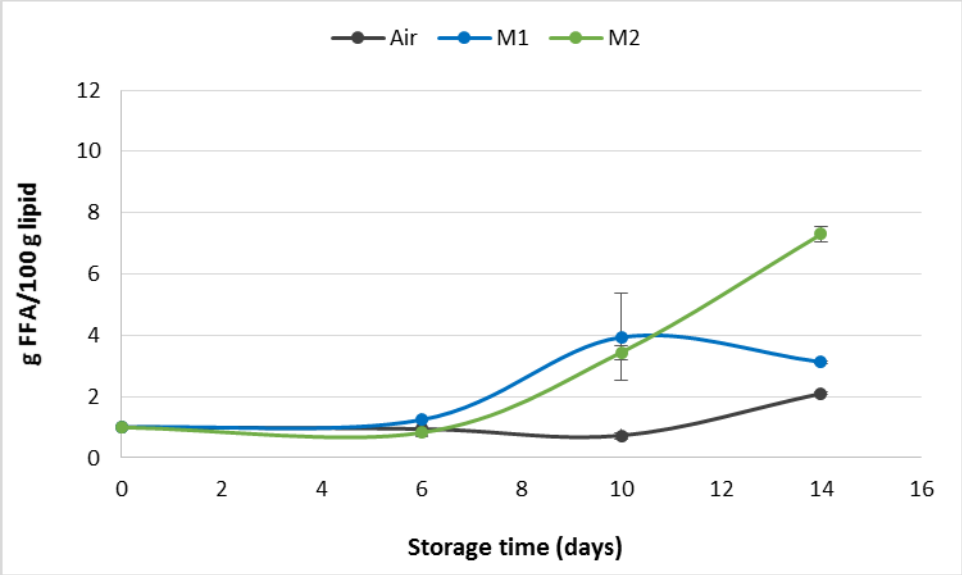

Figure 4.33. *Free fatty acids (FFA) g per 100 g of lipid for the Air, M1, and M2 groups. Samples taken from the lower layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

4.2.9 Lipid Oxidation Analysis

Peroxide Value (PV)

Peroxide evaluation was performed on all groups (A, M1, and M2) on both top and lower layer of the redfish (Figures 4.34 and 4.35, respectively). Similar pattern of PV was observed for both top and lower layer of all groups ($p<0.05$). The Air and M1 groups followed the same pattern with the highest peroxide value measured on day 10 post-packaging. The M2 group showed a different pattern, with the highest PV measured on day 6 post-packaging.

A significant increase in peroxide value was observed in the lower layer of the Air group between days 0 and 10, and significant decrease between days 10 and 14 of the same group ($p<0.05$). On day 10, Air group showed a significantly higher level of peroxide than M2 group ($p<0.05$). Otherwise, M1 and M2 showed no significant change in peroxide value between days of sampling.

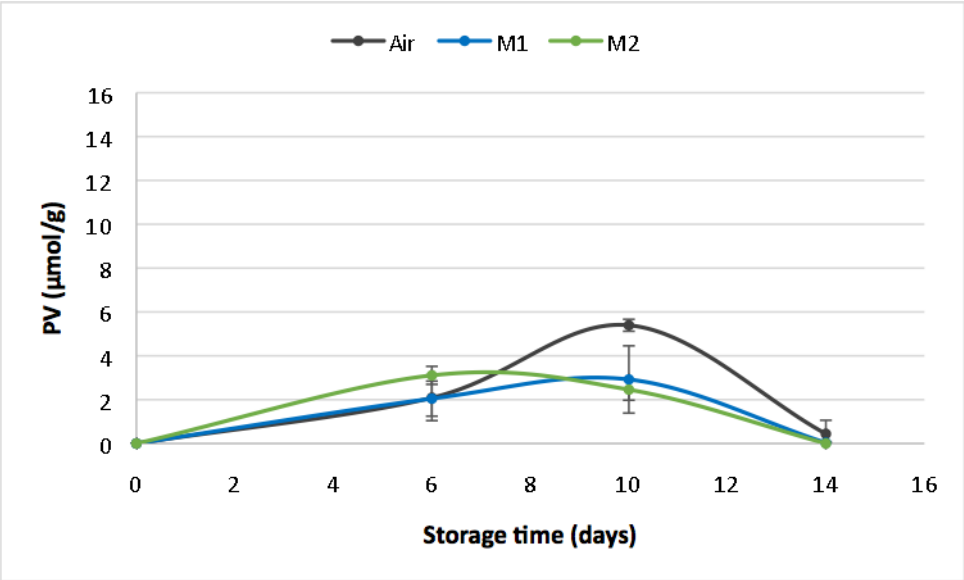

Figure 4.34. *Peroxide value (PV) for the Air, M1 and M2 groups. Samples taken from the top layer of each box. Vertical bars show standard deviation from the mean value (n=8).*

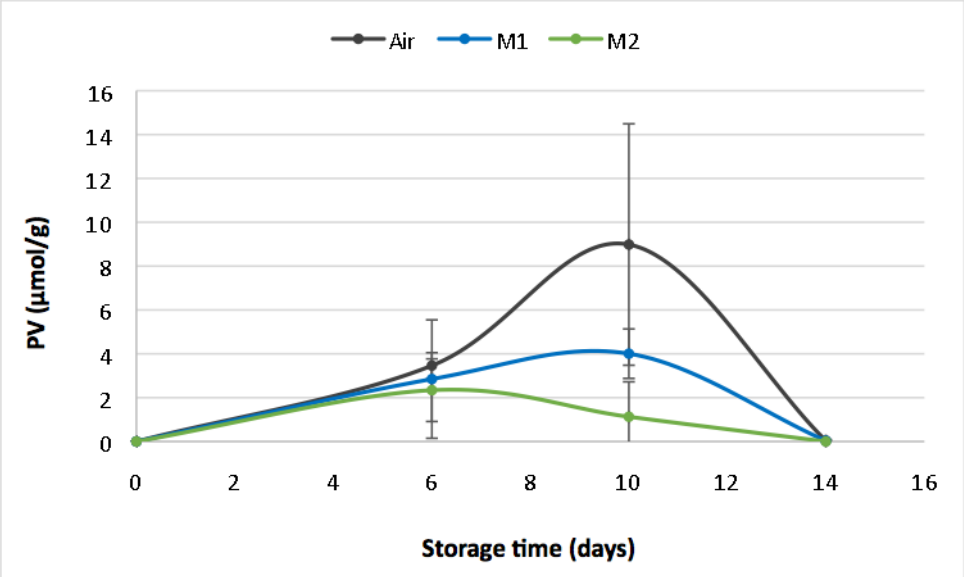

Figure 4.35. *Peroxide value (PV) for the Air, M1 and M2 groups. Samples taken from the lower layer of each box. Vertical bars show standard deviation from the mean value (n=8).*

Thiobarbituric Acid Reactive Substances (TBARS)

Results of TBARS analysis showed similar pattern for all groups throughout storage time, with little changes the first 6 days and the highest point on day 10 post-packaging (Figures 4.36 and 4.37). The highest value, 16.4 µmol/kg, was seen in Air group, lower layer, on day 10.

A significant increase was observed in the lower layer of the Air group from days 6 to 10, and a decrease from days 10 to 14 ($p<0.05$). The top layer of Air group showed a

slower increase in TBARS value than the lower layer, with significant increase from day 6 to 14. A significant increase was observed in the top layer of the M1 group from day 6 to 10 and a decrease from days 10 to 14 ($p<0.05$). The top layer of M2 group showed a significant increase in TBARS value from days 6 to 10 ($p<0.05$). The change in TBARS value in the top layer of the Air group and lower layer of M1 and M2 groups was not significant. The difference between lower and top layers of the different groups was not significant except for Air group on day 10 when the lower layer showed a significantly higher TBARS value ($p<0.05$).

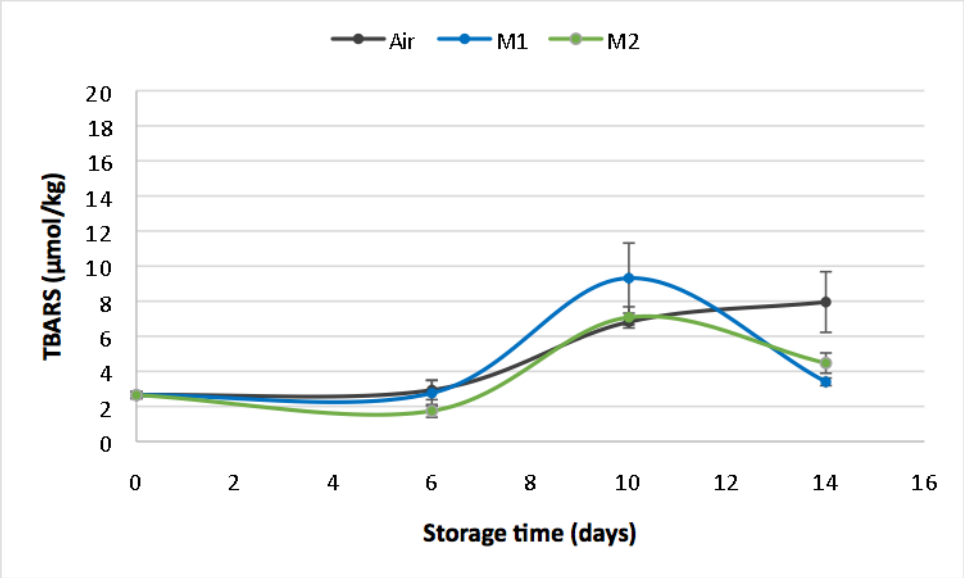

Figure 4.36. *TBARS value for the Air, M1 and M2 groups. Samples taken from the top layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

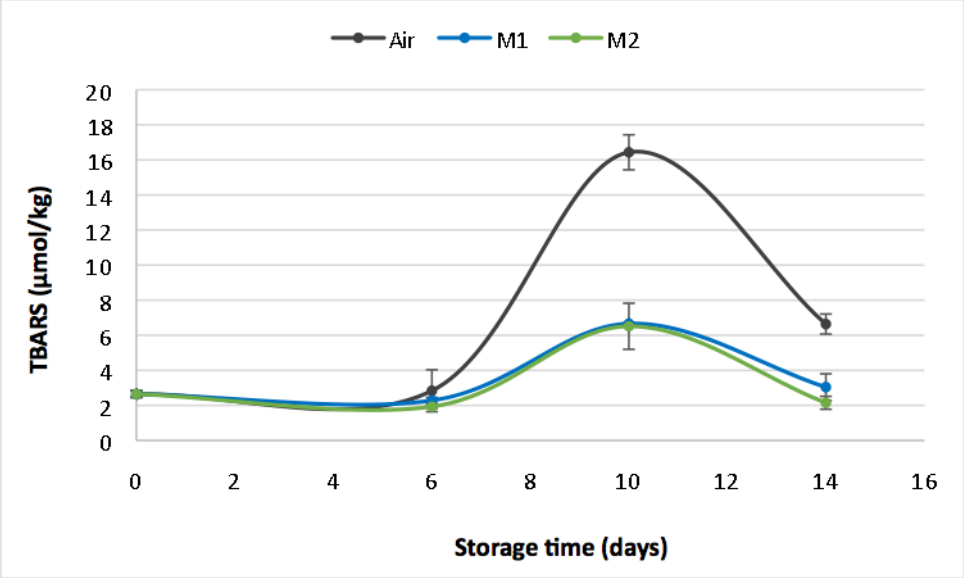

Figure 4.37. *TBARS value for the Air, M1 and M2 groups. Samples taken from the lower layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

Fluorescence Shift (or) Analysis

Results showed the highest value, 2.36, in the top layer of Air group on day 10, when compared to both top and lower layers of M1 and M2 groups (Figures 4.38 and 4.39). The highest value was observed on day 10 in both layers of the Air group. The highest values of M1 and M2 groups, in the top layer, were observed on day 14. Results showed higher values for all groups in the top layer than in lower layer, except for M1 group, day 10. No significant change was observed between days for any of the groups, and no significant difference between the groups.

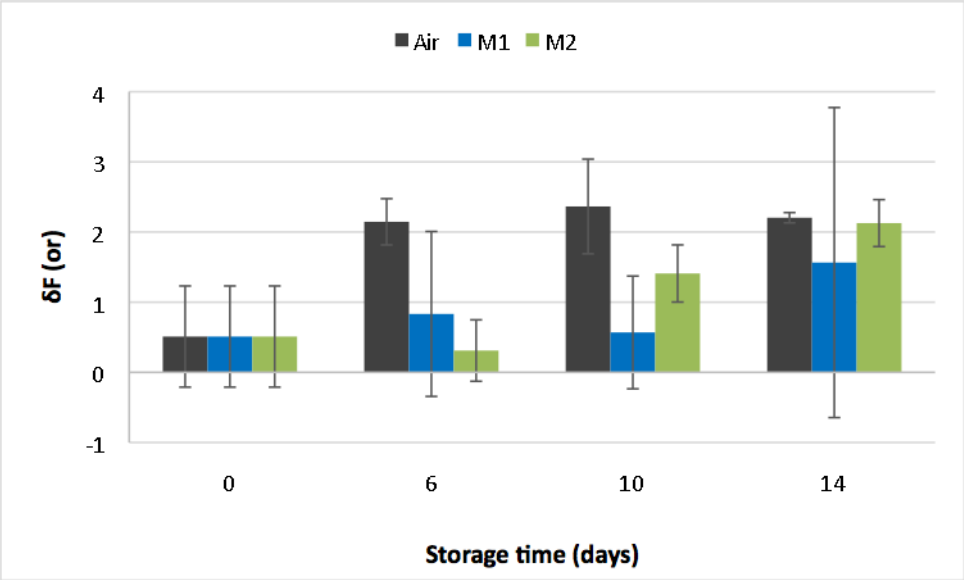

Figure 4.38. *Fluorescence shift (or) for Air, M1 and M2 groups. Samples taken from the top layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

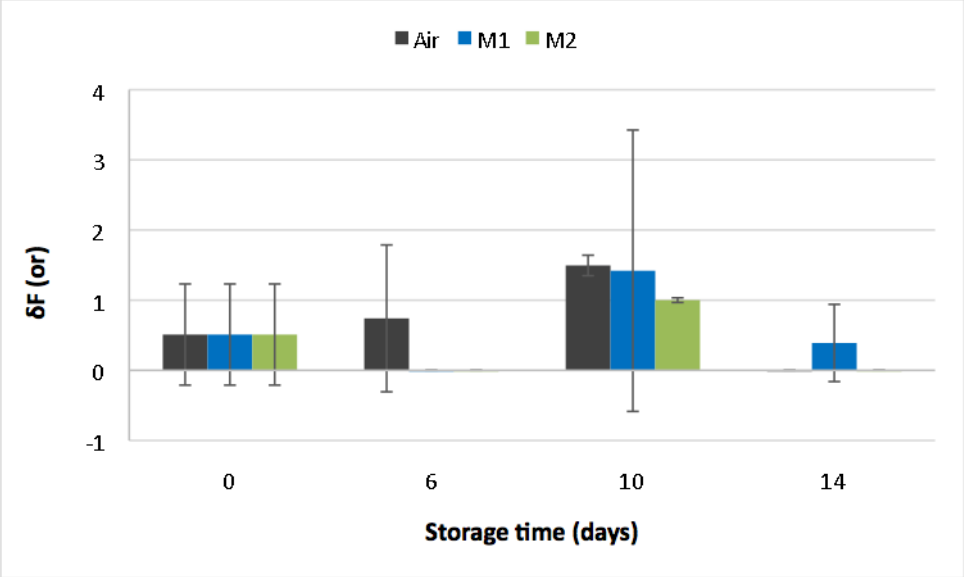

Figure 4.39. *Fluorescence shift (or) for the Air, M1 and M2 groups. Samples taken from the lower layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

4.2.10 Heme Iron

All groups followed the same pattern with the highest value of heme iron on day 6 (Figures 4.40 and 4.41). On day 0, heme iron content measured 26.8 ppm. A significant increase was observed between days 0 and 6, and a decrease between days 6 and 14 in the top and lower layer of all treatments ($p<0.05$). From days 6 to 14, heme iron decreased significantly in the lower layer of M1 and M2 groups ($p<0.05$). A significant increase was observed in the lower layer of Air group from days 0 to 6 ($p<0.05$). On day 6, lower layer of Air group had a significantly higher heme iron than the lower layer of both M1 and M2 groups.

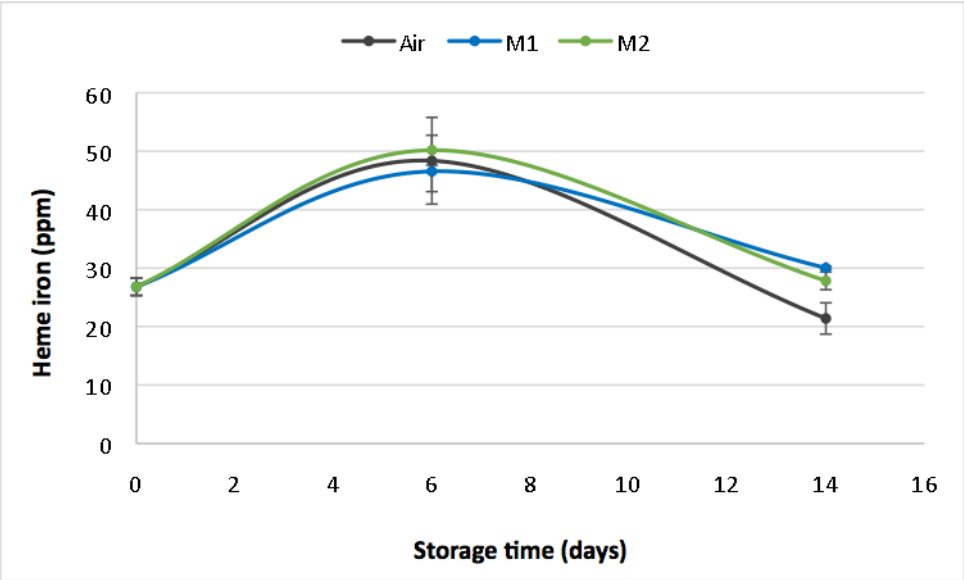

Figure 4.40. *Heme iron (ppm) for the Air, M1, and M2 groups. Samples taken from the top layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

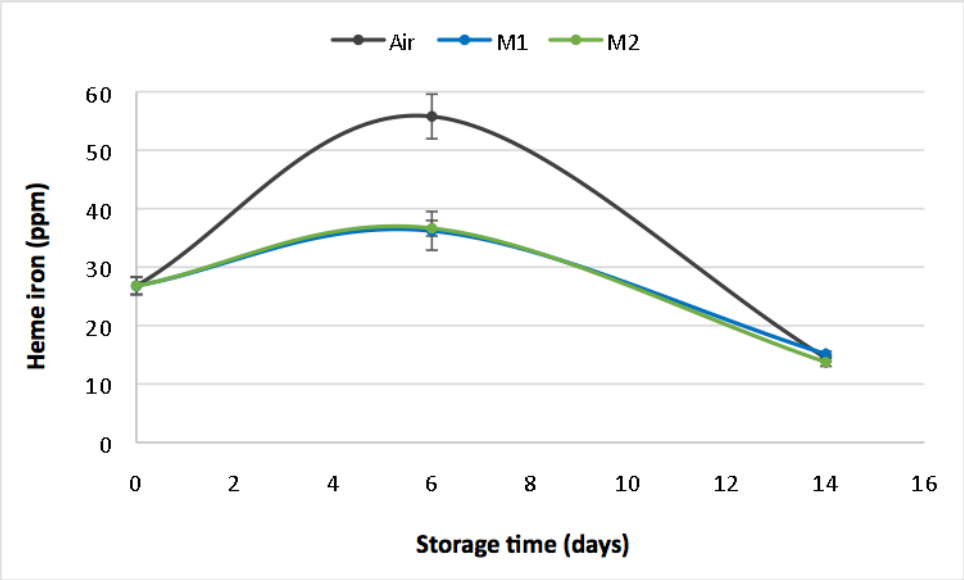

Figure 4.41. *Heme iron (ppm) for the Air, M1, and M2 groups. Samples taken from the lower layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

4.2.11 Non-Heme Iron

All treatment groups followed the same pattern, with the highest value 53.13 ppm non-heme iron on day 0 and a steady decrease in with increased storage time (Figures 4.42 and 4.43). From day 0 to 6, a significant decrease was observed in the top layer of M1, lower layer of Air group, and in the top and lower layer of M2 group ($p<0.05$). No significant difference was observed between the groups.

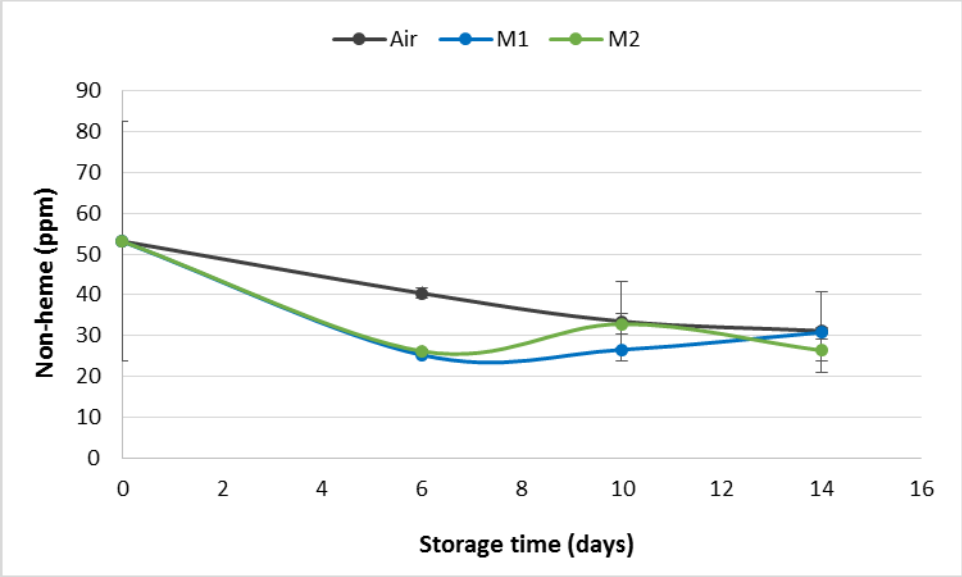

Figure 4.42. *Non-heme iron (ppm Fe) for the Air, M1, and M2 groups. Samples taken from the top layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

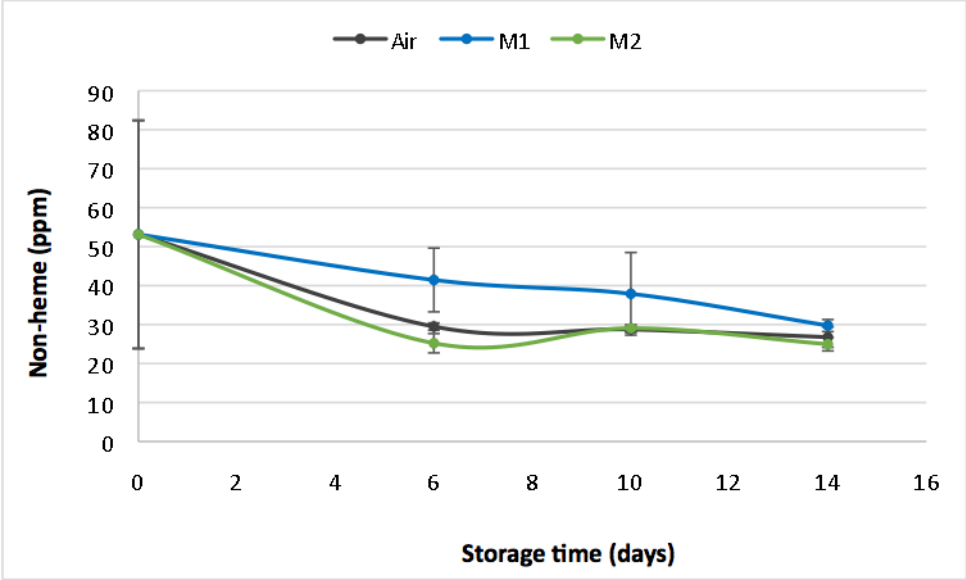

Figure 4.43. *Non-heme iron (ppm Fe) for the Air, M1, and M2 groups. Samples taken from the lower layer of each box. Vertical bars show standard deviation from the mean value (n=4).*

4.2.12 Colour Analysis

Results of colour analysis showed that all groups had similar values for L* (lightness) (Figure 4.44). A small decrease in lightness was observed between days 10 and 14 in all groups, however not significant. The highest value was measured in Air group on day 10. Difference was not significant between groups. Measurements of redness (a* value) showed no significant difference between groups (Figure 4.45). The redness decreased slightly with storage time but the difference was however not significant.

Measurements of yellowness (b* value) measurements showed the Air group with the highest value, 26.14, and M2 with the lowest value, 19.56, on day 10. Still, the difference between groups was not significant (Figure 4.46). A minor decrease in yellowness was noted between the two days in all groups but not significant.

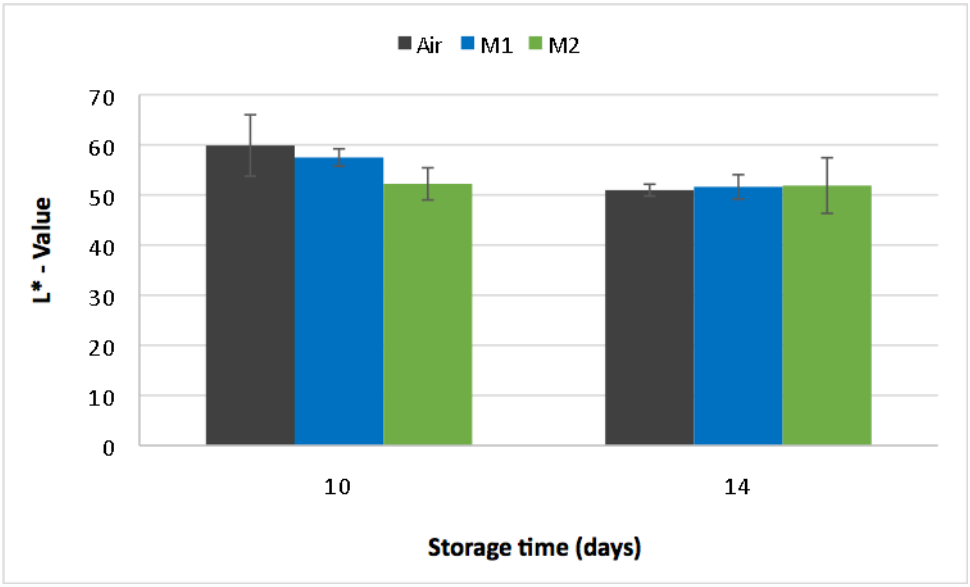

Figure 4.44. *Mean values of lightness (L* value) for the Air, M1, and M2 groups, using the Lens eye computer program. Vertical bars show standard deviation from the mean value (n=6).*

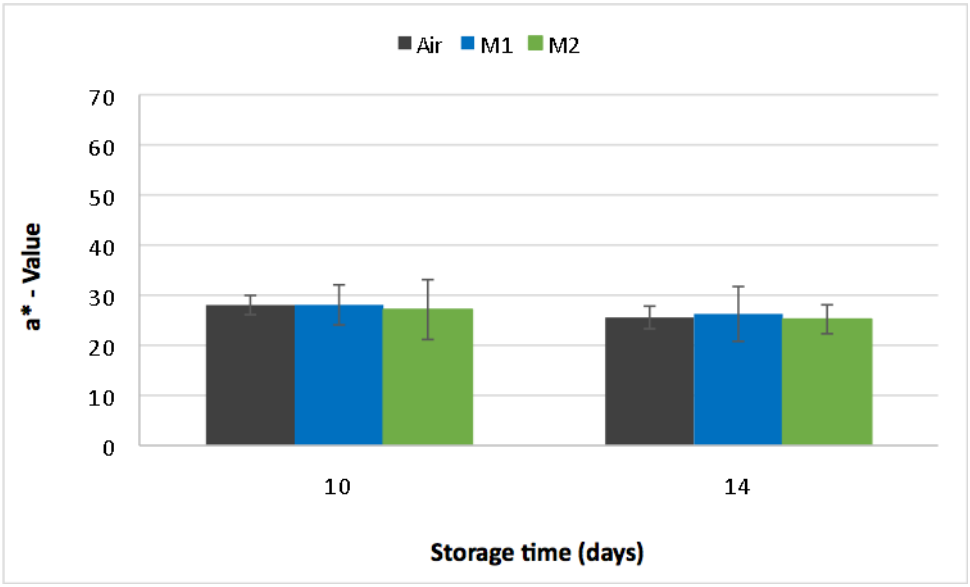

Figure 4.45. *Mean values of redness (a* value) for the Air, M1 and M2 groups, using the Lens eye computer program. Vertical bars show standard deviation from the mean value (n=6).*

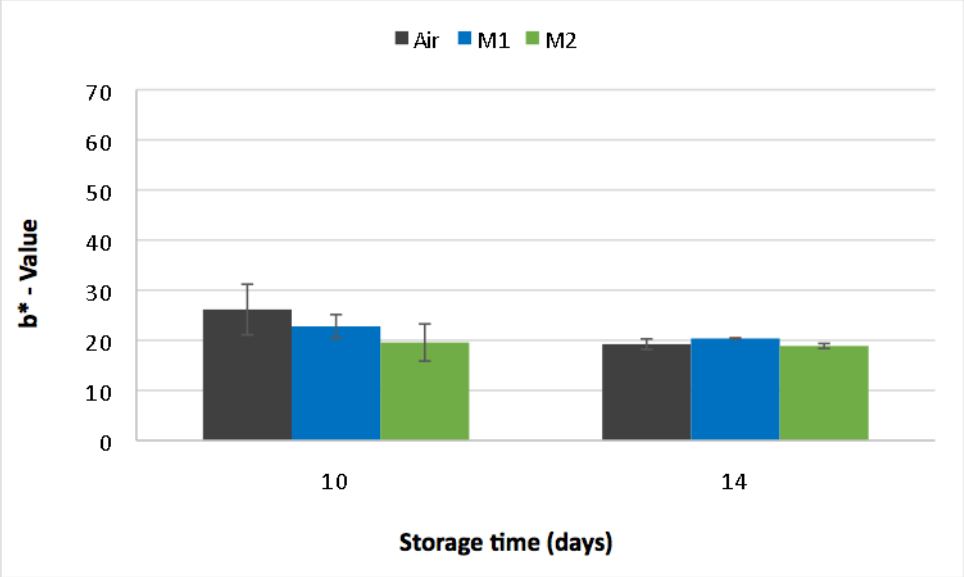

Figure 4.46. *Mean values of yellowness (b* value) for the Air, M1 and M2 groups, using the Lens eye computer program. Vertical bars show standard deviation from the mean value (n=6).*

4.2.13 Sensory Evaluation

Sensory evaluation of cooked redfish was performed by two methods; Torry freshness assessment and the QDA method describing 29 attributes relating to odour, appearance, flavour and texture. Mean Torry score of 5.5 indicates the end of shelf life. In the beginning of the shelf life study all groups were characterised by attributes indicating freshness; sweet odour and flavour, liver odour and flavour, shellfish odour and metallic flavour. No spoilage odour or flavour was detected (Table A.1 in appendix A).

Some difference was seen between groups on day 6. The M1 group had a stronger liver flavour than the other two groups (Table A.1 in appendix A). The Air group had less sweet flavour and more queasy flavour than the M1 and M2 groups, indicating less freshness. No difference was seen in odour, appearance or texture between groups. The Air group received a lower Torry score on day 6 than the other two groups (Figure 4.47).

Differences between groups on day 10 were rather small (Table A.1 in appendix A). Group M1 had a stronger TMA odour than other groups and group A had a more pungent flavour than group M2. The same trend in Torry score was seen as on day 6 but groups were not significantly different. All groups were still considered fit for consumption on day 10 (Figure 4.47).

Only groups M1 and M2 were evaluated on day 14 and no difference was seen between the two groups. The most apparent attribute in both groups was TMA odour (Table A.1 in appendix A). Both groups were also described with a clear TMA flavour, sour odour,

dishcloth odour, pungent flavour and trace of sour flavour and queasy flavour. Both groups received a Torry score of four and were considered spoiled (Figure 4.47).

Changes during storage were seen in odour, appearance and flavour (Figures A.1, A.2, and A.3 in appendix A). Freshness characteristics; sweet odour and sweet flavour, liver odour and liver flavour, shellfish odour and metallic flavour decreased, as spoilage characteristics; dishcloth odour, TMA odour, queasy odour, sour odour and sulphur odour increased. Less change was observed in appearance. The strength of colour increased after day 10, the amount of precipitation increased and flakiness decreased. Very little differences were seen in texture during the storage time (Figure A.4 in appendix A).

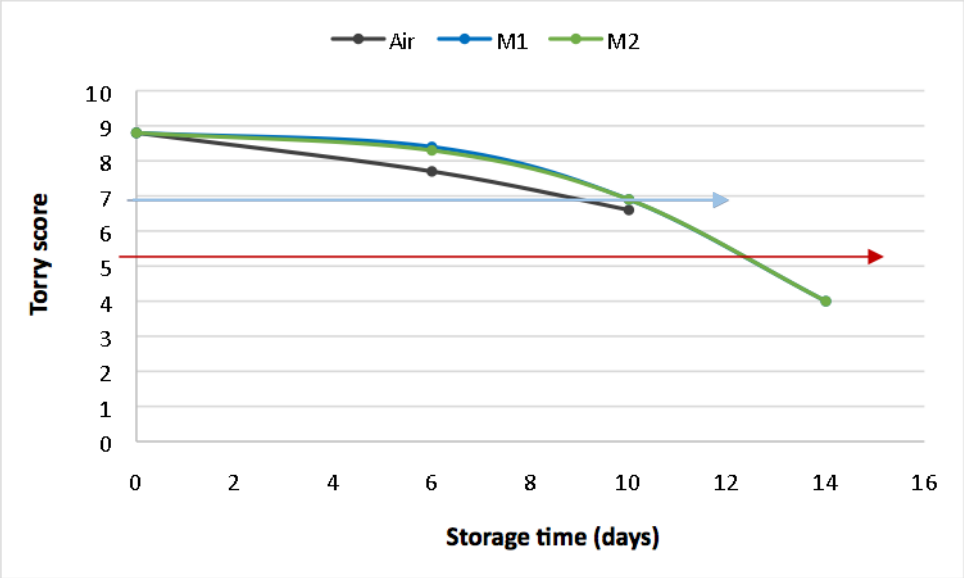

Figure 4.47. *Mean Torry freshness scores for the Air, M1 and M2 groups during storage. The blue horizontal line indicates freshness limit Ttorry score 7); the red horizontal line indicates end of shelf life (Torry score 5.5).*

4.3 Experiment III

The purpose of this study was to compare the following groups with respect to shelf life of fresh redfish fillets by evaluation of lipid oxidation, microbiological-, and sensory analysis:

1. H1 - Gill cut, conventional packing with skin side up.
2. H2 - Unbled fish, conventional packing with skin side up.
3. H3 – Unbled, sandwich packing.
4. H4 – Unbled, sandwich packing with five CO_2 pads in boxes, sandwich packing.

4.3.1 Temperature Monitoring

Temperature of the four groups (H1, H2, H3 and H4) was followed for up to 16 days of storage. The results of ambient temperature measurements showed a sharp decrease in temperature on days 6 and 10 when the storage room was opened (Figure 4.48).
The mean temperature was very similar for all groups (-0.4 °C and -0.5 °C). There was little temperature change despite increased ambient temperature (from initial temperature of -1 °C to +1 °C on day 6, and then again to +2 °C on day 10). The mean product temperature, mean initial temperature as well as the time to reach above 0 °C during storage can be seen in Table 4.5. The mean ambient temperature over the 16 days period was -0.3 ± 1.0 °C. Figure 4.48 shows the ambient temperature change during the entire storage period.

Table 4.5. *Mean initial product temperature (°C), mean temperature throughout storage* and time to reach specific temperatures.*

Treatment	Mean initial temperature (°C)*	Mean temperature (°C) throughout storage time	Stayed below 0 °C throughout storage time	Days to reach above 0 °C
H1	0.71 ± 1.32	-0.5 ± 0.2	H1 (A)	H1 (B) -15 days
H2	-0.39 ± 1.39	-0.5 ± 0.1	H2 (A and B)	
H3	-0.05 ± 0.16	-0.4 ± 0.2		H3 (A, B) - 14.7 days
H4	0.04 ± 0.37	-0.5 ± 0.1	H4 (A and B)	

* calculated from data collected from two loggers (upper and lower) per treatment.

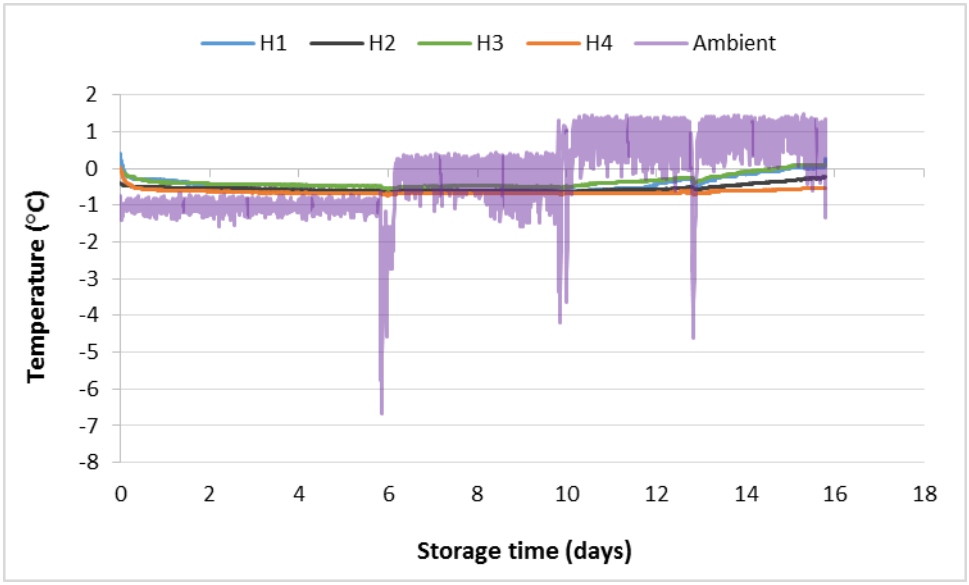

Figure 4.48. *Mean temperature of 4 loggers per group and 3 loggers in storage room, recorded every 10 minutes throughout storage time of 16 days. Temperature in cooling chamber was set at -1 °C on the day 0, increased to +1 °C on day 6, and to +2 °C on day 10.*

4.3.2 Drip Analysis

Water loss was estimated 6, 10, 13 and 16 days post packaging for all treatments groups. Some extreme values were seen for the H1 group on days 10 and 13. Due to the fact that the fish was kept so cold in storage, a thin ice layer was seen on the top layer of the fish on day 10 as well as some ice flakes in between on days 10 and 13, and even some ice flakes on day 16. This ice formation may have affected the drip measurements. Therefore, statistical analysis was not performed.

4.3.3 pH

Measurements of pH were performed on all sampling days (6, 10, and 14) both on a whole fillet and on a fish mince for all groups (Figure 4.49). In addition, measurements that were made on day 0 for the H1 group (bled fish) and the H2 group (unbled fish), showed that both groups had the same average pH of 6.58. Measurements performed on days 6, 10, 14 and 16 showed no significant difference between treatment groups, and no significant change within any of the groups throughout the storage. No significant difference was noted between the top and the lower layers.

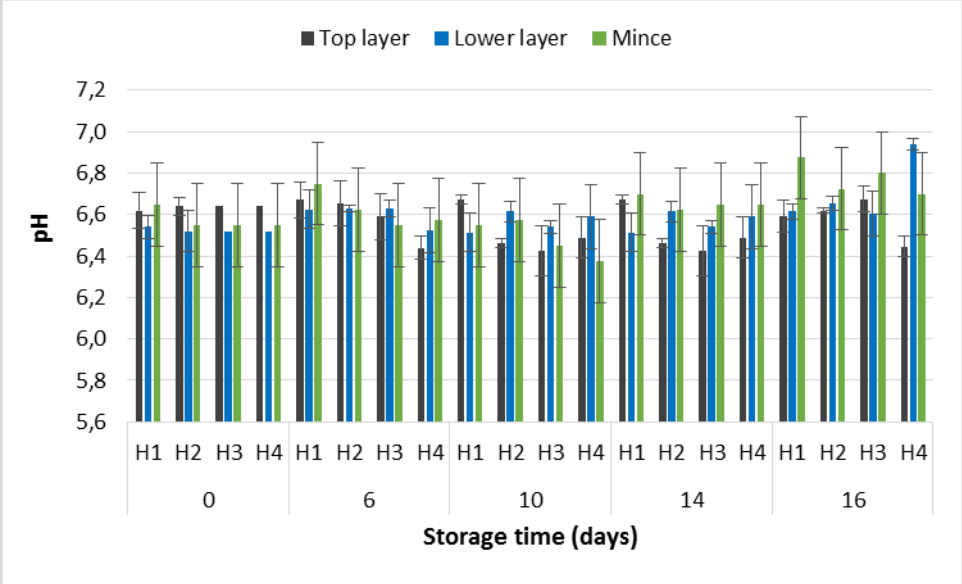

Figure 4.49. *pH in H1, H2, H3, and H4 groups. Samples taken from top layer, lower layer and fish mince. Vertical bars show standard deviation from the mean value (n=2).*

4.3.4 Total Volatile Basic Nitrogen (TVB-N)

TVB-N measurements show similar values for all groups for the first 10 days of the storage (Figure 4.50). However, the lowest value of days 6, 10 and 16 was observed for the H4 group (unbled, sandwich-packed group with CO_2 pads). Measurements of bled (H1) and un-bled (H2) groups on day 0 showed a higher value for the bled fish, 10.7 mg N/100 g, than for un-bled fish, 9.25 mg N/100 g. A significant increase in TVB-N was observed for all groups from days 0, 6, and 10 to day 16 ($p<0.05$). However, difference between the four groups was not significant ($p>0.05$).

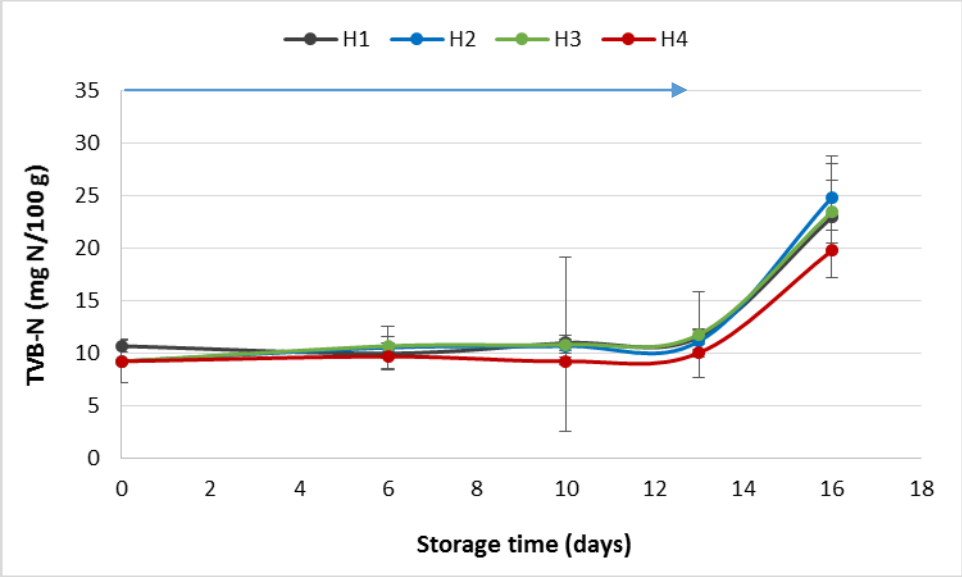

Figure 4.50. *Total Volatile Basic Nitrogen (TVB-N), mg nitrogen per 100 g fish for groups H1, H2, H3, and H4. Vertical bars show standard deviation from the mean value (n=2). Blue horizontal line shows TVB-N limit (EU).*

4.3.5 Microbiological Analysis

Total Viable Counts (TVC)

The analysis of total viable counts (TVC) showed that H1 group (bled fish) tended to have the lowest microbial count throughout storage time when compared to H2, H3, and H4 groups (Figure 4.51). On day 0, bled fish (H1) had a lower TVC, 3.68 log CFU/g, than unbled fish (H2), 4.23 log CFU/g. A significant increase was observed in all treatment groups ($p<0.05$). On day 13, the H1 group had a significantly lower TVC than the H2 and H3 groups, and a significantly lower count than the H3 group on day 10 ($p<0.05$).

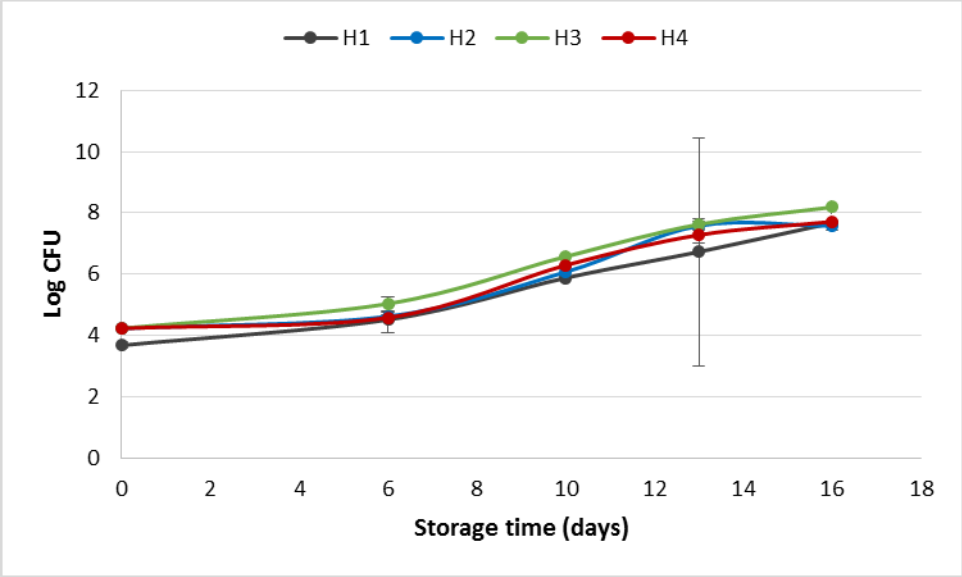

Figure 4.51. *Total viable counts on iron agar (TVC-IA) for groups H1, H2, H3, and H4. Vertical bars show standard deviation from the mean value (n=2).*

H_2S Producing Bacteria

Results from H_2S producing bacteria are shown in Figure 4.52. The same trend was observed in all groups with a significant increase with time for all groups ($p<0.05$). On day 0, the bled fish (H1) had a total H_2S bacterial count of 3.68 log CFU/g whereas the unbled fish (H2) had a total count of 4.23 log CFU/g. On day 13, the H1 group had a significantly lower number than H2 and H3 ($p<0.05$). However, no significant difference was observed between the groups on days 0, 6, 10 and 16.

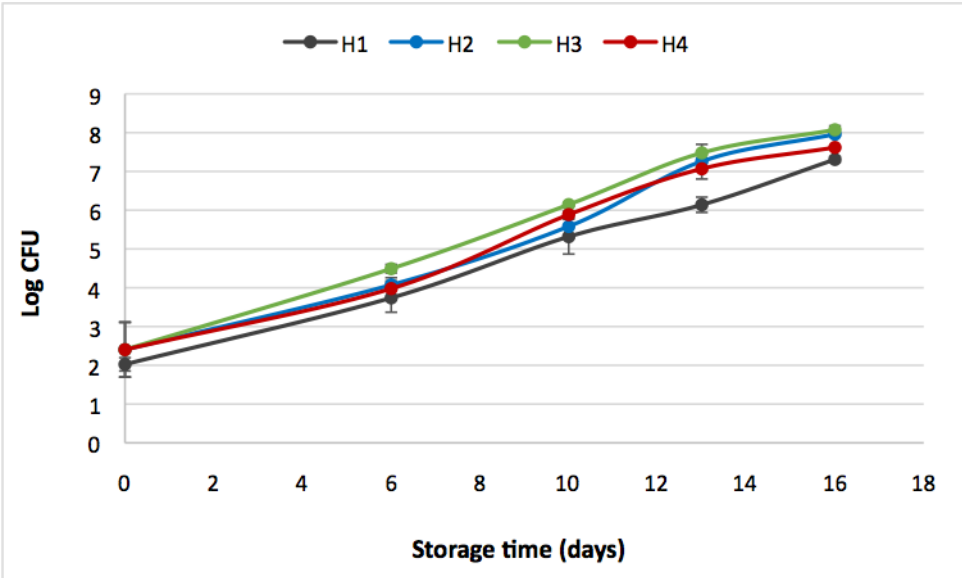

Figure 4.52. *H_2S producing bacteria on iron agar for groups H1, H2, H3, and H4. Vertical bars show standard deviation from the mean value (n=2).*

4.3.6 Water and Lipid Content

The results of water content measurements showed that the redfish contained an average of 78.9% (+/- 1.38%) water. The lipid content measured on day 0 in unbled group showed the average value of 3.42% lipid content. Lipid content was significantly higher in the October experiment than in the May experiment ($p<0.05$) (Figure 4.53). The difference in lipid content is due the life cycle and feeding pattern of the redfish.

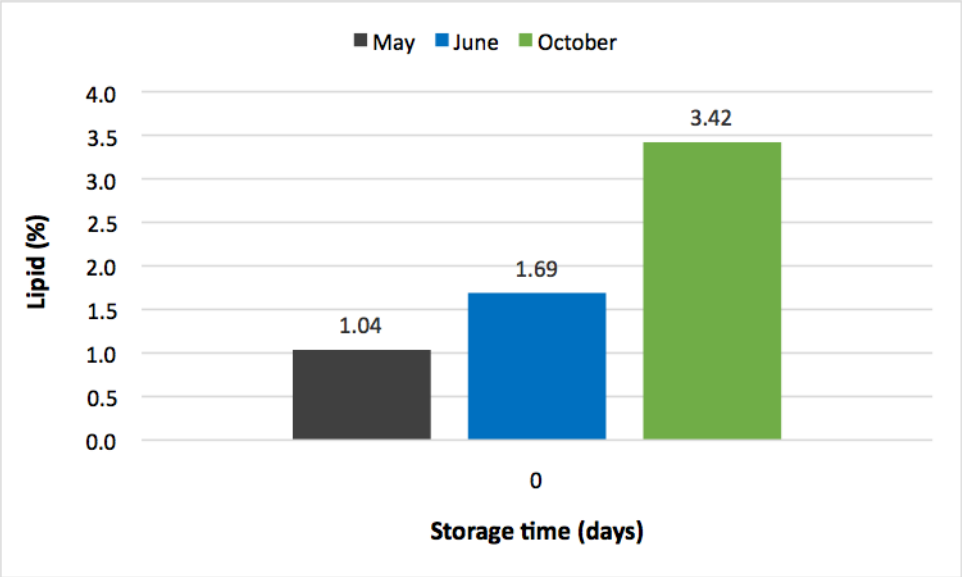

Figure 4.53. *Lipid content (%) in redfish. A comparison of May, June, and October experiments. Vertical bars show standard deviation from the mean value (n=2).*

4.3.7 Fatty Acid (FA) Analysis

Fatty acid composition was analyzed on day 0 for the unbled fish. Results of SFA, MUFA, PUFA as well as EPA and DHA are shown in Figures 4.54 and 4.55 respectively.

A comparison of the fatty acids between experiments I and III, can be seen in Figures E.1, E.2, E.3 and E.4 in appendix E. The results showed that the level of EPA was significantly lower in October than in May, however DHA was not significantly different. The total amount of SFA was significantly lower in October than in May ($p<0.05$). The October redfish had significantly more MUFA than the May redfish, however no significant difference was observed in PUFA between the two months ($p<0.05$).

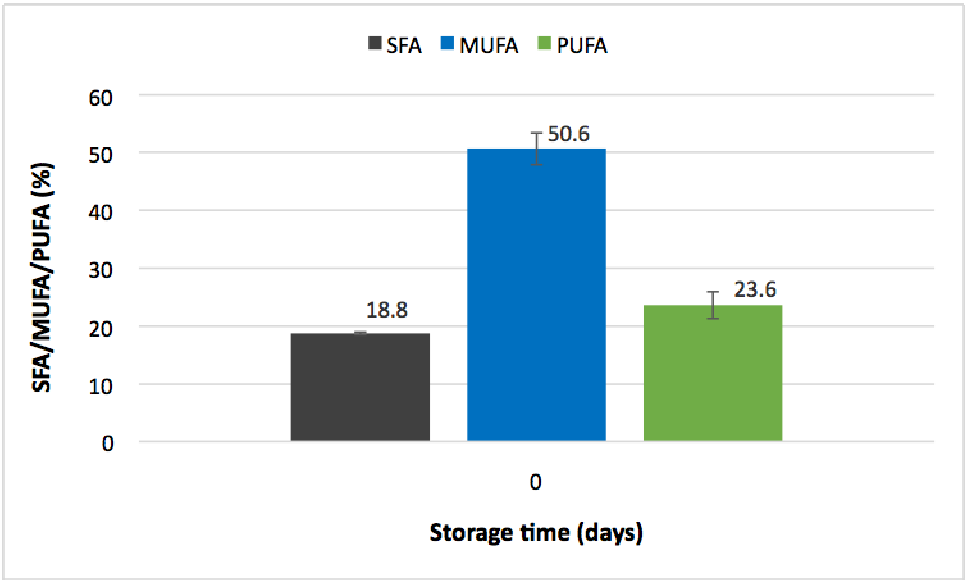

Figure 4.54. *Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (g/100 g lipids) in unbled redfish. Vertical bars show standard deviation from the mean value (n=2).*

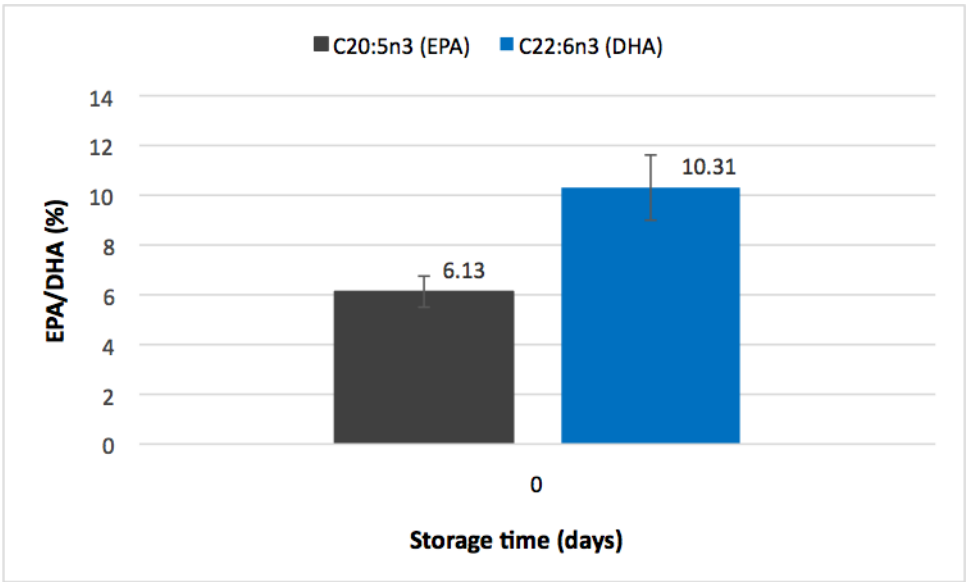

Figure 4.55. *Eicosapentaenoic acid (EPA; g /100 g lipids) and docosahexaenoic acid (DHA; g/100 g lipids) in unbled redfish. Vertical bars show standard deviation from the mean value (n=2).*

4.3.8 Lipid Oxidation Analysis

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS results showed that all groups followed a similar pattern (Figure 4.56). The highest value was observed on day 10 in all groups with very little difference between groups on all sampling days.

A significant increase was observed in all groups from day 6 to 10 and a significant decrease was observed in the H2, H3, and H4 groups from days 10 to 13 ($p<0.05$). On day 16, when TBARS value was increasing again, H4 had a significantly lower value than the H1 group and the H2, and H3 groups showed a significantly lower value than the H2 group ($p<0.05$). No significant difference was observed between groups on days 0, 6, 10, and 13.

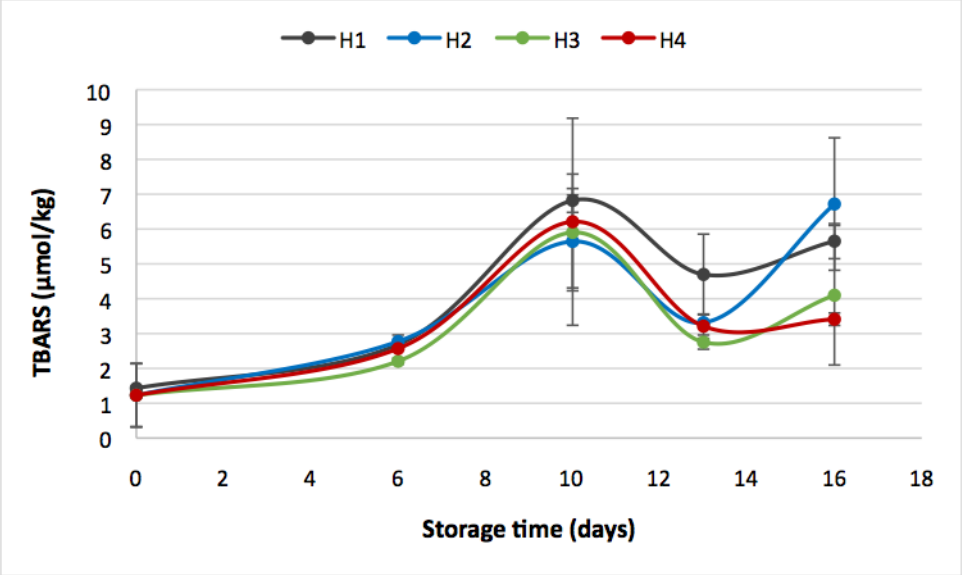

Figure 4.56. *Thiobarbituric acid reactive substances (TBARS) in the H1, H2, H3, and H4 groups. Vertical bars show standard deviation from the mean value (n=4).*

4.3.9 Heme Iron

Heme iron measurements were performed for the bled group (H1) and the unbled groups (H2, H3, and H4) on day 0 (Figure 4.57). Results of two-tailed t-test showed no significant difference between bled and unbled groups of redfish.

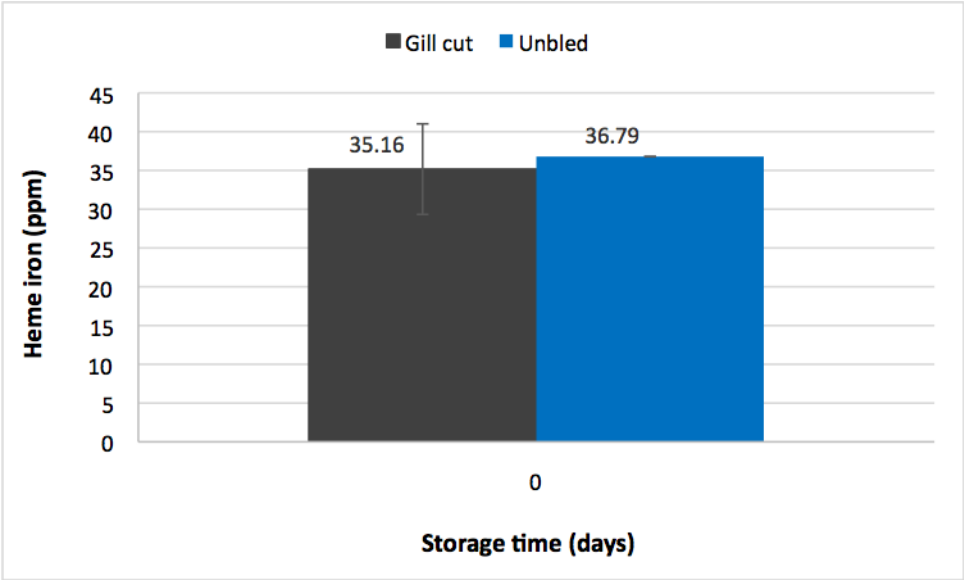

Figure 4.57. *Heme iron content (ppm) in gill cut and unbled redfish. Vertical bars show standard deviation from the mean value (n=4).*

4.3.10 Colour Analysis - Lens Eye

Lightness - L* Value

Results of Lens eye colour measurements showed a significant decrease in lightness from day 0 to day 10 in the H1 and H2 groups (Figure 4.58). A significant increase was observed in the H3 and H4 groups from day 0 to 16, and day 0 to 6, respectively. These results indicate that groups H1 and H2 became darker with time whereas groups H3 and H4 became lighter with time. H1 had the highest value of all the groups throughout the storage time. However, no significant difference was noted between the groups except for day 6 when the H1 group showed a significantly higher value than the H4 group ($p<0.05$).

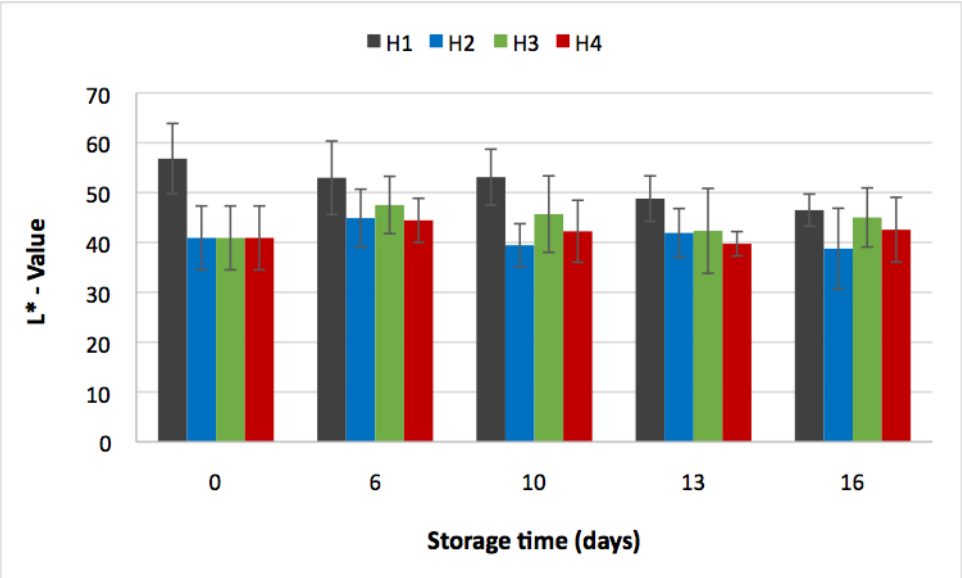

Figure 4.58. *Mean values of lightness (L* value) for the H1, H2, H3 and H4 groups using Lens eye computer program. L=lightness axes, 0=black, 100=white. Vertical bars show standard deviation from the mean value (n=8).*

Redness - a* Value

Colour measurements of redness showed no significant change during the storage for any of the treatment groups (Figure 4.59). The difference between groups was not significant in any of the sampling days except for day 16. On day16, the H1 group showed a significantly lower value than the H3 group and a significantly higher value than the H4 group ($p<0.05$). The H2 group showed a significantly higher value than the H4 group and a significantly lower value than the H3 group ($p<0.05$). All values were positive indicating redness in the fillets.

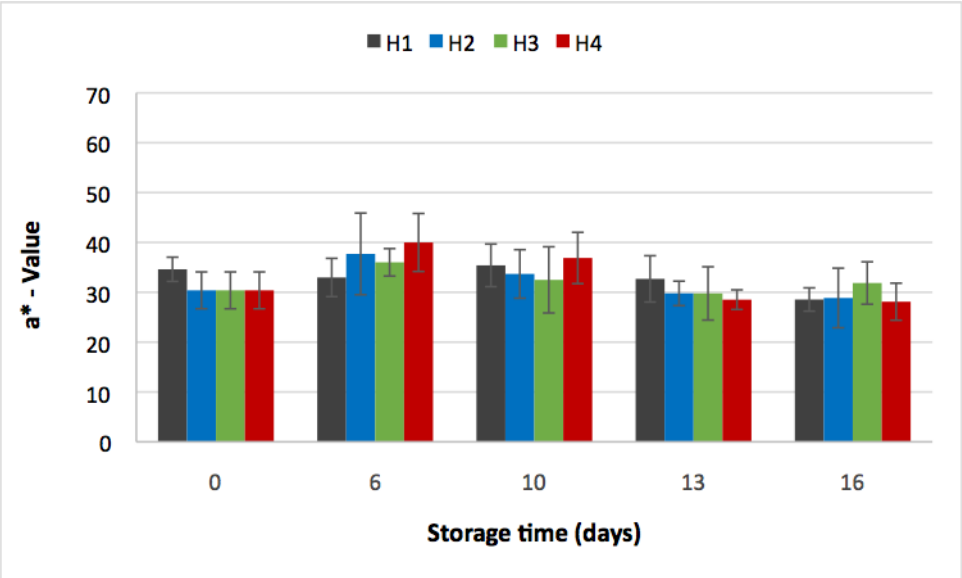

Figure 4.59. *Mean values of red/green colour (a* value) for H1, H2, H3, H4, using lens eye computer program. a=red-green axes, 0=neutral, negative values=green, positive values=red. Vertical bars show standard deviation from the mean value (n=8).*

Yellowness - b* Value

Colour measurements of yellowness showed that the fish had more yellow color than blue (Figure 4.60). Results showed a significant decrease in the H1 group from day 6 to day 16, and a significant increase in the H2 group from day 0 to 10, and a significant decrease in the H4 group from days 0 to 6, and 0 to 10. No significant colour change was found in the H3 group. No significant difference was found between the groups except on day 6 when H4 showed a significantly lower value than the H1, H2, and H3 groups ($p<0.05$). A trend was noted where H1 group showed the highest average value when compared to other treatments on all days of storage.

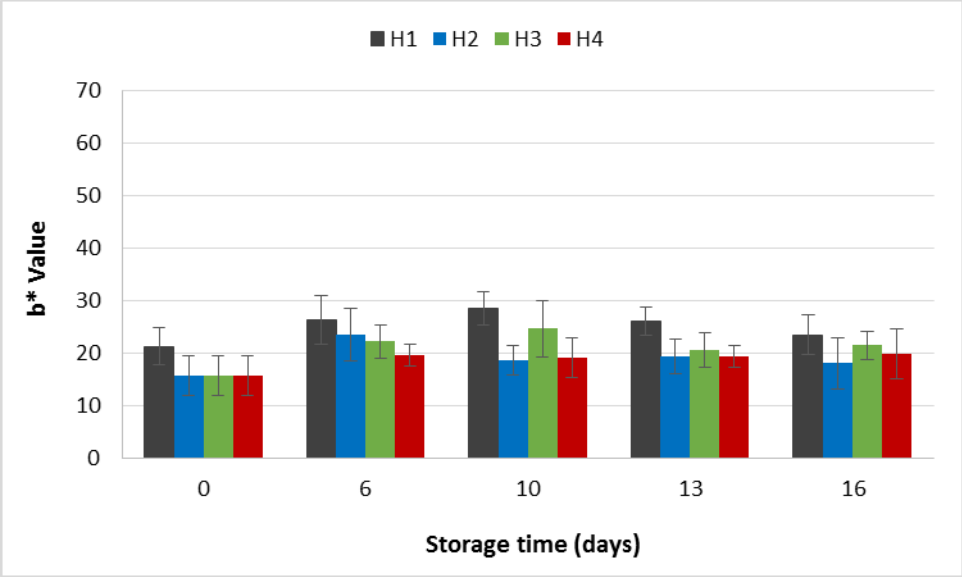

Figure 4.60. Mean values of yellow/blue colour for H1, H2, H3, H4, using lens eye computer program. b=blue-yellow axes, 0=neutral, negative values=blue, positive values=yellow. Vertical bars show standard deviation from the mean value (n=8).

4.3.11 Colour Analysis – Minolta

Lightness - L* Value

Minolta colour measurements showed that on the packaging day (day 0), the bled fish (H1) had a higher L* value, 53.15, than the unbled fish (H2, H3, and H4) (Figure 4.61). A significant increase was observed for the H1 group from day 6 to 10, and for the H2 group from day 6 to 10, and from day 10 to 13 ($p<0.05$). No significant change was observed in the H3 and H4 groups.

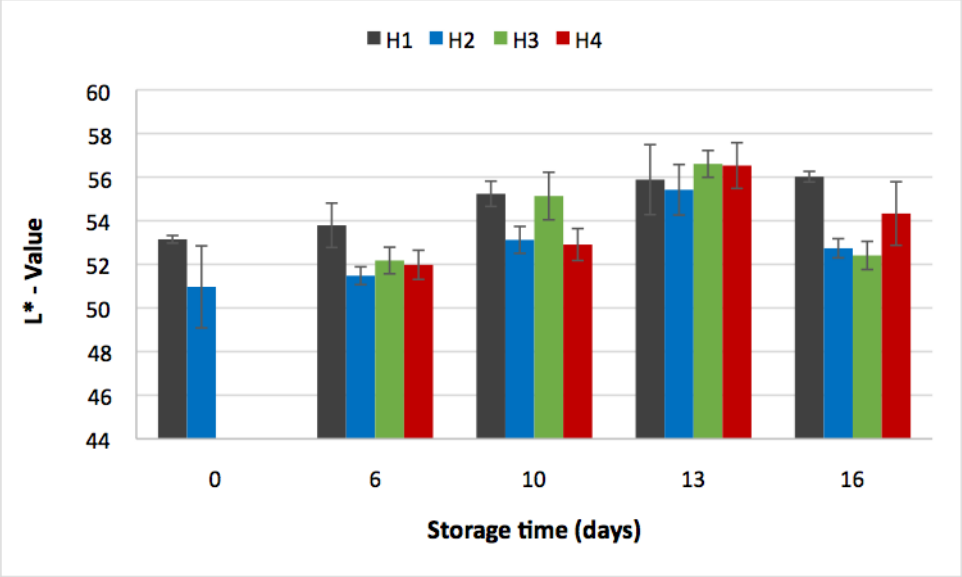

Figure 4.61. Mean values of lightness (L value) for H1, H2, H3, H4, using a Minolta chroma meter. L=lightness axes, 0=black, 100=white. Vertical bars show standard deviation from the mean value (n=16).*

Redness - a* Value

Minolta colour measurements of a* value showed that on day 0, the bled fish (H1) had an a* value of 0.54, and the unbled fish (H2, H3, and H4) had an a* value of -0.48, indicating that the bled fish had more red colour than green, but the unbled had more green colour than red (Figure 4.62). Values of a* decreased in all groups with storage time indicating that more green colour was developing in the fish as storage prolonged. The H1 group had the highest a* value of all the groups throughout the storage time. Results showed a significant decrease in the H2 group from day 6 to 13, in the H3 group from day 0 to 10, and in the H4 group from day 10 to 16 ($p<0.05$). No significant change was found in the H1 group throughout the storage time. Furthermore, no significant difference was found between groups except on day 16 when the H1 group showed a significantly higher a* value than the H3 group ($p<0.05$).

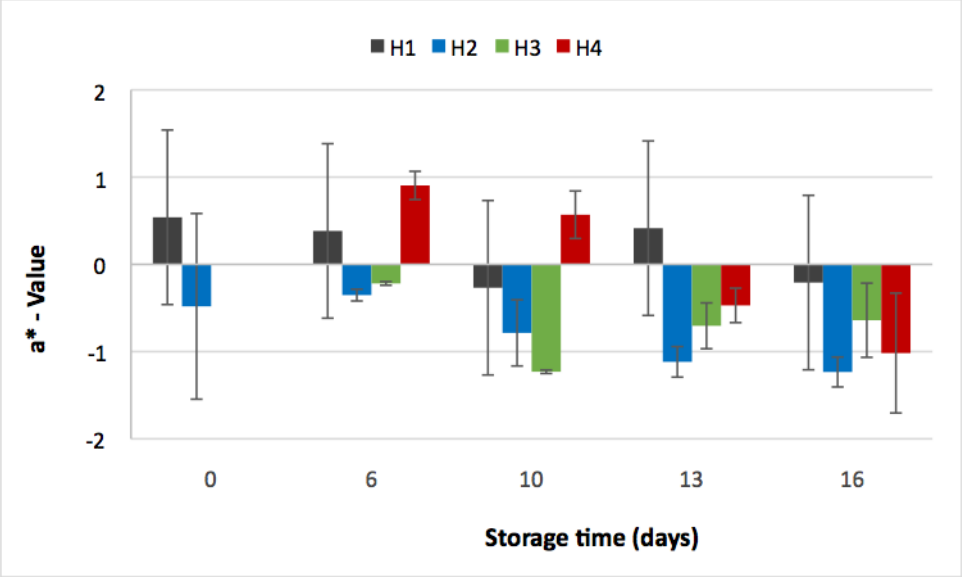

Figure 4.62. *Mean values of red/green colour (a* value) in the H1, H2, H3, H4 groups, using a Minolta chroma meter, (a=red-green axes, 0=neutral, negative values=green, positive values=red). Vertical bars show standard deviation from the mean value (n=16).*

Yellowness - b* Value

Results of Minolta measurements showed that b* value increased in all groups with increased storage time, indicating that the color of the redfish changed from bluish in the beginning of storage to yellowish at the end of storage time (Figure 4.63). A significant increase was observed in all groups from day 6 to 10, and from day 10 to 13 ($p<0.05$).

The H1 group showed a significantly higher value than the H4 group on days 6, 13, and 16, and a significantly higher value than the H3 group on day 6, and the H2 group on day 13 ($p<0.05$). The H4 group showed a significantly higher b* value than the H2 group on days 6, 10, and 13, and the H3 group on days 6 and 13 ($p<0.05$). The H2 group showed a significantly higher lab*b value than the H1 group on day 0, the H3 group on day 6, and the H4 group day 16 ($p<0.05$).

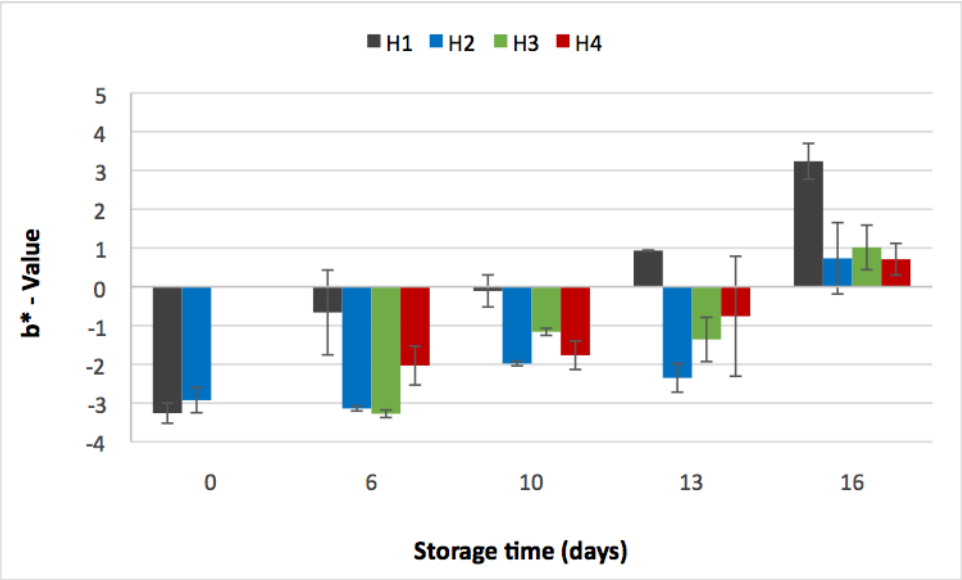

Figure 4.63. *Mean values of yellow/blue colour (b*value) in the H1, H2, H3, H4 groups, using a Minolta chroma meter, (b=blue-yellow axes, 0=neutral, negative values=blue, positive values=yellow). Vertical bars show standard deviation from the mean value (n=16).*

4.3.13 Sensory Evaluation

Sensory evaluation of cooked redfish was performed by two methods; Torry freshness assessment and the QDA method describing 29 attributes relating to odour, appearance, flavour and texture. Mean Torry score of 5.5 indicates the end of shelf life. In the beginning of the shelf life study both groups (bled and unbled) were characterised by attributes indicating freshness; sweet odour and sweet flavour, liver odour and liver flavour, shellfish odour and metallic flavour. No spoilage odour or spoilage flavour was detected (Table B.1 in appendix B).

Minor differences were seen between groups on storage day 6. Group H3 had a sweeter odour than group H2 and a more heterogenous colour than group H1. No differences were seen in flavour or appearance.

Differences between groups on day 10 were small (Table B.1 in appendix B).

Group H1 had a slightly more rancid flavour than other sample groups and group H2 had a more sticky texture than group H4. No difference was seen between groups in odour or appearance. No difference was observed between groups on day 13. Two panellists did not taste one or more samples and evaluated therefore only odour and appearance. No difference was observed in the mean Torry score during the storage period and all groups had a shelf life of 12 days (Figure 4.64).

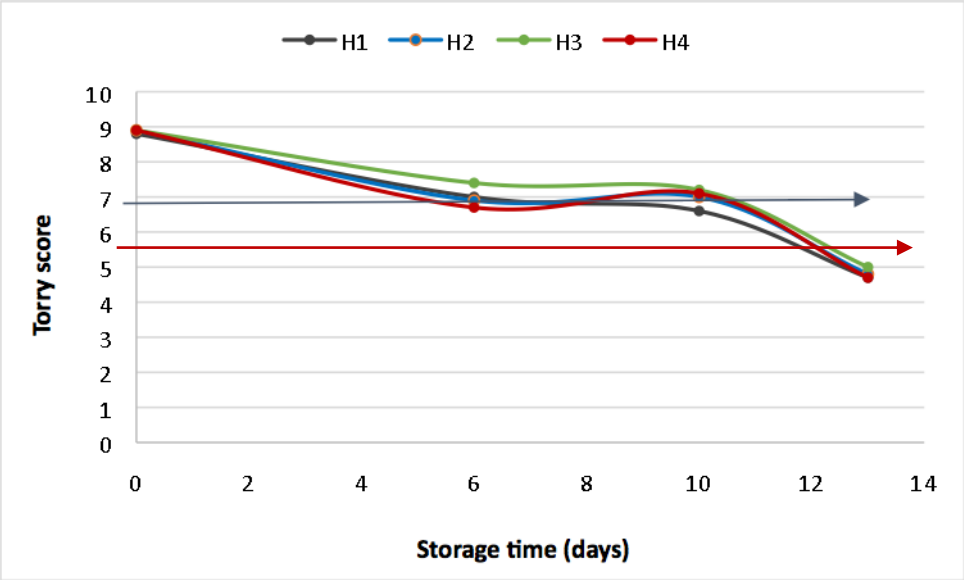

Figure 4.64. *Mean Torry freshness scores for the H1, H2, H3 and H4 groups, during storage. The horizontal blue line indicates freshness limit (Torry score 7); the horizontal red line indicates end of shelf life (Torry score 5.5).*

5 DISCUSSION

5.1 Temperature

The results of temperature measurements in experiment II showed that mean temperature throughout storage was lowest -0.4 °C ±0.3 °C in the Air group (A). The mean temperatures of M1 and M2 were 0.3 °C ±0.9 °C and 0.5 °C ±0.9 °C respectively. Furthermore, it took both MAP groups 6 days to reach 0 °C, while it took the Air group 10 days to reach 0 °C. The reason for this difference in temperature between the Air and the MAP groups is mainly due to the different insulating abilities of the two types of boxes as well as to the ice added on top of the Air group. These results are in line with results from a study by Margeirsson *et al.,* (2009), where it was concluded that corrugated plastic (CP) boxes have much lower insulation than expanded polystyrene (EPS) boxes.

Results of temperature measurements in experiment III showed that fish packed in EPS boxes with ice and dry ice can maintain below 0 °C for days as most groups stayed below 0 °C the entire time, despite fluctuations in ambient temperature from -1 °C to above +1 °C. The mean temperature of the groups was -0.4 °C (H3) and -0.5 °C (H1, H2, and H4) throughout the storage time. These results confirm that EPS boxes are great insulators and an effective packaging method in terms of keeping the fish cold despite ambient temperature fluctuations.

5.2 Headspace Gas, pH and drip Analysis

Results of headspace gas composition in the two MAP groups in experiment II showed that CO_2 was significantly higher in the boxes of M2 group than in the boxes of M1 group throughout the entire storage time ($p<0.05$). This difference was expected as CO_2 pads release their CO_2 gas, as the pads get wet. The headspace level decreased somewhat after day 6 during storage, which was possible caused by the dissolution of CO_2 into the water phase of the fish muscle. However, it should be noted that it is not only the amount of CO_2 in the package but rather the temperature, which decides the amount of CO_2 absorbed into the fish muscle.

Greater pH reduction has been noted with high CO_2 concentration at low temperature (0 °C) as CO_2 dissolves more readily under such conditions (Umbreit *et al.*, 1972). This was confirmed in a study on haddock comparing air stored fish to MAP stored fish showing that temperature is indeed an important factor. No benefit was observed in the MAP packed haddock, 40 or 60% CO_2 atmosphere, when compared to air packed at 5 to 10 °C, whereas MAP packed haddock had 2 to 4 days longer shelf life when stored at 0 °C (Dhananjaya & Stroud, 1994).

Results of pH analysis showed that MAP packaging decreased the pH of the fish. Lower pH in the MAP groups was expected as the CO2 dissolved into the fish muscle, therefore lowering the pH. A significant increase in pH was observed in both layers of M1 group, and in the top layer of the M2 group as the fish got older ($p<0.05$). The increase in pH during storage is due to the production of basic volatile compounds as microbes reduce TMAO to TMA. The Air group had a significantly higher pH than M2 on days 6 and 10. The pH of the mince, was generally higher, for all groups, than in whole loin. A higher pH was expected in the mince, as the middle of the fillet would have higher pH than the surface of the fillet.

Results of drip analysis, in experiment II, showed a higher drip loss in both MAP groups than in the air group. On day 6, about 2% difference was noted between sampling groups with Air group the lowest and M2 the highest. The increase of drip in MAP packed fish, is due to the fact that as CO_2 dissolves in the water phase of the fish, pH level drops, which again results in decreased water holding capacity of the fish muscle. These results are in line with other studies including one, done by Dalgaard *et al*. (1993), which reported an increased liquid loss with increasing CO_2 level.

No significant difference was observed between groups in the third experiment and no significant change within each group was observed. Even though group H4 had 8 CO_2 pads in the bottom of the box, pH of the fish was not affected. This is most likely due to the fact that in this experiment EPS boxes were used and no sealed plastic bag to keep the gas inside the box. As EPS boxes are not airtight, the gas must have leaked out. Therefore, EPS boxes are not a suitable packaging method to lower the pH of the fish by using CO_2 pads. Results of drip analysis in experiment III were inconclusive due to low temperature of the fish and ice formation.

5.3 TVB-N and TMA

Results from experiment II showed a significant increase in both M1 and M2 from day 10 to 14. All treatment groups were tested the first 10 days of storage, however as the Air group was determined spoiled on day 14 it was not tested further than to day 10. Therefore, the only comparison possible is between the two MAP groups after day 10. However, the bacterial reduction of TMAO to TMA has been shown to occur at a faster rate in oxygen-reduced packaging of fish (Huss, 1972). On day 10, none of the three groups had passed neither the EU limit of TVB-N for safe consumption (35 mg N/100 g) nor the TMA limit proposed (15 mg N/100 g) (Connell, 1995). However, on day 14 both MAP groups had surpassed both TVB-N and TMA limits. No significant difference was noted between any of the groups throughout the entire storage time.

It can be concluded that adding the CO_2 pads in the box of M2 group was not effective in reducing TVB-N or TMA when compared to gas packaging without the CO_2 pads (M1). This is probably due to the fact that *Photobacterium phosphoreum*, which tolerates well MAP packaging, is also a great producer of TMA in the absence of oxygen.

The different treatments of the redfish in experiment III did not prove to have a significant effect on the redfish in terms of TVB-N level. However a significant increase was noted in all treatments.

5.4 Microbiological Analysis

Overall, MAP packaging was effective in slowing the growth of *Pseudomonads*. However, MAP packaging did not reduce the TVC, the growth of H_2S producers or *P. phosphoreum*. Adding the five CO_2 pads to the MAP group (M2) did not reduce the number of microbes significantly. It should be noted that the temperature control in the MAP groups was not as efficient as in the Air group. A review study by Sivertsvik *et al.* (2002) concluded that without proper temperature control, the benefits of MAP can be lost. Therefore, the benefits of MAP packaging may have been greater if temperature of the fish had been lower throughout the storage. Studies have shown that, *P. phosphoreum* tolerates well CO_2 packaging and has been found to be the main specific spoilage organism (SSO) in MAP packaging (Dalgaard *et al*., 1993; Dalgaard, 1995).

Results from experiment III showed a significant increase in all treatment groups ($p<0.05$). A comparison of the different groups showed only a minor variation during storage. Looking at the results of TVC and H_2S producing bacteria, it can be noted that the TVC in the redfish was mostly based on H_2S producing bacteria.

5.5 Water and Lipid Content

The average water content was 81.1% (+/-0.6%) in experiment I, and 78.9% (+/-1.4%) in experiment III.

Lipid content measured highest in the middle section of the redfish. Generally, difference between the groups was not significant. The highest values of lipid content were observed on day 8. Increasing values for lipid (%) are noted due to variation of the fish.

The results of lipid content analysis in experiment II showed some difference between sampling days as well as between groups, indicating the variation between the redfish samples.

The lipid content of the fish was significantly higher in October, experiment III, than in May, experiment I. The higher lipid content in October would be expected as the fish has been feeding the whole summer and gaining energy for the winter months.

5.6 Fatty Acid Analysis

Results from the fatty acid analysis in experiment I, showed no significant difference in EPA, DHA, MUFA, or PUFA between the three treatment groups, throat cut, gill cut and unbled. Significant decrease was observed during storage in SFA for the middle section of the unbled group ($p<0.05$). It can be concluded that bleeding does not affect level of unsaturated or saturated fatty acids.

Fatty acid analysis in experiment III was only performed on unbled fish on day 0. Results showed that, EPA was significantly lower in October than in May, however DHA was not significantly different. The total amount of SFA was significantly lower in October redfish than in May redfish. October redfish had significantly more MUFA than May redfish, however no significant difference was noted in PUFA between the two months.

5.7 Free Fatty Acids

Results of free fatty acids measurements in experiment I showed that there is a significant increase in all treatment groups during storage, with the highest value on day 12 ($p<0.05$). No significant difference was noted between groups.

Looking at different packaging treatments in experiment II, it was noted that the Air group did not have a significant increase in FFA value during storage. However, significant increase was observed in the both M1 and M2 treatments ($p<0.05$). No significant difference was observed between M1 and M2. The amount of FFA was significantly higher in M2 than in Air group on day 14. The higher level of FFA in MAP groups, could be caused by lower pH of the MAP packed fish, as enzymatic action is enhanced by lower pH.

5.8 Lipid Oxidation

Results from lipid oxidation measurement in experiment I, showed a significant increase in all sections of all groups ($p<0.05$), except for loin section of throat cut fish where no significant increase was observed. The highest peroxide values for throat cut and unbled groups were observed on day 8 whereas the highest value for the gill cut group was observed on day 5. This indicates faster primary oxidation rate for the gill cut group than the other two. Difference between sections of the fish were not significant except on day 8 for the throat cut group where the loin section had a significantly lower value than the middle and tail sections. In fact, as no significant change was noted in the loin section of the throat cut group, it can be assumed that little or no primary oxidation was taking place in that group.

Results of PV measurements in experiment II, showed no significant change in MAP groups (M1 and M2) indicating little or no oxidation. However, a significant increase was observed in the Air group ($p<0.05$). Difference between top and lower layer were not significant. As it is known, O_2 is needed for lipid oxidation. MAP packaging of the fish was effective in almost eliminating O_2 during storage therefore these results were as expected with no primary oxidation in the MAP groups.

Results from secondary oxidation (TBARS) measurements in experiment I, showed no significant increase between days in either of the bled groups, throat cut and gill cut, indicating little or no secondary oxidation in those two groups. However in the unbled fish, there was a significant increase in TBARS value, with the highest value on day 8, indicating secondary oxidation ($p<0.05$). Tail section of unbled fish came out the worst as it showed the highest value when compared to other sections. It should be noted that on day 8, the unbled group had the highest value of heme iron, which may have acted as a pro-oxidant.

Results from secondary oxidation (TBARS) measurements in experiment II, showed no significant change in the lower layer of MAP groups indicating little or no secondary oxidation. Significant increase was observed in the top layer of the MAP groups and in both layers of the Air group ($p<0.05$). The top layer of MAP fish was not as good as the lower layer, which may have been the result of lower pH of the top layer. These results indicate no secondary oxidation in the lower layer of the MAP groups. A secondary oxidation was taking place in the top layer of the MAP groups indicating lower quality of the top layer than the lower layer of the MAP fish. This difference in layers may have been due to the fact that the top layer of the MAP groups had a lower pH than the lower layer. Secondary oxidation was observed in both Air groups.

Results from secondary oxidation (TBARS) measurements in experiment III, showed no significant difference between the groups. All groups showed a significant increase in TBARS indicating secondary oxidation in all groups. Therefore, bleeding did not have a limiting effect on secondary oxidation. However, it should be noted that bled fillets (H1) were noticeably smaller than fillets in other groups, which may have affected the outcome of this experiment. On day 10 the average weight of the H1 group was 92.74 g whereas the H2 group was 154.22 g, the H3 group was 141.04 g, and the H4 group was 131.15 g.

Results from tertiary oxidation in experiment I, showed a significant increase for the loin and middle sections of gill cut fish with the highest value observed on day 12 ($p<0.05$. The results revealed that tertiary oxidation in throat cut fish reached the top on day 8 whereas unbled and gill cut fish were still increasing on day 12, indicating a faster rate of spoilage for the throat cut fish than the other two groups.

Results from tertiary oxidation in experiment II, showed that there was a significant increase in tertiary oxidation products in all treatment groups ($p<0.05$). It was observed that no difference was between the two bleeding methods. The throat cut group had a significantly higher value than unbled group on day 8 ($p<0.05$).

5.9 Heme Iron and Non-Heme Iron

Results of heme iron analysis in experiment I, showed a significant decrease in all groups during the storage period ($p<0.05$). This decrease is most likely due to the breakdown of heme, which would result in an increase in non heme content (Benjakul & Bauer, 2001). No significant difference was noted between the different sections (loin, middle and tail) of the fish. Unbled fish had a significantly lower heme iron than both gill cut, and throat cut fish indicating that bleeding was not effective in reducing the heme iron ($p<0.05$). This may have been the result of improper bleeding of the fish. Comparing the different packaging methods (experiment II), a significant increase in heme iron was noted in all groups from day 0 to day 6, except in the lower layer of M1 and M2 groups ($p<0.05$). The increase in heme iron during storage was probably due to the deterioration of the muscle, making it easier to extract the heme iron from the muscle. In October experiment (III), no significant difference was noted between bled and unbled groups. This supports the results of experiment I, that bleeding of the fish was not effective in reducing the heme iron content.

Results of non-heme iron analysis in experiment I showed no significant difference between the groups. A significant decrease was observed in the throat cut fish from day 0 to day 8 ($p<0.05$), however no significant change was observed in other groups (unbled and gill cut). These results indicate that bleeding did not affect the non-heme content in the fish muscle.

Results of non-heme iron analysis in experiment II showed a significant decrease in all groups (Air, M1 and M2) from day 0 to day 6 ($p<0.05$). However, no significant difference was noted between groups or between layers in the box. Therefore, it can be concluded that MAP packaging did not affect the non-heme content in the fish.

5.10 Colour

5.10.1 Lens Eye

Results of colour analysis (Lab*) in experiment I showed no significant difference between the groups in L* and a* and b* values. A significant increase in b* value was noted in both gill cut and throat cut fish ($p<0.05$). The results indicated that lightness in the fillets was not significantly lost in any of the groups throughout the storage time. All the groups were redder than green with no significant loss in redness. However, a significant increase was seen in b* values in both gill cut and throat cut fish ($p<0.05$). Therefore, bleeding of the fish did not affect the lightness or redness of the fillets but a significant more yellow color developed with time in both throat cut and gill cut fish.

Different packaging methods in experiment II, did not affect the color of the redfish fillets as no significant difference was noted in L* a* b* values.

Results from experiment III, showed a significant decrease in lightness in both H1 (gill cut) and H2 (unbled) groups and a significant increase in lightness in groups H3 (unbled, sandwich placement) and H4 (unbled with CO_2 pads) ($p<0.05$). Group H1 was significantly lighter than the H4 group on day 6 ($p<0.05$). No significant change was noted in A* value between sampling days in any of the groups. A significant difference between groups was only observed on day 16, when the H4 group was significantly redder than the other groups and the H4 group was significantly less red than the other groups ($p<0.05$). A significant decrease in b* value was noted in the H1 and H4 groups indicating that the color was becoming less yellow ($p<0.05$). A significant increase was noted in the H2 group indicating that the color was becoming more yellow ($p<0.05$). No significant change was observed in b* value for the H3 group. The H4 group was significantly less yellow than other groups on day 6 ($p<0.05$).

Looking at the fillets, it was obvious that the bled fish was distinctively lighter than the unbled fish. The colour difference was greatest on day 0, however as storage time prolonged the colour difference evened out and darker spots appeared in the bled fish as well (Figure 1, appendix C).

5.10.2 Minolta

The colour analysis by Minolta chroma meter in experiment II did not show the same

results as the colour analysis by the lens eye computer program.

Results of the colour analysis of L* value showed a significant increase in lightness of the H1 group and the H2 group from day 6 to 10. No significant change in lightness was observed in the H3 and the H4 groups. The H1 group was significantly lighter than the H3 group on days 10 and 16 and significantly lighter than the H4 group on day 6 ($p<0.05$). The H3 group was significantly lighter than the H4 group on days 6 and 16 and significantly lighter than the H2 group on day 10 ($p<0.05$). No significant difference was observed between the groups during storage up until day 16 when the H1 group showed a significantly higher a* value than the H3 group ($p<0.05$). A significant decrease in redness (a* value) was observed in groups H2, H3, and H4 ($p<0.05$). A* values were much lower, indicating more green color, when measured by minolta chroma meter than when measured by the lens eye program which showed more red color in the fillets.

On day 0, b* values were negative, indicating blue color of the fillets. A significant increase in b* value was noted in all groups, indicating that all groups became less blue and more yellow with time, with positive values noted in all groups on day 16 ($p<0.05$).

As the redfish fillets are not uniform in color, using minolta chroma meter as a colour analysis tool proved difficult as only a small part of the fillet is analyzed.

A computer vision system covers greater area of the sample for the color analysis than a minolta chroma meter. Therefore if the sample is not uniform in color, a computer system would be more accurate in the colour measurements (Oliveira & Balaban, 2006).

It should be noted that any difference in color between group H1 and H2, H3 and H4 observed from the colour analysis (by lens eye and by minolta chroma meter) might be due to the smaller size of the fillets in the H1 group. Therefore, making it hard to compare the colour of the H1 group to the colour of other groups.

5.12 Sensory Analysis

Results of sensory analysis in experiment II showed that TMA odour seems to have developed more rapidly in the M1 group than in the other two groups. Groups M1 and M2 retained their freshness longer than the Air group. After 6 days of storage, Air group received a lower Torry score than the other two groups. However difference in

Torry score on day 10 is not significantly different. A lower and steadier temperature of the Air group than of the other two groups might explain slower deterioration in the Air group. It is difficult to estimate the shelf life of the Air group since it was not evaluated on storage day 14, but groups M1 and M2 had a shelf life of 12 days from processing. TMA odour was the most apparent spoilage attribute on storage day 14.

Results of sensory analysis in experiment III showed a very little difference between the four groups during the storage time. All groups had a shelf life of 12 days. The H3 group might have a tendency to retain freshness a little longer than other groups and results indicate a very slight increase of rancid flavour with storage in the H1 group.

6 CONCLUSIONS

Lipid oxidation measurements in experiment I, showed some conflicting results as primary and tertiary oxidation measurements showed no benefit of bleeding whereas secondary measurements showed no secondary oxidation in either of the bled groups. However, as heme iron content measured higher in both bled groups it can be assumed that the bleeding was not effective. No clear pattern was observed when looking at the different sections of the fish. However, when compared to the middle and loin sections, the tail section had the highest values of FFA, TBARS on day 8 post-packaging. This may have been caused by higher enzymatic, and/or microbial action in the tail than in the other two sections. The colour measurements showed that bleeding the fish did not significantly affect the colour of the fillets. However, visual observations showed that bled fillets were lighter in colour than unbled fillets on the day of packaging. This colour difference evened out with storage time.

Overall the greatest effect of the MAP packaging in experiment II was observed in the sensory analysis as the freshness period increased by about 1.5 days. MAP was effective in reducing lipid oxidation to some degree. Microbiological observations revealed no difference between the Air group and the two MAP groups in terms of TVC, and the growth of *Photobacterium phosphoreum*. However, MAP was beneficial in reducing the number of *Pseudomonads*. The benefits of adding CO_2 pads to the gas packed fish were not significant with respect to lipid oxidation and microbial growth. The colour of the fish was not affected by MAP packaging. MAP fish had lower pH than air packed fish with further reduction in pH in the M2 group. Results of drip loss measurements showed an increase in drip as pH was lowered with the highest drip loss observed in the M2 group and the lowest drip loss in the Air group. The sensory shelf life of the two MAP groups was determined to be about 12 days, counting from the day of packaging. Therefore, the total shelf life was 18 days counting from the day of catch.

In experiment III, the addition of the CO_2 pads to EPS boxes in was not successful in lowering the pH of the fish to a significant level. As EPS boxes are not airtight, it is likely that much of the CO_2 gas escaped during storage. Therefore, EPS boxes without inner, airtight seal cannot be recommended for gas packaging. It should be noted that the fillets in bled group, H1, were much smaller than the unbled fillets in groups H2,

H3, and H4. This may have affected the outcome of this experiment, as the spoiling pattern may be affected by size of the fish. As in experiment I, the bleeding of the fish in experiment III did not prove to be effective in reducing heme iron in the fish muscle. The different treatments and packaging methods did not affect the rate of lipid oxidation or microbial growth. The bled fish had more lightness than the other groups throughout storage time. However the difference in lightness evened out with storage time. Fish placed in boxes by sandwich lining, H3 group, had more lightness than fish placed in boxes with skin side up, H2 group. Redness was similar in all groups throughout storage time. Yellowness was similar in all groups throughout storage however a trend was noted with the H1 group being more yellow than the other three groups. The sensory shelf life of the four groups was between 12 and 13 days, counting from the day of packaging. Therefore, the total shelf life was 15 to 16 days, counting from the day of catch.

7 FUTURE PERSPECTIVES

The findings in this study show a promising benefit of bleeding the redfish, which would increase the overall quality of the redfish. However, bleeding methods must be optimized in order to reduce the heme iron content of the fish. Modified atmosphere packaging (MAP) proved to reduce lipid oxidation and increase the freshness period of the fish. However, overall shelf life was not increased. MAP induces greater costs for the fishing industry, which may not be feasible for the export of fresh redfish fillets. EPS boxes are great insulators, and have proven effective in keeping the product below 0 °C. However the terms of environmental aspects, they are not reusable and therefore contribute to a large waste. In the near future new reusable boxes must be designed, perhaps plastic covered EPS boxes, which can be sanitized, and used again in the fishing industry.

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Appendix A

June sensory score.

Table A.1. *Mean scores for sensory attributes for groups A, M1 and M2. Different letters within a column each day represent statistical difference between groups.*

Group	Torry	O-sweet	O-liver	O-shellfish	O-vanilla	O-potatoes	O-rancid	O-cloth	O-TMA	O-queasy	O-sour	O-sulphur	A-colour	A-heterog.	A-precipit.	A-flakes
Day0	8,8	45,3	38,9	22,3	14,1	4,1	0,3	1,6	0,3	0,2	0,2	0,4	27,6	31,9	33,1	51,9
Day6																
A	7,7 **b**	40	29	20	11	9	0	2	0	2	0	0	35	35	43	39
M1	8,4 **a**	42	32	21	13	4	0	0	0	1	1	0	29	29	44	40
M2	8,3 **a**	40	27	19	10	8	1	2	1	0	1	0	29	35	45	40
p-value	0,023	0,736	0,507	0,597	0,382	0,098 **ms**	0,254	0,173	0,280	0,143	0,535	0,185	0,109	0,263	0,954	0,966
Day10																
A	6,6	32	27	19	12	14	2	6	1 **b**	4	2	0	31	34	45	38
M1	6,9	33	27	21	11	14	2	8	7 **a**	5	3	2	32	36	38	46
M2	6,9	31	25	19	10	14	1	5	2 **b**	4	2	1	33	35	41	40
p-value	0,437	0,586	0,659	0,497	0,636	0,964	0,376	0,595	0,010 **	0,715	0,645	0,173	0,838	0,908	0,172	0,152
Day14																
M1	4,0	13	12	9	7	12	3	22	44	7	22	18	43	39	48	38
M2	4,0	16	11	10	11	17	7	24	39	10	26	13	46	41	49	44
p-value	1,000	0,437	0,690	0,674	0,187	0,196	0,061 **ms**	0,447	0,539	0,224	0,346	0,178	0,670	0,545	0,737	0,174

Group	F-liver	F-metallic	F-sweet	F-rancid	F-pungent	F-queasy	F-sour	F-TMA	F-off	T-soft	T-juicy	T-tender	T-mushy	T-sticky
Day0	46	51	46	0	2	0	0	0	0	47	44	55	26	45
Day6														
A	30 **b**	34	27 **b**	4	6	6 **a**	1	0	0	48	46	55	26	39
M1	37 **a**	39	38 **a**	0	2	0 **b**	0	1	0	50	46	54	26	37
M2	30 **b**	38	35 **a**	2	4	3 **b**	0	1	0	50	49	54	27	38
p-value	0,025 *	0,323	0,003 **	0,084 **ms**	0,329	0,005 **	0,361	0,842	0,612	0,874	0,566	0,964	0,950	0,844
Day10														
A	29	35	24	6	10 **a**	7	3	4	1	57	47	57	30	35
M1	29	39	28	3	9	5	1	5	1	55	51	58	30	37
M2	31	34	28	2	5 **b**	4	2	1	0	53	44	52	33	44
p-value	0,668	0,078 **ms**	0,344	0,147	0,043 *	0,601	0,135	0,124	0,308	0,569	0,145	0,165	0,766	0,089 **ms**
Day14														
M1	17	16	12	5	21	10	17	26	15	57	51	57	32	32
M2	18	18	12	7	20	15	17	21	13	55	50	55	38	33
p-value	0,769	0,591	0,954	0,520	0,908	0,193	0,961	0,566	0,583	0,815	0,876	0,592	0,387	0,906

Table A.2. *Torry score sheet for freshness evaluation of cooked medium fat fish such as redfish.*

Odour	Flavour	score
		10
Initially weak odour of boiled cod liver, fresh oil, starchy	Boiled cod liver, watery, metallic	
		9
Shellfish, seaweed, boiled meat, oil, cod liver	Oily, boiled cod liver, sweet, meaty, characteristic	
		8
Loss of odour, neutral odour	Sweet and characteristic flavours, but reduced in intensity.	
		7
Woodshavings, woodsap, vanillin	Neutral	
		6
Condensed milk, boiled potato	Insipid	
		5
Milk jug odours, boiled clothes- like	Slight sourness, trace of "off"-flavours, rancid	
		4
Lactic acid, sour milk, TMA	Slight bitterness, sour, "off"-flavours, TMA, rancid	
		3
Lower fatty acids (eg acetic or butyric acids) composed grass, soapy, turnipy, tallowy	Strong bitter, rubber, slight sulphide, rancid	

Appendix B

October sensory score.

Table B.1. *Mean scores for sensory attributes for groups H1, H2, H3 and H4. Different letters within a column each day represent statistical difference between groups.*

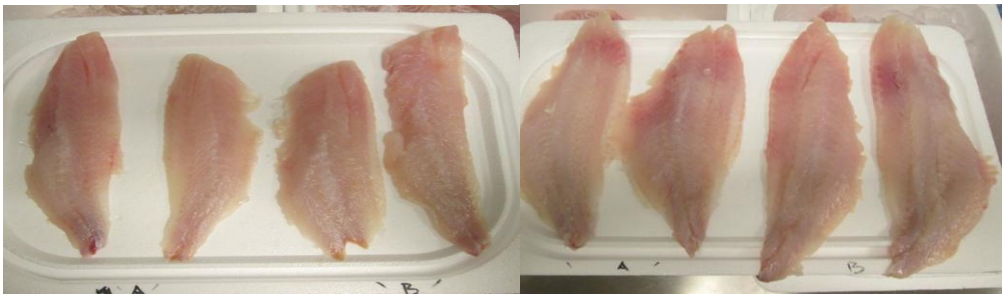
Group	Torry	O-sweet	O-liver	O-shellfish	O-vanilla	O-potatoes	O-rancid	O-cloth	O-TMA	O-queasy	O-sour	O-sulphur	A-colour	A-heterog.	A-precipit.	A-flakes
D0																
Control	8,9	47	44	31	10	6	1	0	1	1	0	1	33	33	32	47
Bled	8,8	49	43	28	9	3	2	0	1	0	1	0	21	20	31	44
p-value		0,257	0,863	0,385	0,445	0,185	0,507	0,663	0,337	0,663	0,430	0,011	0,079	0,039	0,637	0,592
D6																
H1	7,0	32	23	11	10	4	1	1	0	1	**0 b**	0	38	**25 b**	40	43
H2	6,9	**27 b**	19	13	9	6	1	1	1	**0 b**	**0 b**	0	37	38	38	43
H3	7,4	**37 a**	25	19	9	3	0	0	0	**1 b**	**0 b**	0	38	**42 a**	44	46
H4	6,7	31	22	13	9	6	1	2	1	**5 a**	**2 a**	0	37	36	45	43
p-value		0,039	0,329	0,060	0,933	0,550	0,627	0,084	0,606	0,044	0,001	0,330	0,997	0,018	0,660	0,930
D10																
H1	6,6	26	18	19	14	13	3	5	1	6	2	**1 a**	34	36	43	49
H2	7,0	28	21	21	13	15	2	**2 b**	1	4	0	**0 b**	30	35	43	48
H3	7,2	32	20	20	16	15	1	5	1	5	2	1	31	34	45	49
H4	7,1	30	18	20	13	19	1	**6 a**	1	4	1	**0 b**	30	31	42	49
p-value		0,475	0,892	0,971	0,806	0,268	0,361	0,026	0,356	0,404	0,379	0,022	0,686	0,372	0,835	0,982
D13																
H1	4,7	13	8	10	10	25	4	17	10	14	9	**1 b**	39	41	43	43
H2	4,8	15	10	11	12	22	3	18	9	16	12	**5 a**	36	40	43	41
H3	5,0	11	7	10	7	21	1	22	13	13	13	2	40	37	46	47
H4	4,7	10	7	10	11	20	7	24	14	16	14	**1 b**	39	40	43	43
p-value		0,305	0,646	0,953	0,269	0,629	0,361	0,454	0,774	0,830	0,792	0,007	0,782	0,827	0,649	0,464

Group	F-liver	F-metallic	F-sweet	F-rancid	F-pungent	F-queasy	F-sour	F-TMA	F-putrid	T-soft	T-juicy	T-tender	T-mushy	T-sticky
D0														
Control	46	54	45	1	1	1	0	0	1	62	53	57	21	31
Bled	47	51	52	1	2	0	0	0	0	59	55	55	22	30
p-value	0,648	0,376	0,184	0,394	0,953	0,001	1,000	1,000	0,337	0,622	0,721	0,767	0,750	0,863
D6														
H1	30	32	26	4	10	3	1	0	0	52	43	53	30	29
H2	24	28	23	1	4	3	1	1	0	47	43	49	20	28
H3	28	33	29	2	5	2	0	2	0	47	46	48	25	29
H4	26	31	24	6	6	6	1	2	0	44	42	46	22	30
p-value	0,888	0,870	0,722	0,362	0,451	0,696	0,722	0,499	0,218	0,730	0,932	0,837	0,332	0,973
D10														
H1	20	32	23	**10 a**	8	8	3	1	**1 a**	58	54	59	26	30
H2	22	36	21	**2 b**	5	7	1	1	**1 a**	56	50	58	26	**35 a**
H3	26	33	24	**2 b**	5	7	1	1	**1 a**	56	50	57	25	32
H4	24	35	21	**2 b**	8	8	1	0	**0 b**	56	49	57	24	**26 b**
p-value	0,268	0,495	0,808	0,021	0,494	0,820	0,314	0,328	0,004	0,829	0,461	0,964	0,900	0,086
D13														
H1	11	13	11	14	23	11	13	10	7	50	49	49	23	24
H2	11	17	12	6	16	14	7	10	4	53	52	54	30	25
H3	16	15	10	2	20	13	8	5	8	60	55	58	32	25
H4	9	11	13	6	16	19	9	10	14	57	55	58	24	26
p-value	0,516	0,390	0,932	0,215	0,590	0,576	0,324	0,764	0,278	0,156	0,149	0,076	0,697	0,874

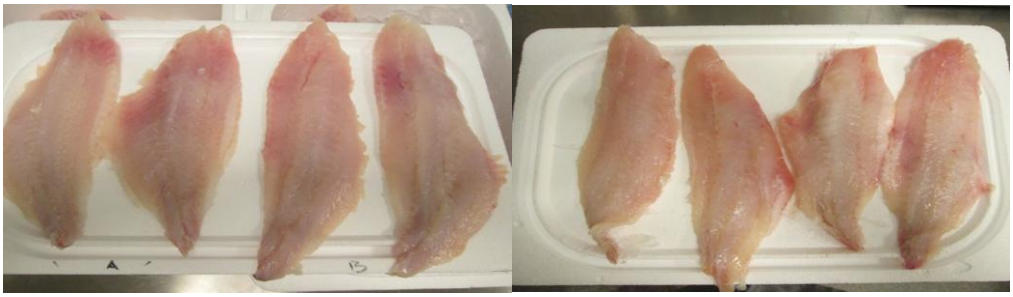
Appendix C

Pictures from experiment III showing the colour of bled and unbled fillets.

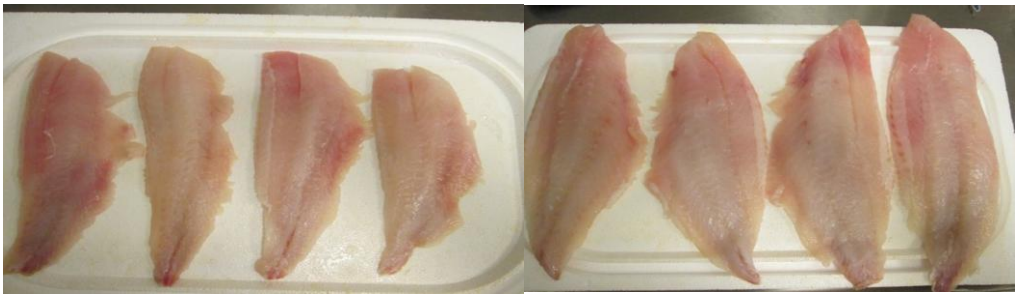
Dagur 0



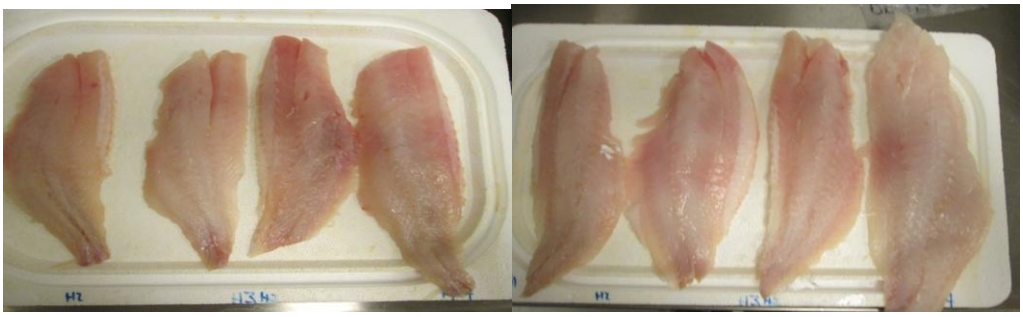
Dagur 6



Dagur 10



Dagur 13



Dagur 16

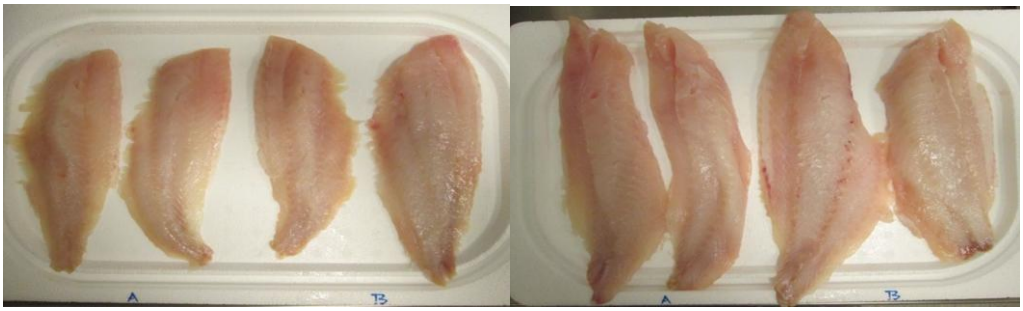


Figure C.1*. The colour of bled redfish fillets on the left side and unbled redfish fillets on the right side on all sampling days in experiment III.*

Appendix D

Results of TVC on Long and Hammers (LH) agar from experiment II.

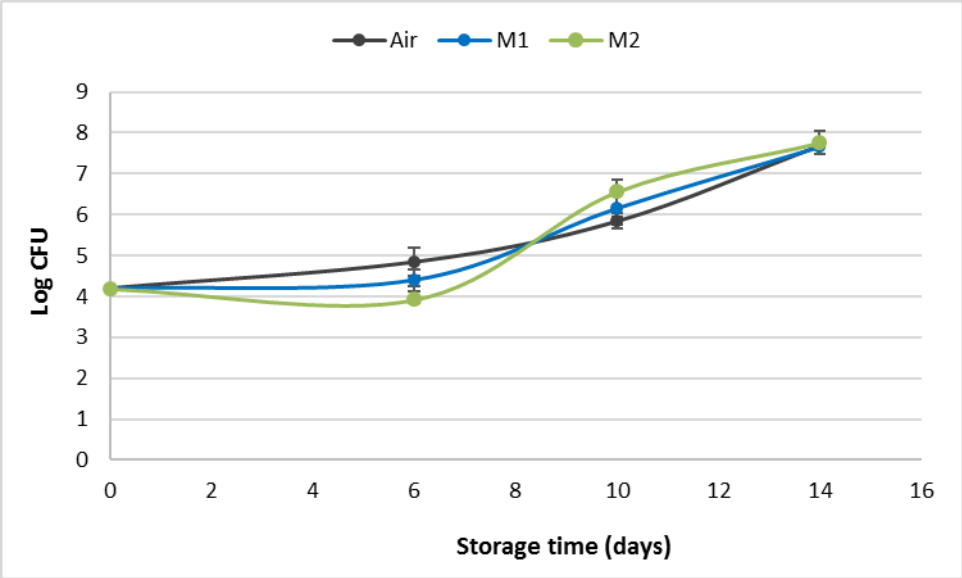


Figure D.1. *TVC on LH agar for the Air, M1 and M2 groups. Vertical bars show standard deviation from the mean value (n=2).*

Appendix E

A comparison of fatty acid composition of experiments I and II.

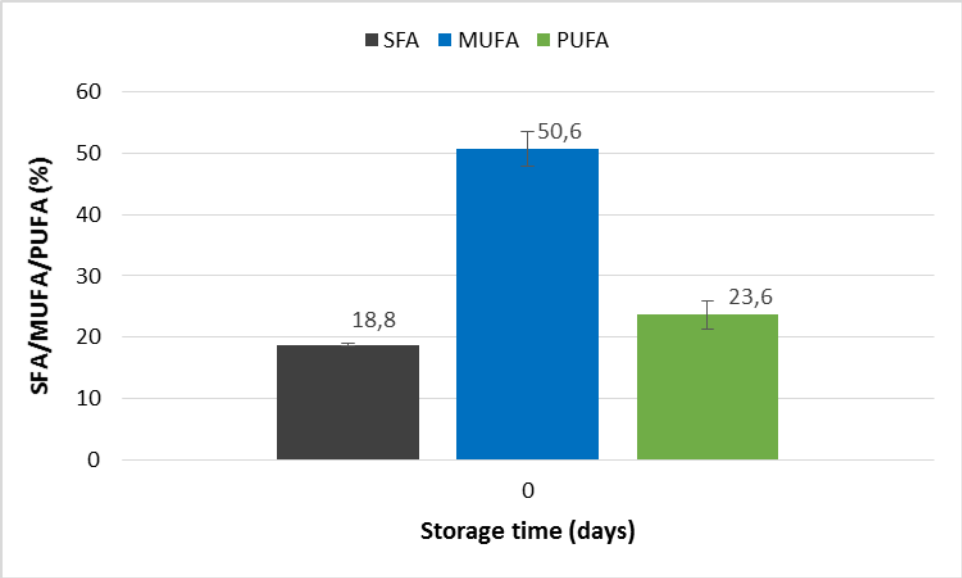


Figure E.1. *Average value of SFA, MUFA and PUFA in October fish (experiment III). Vertical bars show standard deviation from the mean value (n=2).*

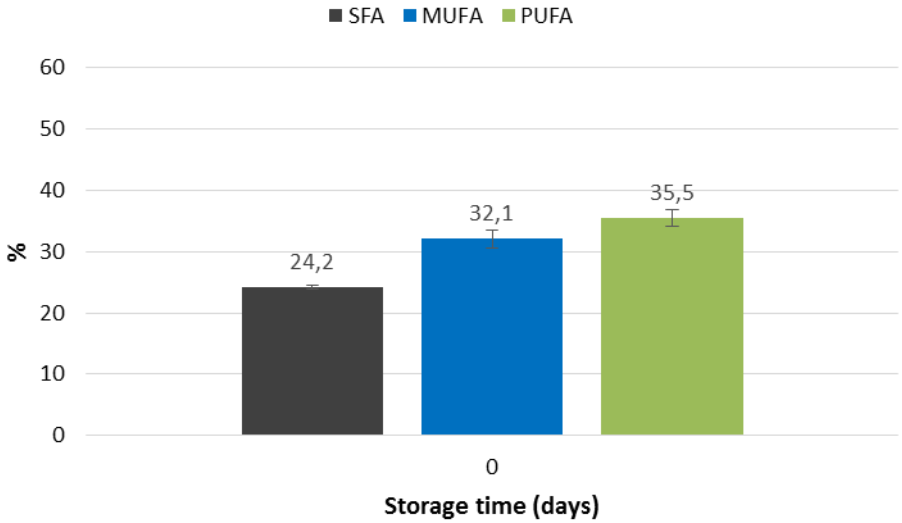


Figure E.2. *Average value of SFA, MUFA and PUFA in May fish (experiment I). Vertical bars show standard deviation from the mean value (n=2).*

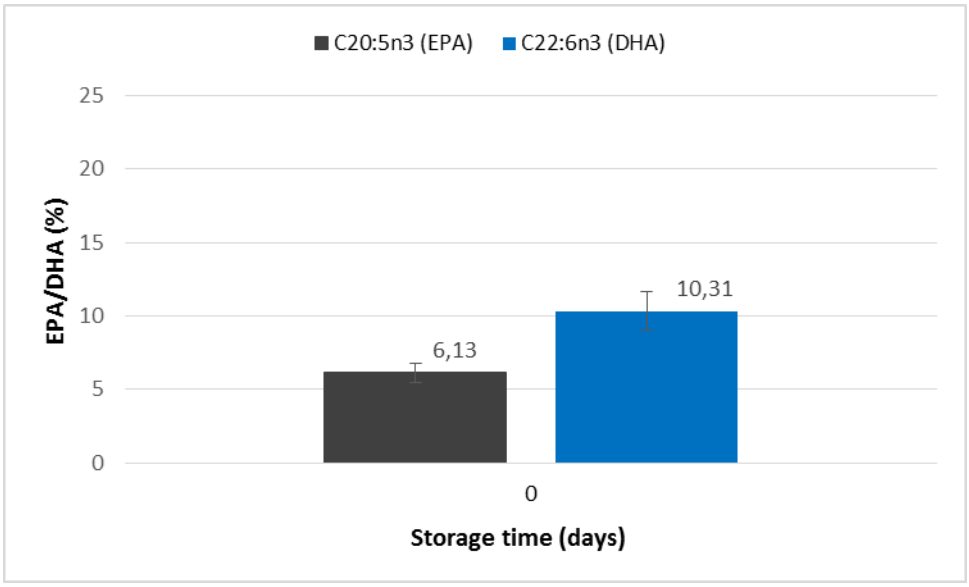


***Figure E.3**. Average value of EPA and DHA in unbled fish (experiment III – October). Vertical bars show standard deviation from the mean value (n=2).*

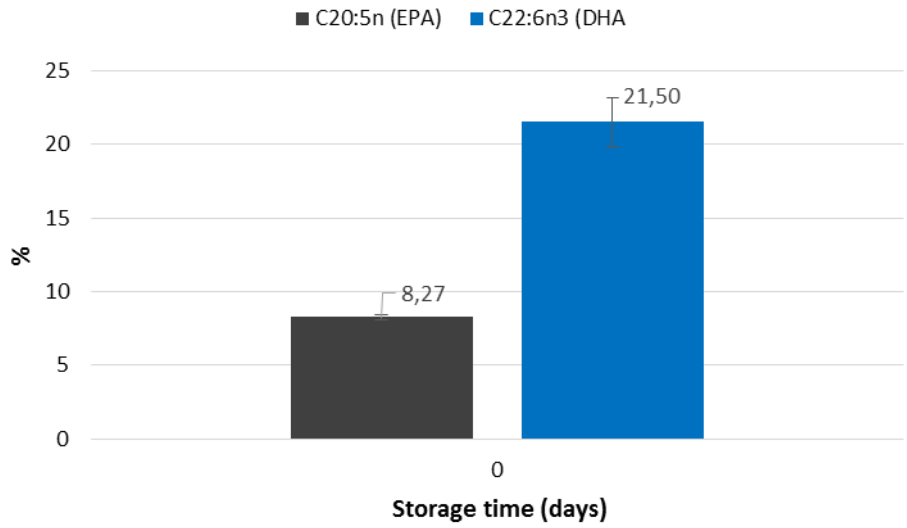


***Figure E.4.** Average value of EPA and DHA in unbled fish (experiment I – May). Vertical bars show standard deviation from the mean value (n=2).*